

Научном већу Института за физику Београд

Београд, 20. април 2021.

Предмет:

Молба за покретање поступка за избор у звање научни сарадник

С обзиром да испуњавам критеријуме прописане од стране Министарства просвете, науке и технолошког развоја и Научног веће Института за физику у Београд за стицање звања научни сарадник, молим Научно веће Института за физику у Београду да покрене поступак за мој избор у наведено звање.

У прилогу достављам:

1. Мишљење руководиоца лабораторије са предлогом чланова комисије за избор у звање
2. Стручну биографију
3. Преглед научне активности
4. Елементе за квалитативну и квантитативну оцену научног доприноса са доказима
5. Списак објављених научних радова и њихове копије
6. Податке о цитираности
7. Уверење о одбрањеној докторској дисертацији

Са поштовањем,

др Ненад Селаковић

Научном већу Института за физику Београд

Београд, 20. април 2021. године

Предмет:

Мишљење руководиоца лабораторије о избору др Ненада Селаковића у звање научни сарадник

Ненад Селаковић је запослен у Лабораторији за неравнотежне процесе и примену плазме и ангажован у раду Центра изузетних вредности Центра за неравнотежне процесе Института за физику у Београду. У истраживачком раду бави се дијагностикама нискотемпературних плазми на атмосферском притиску као и њиховим применама у биомедицини.

С обзиром да испуњава све предвиђене услове у складу са Правилником о поступку, начину вредновања и квантитативном исказивању научноистраживачких резултата Министарства просвете науке и технолошког развоја, сагласна сам са покретањем поступка и предлажем избор др Ненада Селаковића у звање научни сарадник.

За састав комисије за избор др Ненада Селаковића у звање научни сарадник предлажем:

- 1) др Невена Пуач, научни саветник, Институт за физику у Београду
- 2) др Гордана Маловић, научни саветник, Институт за физику у Београду
- 3) проф др Срђан Буквић, редовни професор, Физички факултет Универзитета у Београду

др Гордана Маловић

Научни саветник

Руководилац Лабораторије за неравнотежне процесе и примену плазме

1. Стручна биографија кандидата

Ненад Селаковић је рођен 14.11.1981. године у Београду где је завршио основну и средњу школу. Основне академске студије на Физичком факултету Универзитета у Београду – смер Примењена физика и информатика је уписао школске 2000/2001. године и завршио са просечном оценом 8.00. Физички факултет је завршио 11.10.2011. одбраном дипломског рада на тему „*Електрична карактеризација и просторно-временски разложена мерења атмосферског пражњења у режиму плазма метка*” са оценом 10, под менторством др Невене Пуач и др Гордане Маловић у Лабораторији за гасну електронику под руководством др Зорана Љ. Петровића. Добитник је награде “Проф. др Љубомир Ћирковић” за најбољи дипломски рад одбрањен на Физичком факултету у периоду 2010/2011.

Од 2011. године је студент докторских студија на Физичком факултету, Универзитета у Београду – смер Физика јонизованог гаса и плазме. Положио је све изборне испите са просечном оценом 10.00. Докторска дисертација под називом „*Масена спектрометрија плазменог млаза и примене електричних пражњења на атмосферском притиску у биомедицини*“ урађена је под менторством др Невене Пуач у Лабораторији за неравнотежне процесе и примену плазме у Институту за физику и одбрањена 19. 2. 2021. на Физичком факултету, Универзитета у Београду.

Ненад Селаковић је у радном односу од 31.12.2011. године у Институту за физику у Београду у Лабораторији за гасну електронику (сада Лабораторија за неравнотежне процесе и примену плазме) под руководством проф. др Зорана Љ. Петровића до децембра 2019. а од јануара 2020. под руководством др Гордане Маловић. На седници Научног већа Института за физику одржаној 23.9.2014. године изабран је у звање Истраживач сарадник, а реизабран у исто звање на седници Научног већа Института за физику одржаној 19.9.2017. године.

Аутор и коаутор је шест научних радова који су објављени у међународним часописима категорија M21a, M21 и M23. Резултати његових истраживања су презентовани на великом броју међународних конференција. Био је коаутор већег броја предавања по позиву (12) и већег броја радова штампаних у изводу (9) и у целини (9).

2. Преглед научне активности кандидата

Кандидат Ненад Селаковић се бави истраживањима која спадају у област физике јонизованих гасова и плазми, у Лабораторији за неравнотежне процесе и примену плазме под руководством др Гордане Маловић (раније Лабораторија за гасну електронику коју је водио академик Зоран Љ. Петровић) у Институту за физику у Београду. Главна тематика његових истраживања су дијагностика и примене електричних пражњења на атмосферском притиску у биомедицини. Посебан фокус његових истраживања представља масена спектрометрија атмосферских плазми. До сада је радио на више извора атмосферских плазми: плазмени млаз, плазма игла, многоструки плазмени млаз и диелектрична копланарна баријерна пражњења. Дијагностичке методе које је користио у својим истраживањима су: масена спектрометрија, снимање брзом ICCD камером, електрична карактеризација извора електричних пражњења и испитивање промене контактеног угла третираног узорка.

Са аспекта примена нискотемпературних пражњења на атмосферском притиску, рађен је третман ћелија биљног порекла (у сарадњи са Биолошким институтом “Синиша Станковић”) као и МРСА бактерија (у сарадњи са Институтом за Микробиологију и имунологију, Медицинског факултета, Универзитета у Београду), третман људског дентина (у сарадњи са Стоматолошким факултетом, Универзитета у Београду) и третман канцерогених ћелија (у сарадњи са Институтом за Онкологију и Радологију Србије) коришћењем плазма игле.

Истраживачки рад и научни резултати које је до сада остварио др Ненад Селаковић могу се груписати у следеће теме:

1. Масена спектрометрија и ICCD снимање временске еволуције простирања плазменог млаза:
 - Утицај влажности на простирање пражњења и њен масени састав
 - Синхронизована мерења временске еволуције простирања плазме и позитивних јона
2. Масена спектрометрија многоструког плазменог млаза и ICCD снимање временско-просторне еволуције његовог простирања
3. Масена спектрометрија диелектричног копланарног баријерног пражњења
4. Примена плазма игле на узорке биолошког порекла

У првој теми кандидат је испитивао утицај влажности унутар проточног система радног гаса на формирање дискретног пакета плазме који се креће великом брзином у односу на брзину протока радног гаса, тзв. PAPS-a (*eng.* Pulsed Atmospheric pressure Plasma Streamer). При мерењима у експерименталној поставци је коришћена испираница за вештачко додавање влажности у проточни систем радног гаса као и варијанта без ње. Установљено је да без влажности унутар проточног система, тј. када је он скроз просушен, PAPS губи свој облик и пражњење је дифузно. Такође, констатовано је да вештачки додата водена пара враћа форму PAPS-a али му смањује диметр. Састав плазме зависи од параметара који су коришћени, што је показано масеном спектрометријом. Вештачко додавање влажности значајно утиче на хидратацију и повећање продукције врста као што су HeH^+ , H_3O^+ , OH^+ , H_2O^+ и доводи до смањења одброја, N_2^+ , O_2^+ , N , O , He , итд.

У првој теми је кандидат радио, такође и на испитивању временске еволуције PAPS-а коришћењем синхронизованих мерења масеним спектрометром, ICCD камером и напонским сондама. Из добијених мерења је, поред праћења разложених снимака интензитета светлости плазменог млаза, одређен и масени састав најзаступљенијих врста које сачињавају пражњење а самим тим и PAPS. Урађено је поређење за два различита протока радног гаса на фиксном растојању плазменог млаза у односу на масени спектрометар и при фиксном примењеном напону. Установљено је да промена протока не утиче значајно на простирање тежих врста која се крећу прилично синхронизовано, док су лакше атомске врсте попут водоника фазно померене. Одређивање и варирање састава PAPS-a овом методом може бити од великог значаја за потенцијалне будуће примене. Описани резултати су приказани у дисертацији др Ненада Селаковића:

- **N. Selaković**, “Masena spektrometrija plazmenog mlaza i primene električnih pražnjenja na atmosferskom pritisku u biomedicini”, Fizički fakultet, Univerzitet u Beogradu, 2021.

У другој теми др Ненад Селаковић је радио на масеној спектрометрији и ICCD оптичко емисионој спектроскопији интензитета светлости многоструког плазменог млаза - МПМ. Испитивана је међусобна интеракција млазница многоструког млаза. Варијацијом брзине протока радног гаса могуће је комплетно променити природу пражњења овог извора. Правим избором протока могуће је добити спојени или неспојени режим који имају међусобно различит број јонизационих фронтова, различиту временску еволуцију и масени

састав. Примећена је и појава вишеструких секундарних стримера који се крећу дуж понављајућих јонизационих канала током рада МПМ-а. Из електричне карактеризације МПМ у спојеном режиму исказује нелинеарност током пражњења услед повећања напона побуде коришћењем сигнал генератора. Описани резултати су објављени у врхунском међународном часопису:

- Stancampiano, A., **Selaković, N.**, Gherardi, M., Puač, N., Petrović, Z. Lj., & Colombo, V. (2018). Characterisation of a multijet plasma device by means of mass spectrometric detection and iCCD imaging. *Journal of Physics D: Applied Physics*, 51(48), 484004. (M21, IF: 2.373)

У трећој теми кандидат је радио на масеној спектрометрији диелектричног копланарног баријерног пражњења малих димензија. За шест различитих смеша кисеоника и азота (највећа пажња је посвећена односу 80/20 што представља однос који је у атмосфери) и три различите фреквенције (15 kHz, 30 kHz и 50 kHz) при констатном високом напону (11.2 kV) посматрана је продукција реактивних кисеоничних и азотних врста, RONS-а (*eng.* Reactive Oxygen and Nitrogen Species) (N, O, OH, N₂, NO, O₂, NO₂, N₂O или CO₂ и O₃) у електричном пражњењу. Посебна пажња је посвећена врстама од значаја као што су N, NO_x или O₃, јер ове врсте играју важну улогу у интеракцији плазме са живим организмима и ткивима. Варирање експерименталних услова је показало значајне разлике. При вишим фреквенцијама од 30 kHz и 50 kHz примећен је максимум одброја за NO радикале у условима 80/20 односа азота и кисеоника. За најнижу фреквенцију од 15 kHz производња молекула O₃ и NO била је највећа у испитиваном распону од 15 kHz –50 kHz. У зависности од типа примене продукција ових врста се варирањем параметара може смањити или повећати. Описани резултати су објављени у међународном часопису:

- Šech, J., Brablec, A., Černák, M., Puač, N., **Selaković, N.**, & Petrović, Z. Lj. (2017). Mass spectrometry of diffuse coplanar surface barrier discharge: influence of discharge frequency and oxygen content in N₂/O₂ mixture. *The European Physical Journal D*, 71(2), 27. (M23, IF: 1.393)

У четвртој теми кандидат је користио плазма иглу за третман биљних ћелија калуса – *lat. Daucus Carota*. Значајни ефекти на узорцима су добијени одмах након третмана као и две недеље касније. За ово су заслужне реактивне кисеоничне врсте – ROS које се стварају у ћелијама и у пражњењу плазма игле и они изазивају активност ензима унутар ћелија.

Ензими су део одбрамбеног механизма од оксидативног стреса, до којег долази уколико је вредност ROS-а високе концентрације. Активност SOD ензима, иако је и даље била знатно већа од активности у нетретираним ћелијама, смањила се после две недеље у поређењу са активношћу непосредно после третмана. Активност CAT ензима се значајно повећава након две недеље третмана, што доводи до веће концентрације H₂O₂ у самој ћелији. Већа концентрација водоник-пероксида може да изазове појаву морфогенезе код саме биљке. Описани резултати су објављени у врхунском међународном часопису:

- Puač, N., Živković, S., **Selaković, N.**, Milutinović, M., Boljević, J., Malović, G., & Petrović, Z. Lj. (2014). Long and short term effects of plasma treatment on meristematic plant cells. *Applied Physics Letters*, 104(21), 214106. (M21, IF=3.515)

У истој теми кандидат је такође користио плазма иглу за третман MRSA (*meticilin rezistivne lat. Staphylococcus aureus*) бактерија где су варирани следећи параметри: растојање плазма игле од површине узорка (2 mm, 4 mm и 6 mm), снага плазме (1.0 W и 2.5 W), време експозиције, тј. време излагања узорка електричном пражњењу (30 s, 60 s, 90 s и 180 s). Третиране су бактерије на хранљивој подлози у Петри посуди и биофилм бактерија у миктортитар плочицама. Добијени резултати третмана имплицирају да је најефикаснији сет параметара најдуже време експозиције и најмање растојање од плазма игле у односу на узорак. Плазма игла без обзира што формира видљиво пражњење дијаметра 3-4 mm за одређени сет параметара својим деловањем формира инхибиционе зоне MRSA бактерија значајно већег дијаметра (за најдуже време експозиције 180 s и снагу плазме од 2.5 W је она износила 9 mm). Претпоставка је да се ширење дешава под утицајем реактивних врста генерисаних у електричном пражњењу на атмосферском притиску.

Такође, у овој теми кандидат је радио на примени плазма игле у третману канцерогених ћелија: аденокарцином плућа човека (A549), ћелије карцинома хуманог грлића материце (ХелПа), 3Д модел културе вишећелијских туморских сфероида (A549) као и нормалне људске ћелије: ћелије бронхијалног епителија људског порекла без рака (БЕАС-2Б). Показано је да у већој или мањој мери плазма игла свакако смањује вијабилност код канцерогених ћелија. У случају људских неканцерогених ћелија тај ефекат је слабији и те ћелије се брже опорављају. Овај тип пражњења при лечењу канцерогених ћелија може да изазове поремећај у фазама ћелијског циклуса и да смањи њихову продукцију. Приказани су и прелиминарни резултати третмана 3Д модела, тј. кластера канцерогених ћелија који за испитивање представљају велики корак ка ин vivo третманима јер се понашају као ткива

канцера. За сад се нису добили већи ефекти у покушају њиховог смањења након третмана и потребна су додатна истраживања. Ови резултати су приказани у већ наведеној докторској дисертацији кандидата.

3. Елементи за квалитативну оцену научног доприноса кандидата

3.1. Квалитет научних резултата

3.1.1. Значај научних резултата

Кандидат се у току досадашњег рада бавио проучавањем масеног састава као и простирањем неравнотежних плазми на атмосферском притиску. Испитивао је утицај влажности у протоку радног гаса плазменог млаза на понашање и састав плазме. Овде се може истаћи да утицај влажности у протоку радног гаса на простирање електричног пражњења није детаљније испитиван у литератури. Показано је да услед просушивања проточног система радног гаса PAPS плазменог млаза губи своју форму, да би при додавању малог процента водене паре у проток вратио свој првобитан облик али са краћим дометом.

Временски разложеним мерењима масеном спектрометријом може се одредити састав плазме током временске еволуције пражњења. Позиција самог извора у односу на масени спектрометар снажно утиче на само праћење простирања пражњења што је за ову технику било од посебног значаја. Овај резултат је битан при дизајнирању самог хемијског састава варирањем експерименталних параметара плазменог млаза, који има велики потенцијал у биомедицинским применама. Приликом третмана бактерија плазма иглом долази до њихове значајне редукције, што се може закључити и код третмана канцерогених ћелија. Третман биљних ћелија плазма иглом, која производи реактивне кисеоничне врсте, тригерује активност ензима унутар физиолошког система и самим тим може да утиче на повећање брзине раста биљке.

3.1.2. Параметри квалитета часописа

Кандидат др Ненад Селаковић је објавио укупно 6 радова у међународним часописима и то:

- 1 рад у међународном часопису изузетних вредности (M21a) Plasma Sources Science and Technology (IF= 3.591 SNIP= 1.951 (вредности за 2014. годину))
- 1 рад у врхунском међународном часопису (M21) Applied Physics Letters (IF= 3.515, SNIP= 1.634 (вредности за 2013. годину))
- 2 рада у врхунском међународном часопису (M21) Journal of Physics D: Applied Physics (IF2016=2.588 SNIP2016=1.135, IF2015= 2.772, SNIP2015= 1.329)
- 1 рад у врхунском међународном часопису (M21) Clinical Oral Investigations (IF= 2.453, SNIP= 1.269 (вредности за 2018. годину))
- 1 рад у међународном часопису (M23) European Physical Journal D: Atomic, Molecular, Optical and Plasma Physics (IF= 1.393, SNIP= 0.684 (вредности за 2017. годину))

Укупан импакт фактор објављених радова др Ненада Селаковића износи 16.312. Додатни библиометријски показатељи према упутству о начину писања извештаја о изборима у звања које је усвојио Матични научни одбор за физику су:

	ИФ	М	СНИП
Укупно	16.312	45	8.002
Усредњено по чланку	2.72	7.5	1.334
Усредњено по аутору	2.33	6.43	1.143

3.1.3. Позитивна цитираност научних радова кандидата

Према бази Web of Science радови др Ненада Селаковића су цитирани 67 пута (без аутоцитата 61 цитата), а Хиршов индекс је 5. Према бази Google Scholar-а радови др Ненада Селаковића су цитирани 88 пута (без аутоцитата 82 цитата), а Хиршов индекс је 5.

3.1.4. Међународна сарадња

- 2016 учешће у европској COST акцији TD1208 кроз STSM под називом „LIF (TALIF) of atmospheric pressure plasma jet” у периоду од 7.3.2016. – 25.3.2016. у лабораторији др Павела Дворжака на Масарик универзитету у Брну, Република Чешка
- Учесће у школи одржаној у Београду у септембру 2016. године – 3rd Training School: Advanced Diagnostics of Discharges with Liquids and Plasma Treated Liquid Phase у оквиру COST акције TD1208 Electrical discharges with liquids for future applications
- Учесће у летњој плазма школи - Plasma school Bad Honnef, одржаној у октобру 2019. године под називом „International School on Low Temperature Plasma Physics: Basics and Applications“ у Бад Хонефу, Савезна Република Немачка

3.2. Нормирање броја коауторских радова

Сви публиковани радови др Ненада Селаковића спадају у радове експерименталне природе. Према Правилнику о поступку и начину вредновања и квантитативном исказивању научноистраживачких резултата истраживача у случају експерименталних радова предвиђено је до 7 коаутора. Укупан ненормиран број бодова је 64.5, док је нормиран број М бодова 61.27 што је знатно више у односу на захтеваних 16 бодова за избор у научног сарадника.

3.3. Учесће на пројектима МПНТР Републике Србије

Др Ненад Селаковић је учествовао на пројекту Министарства просвете, науке и технолошког развоја:

2012.–2019. „Примене нискотемпературних плазми у биомедицини, заштити човекове околине и нанотехнологијама“ (ИИИ41011).

Учесник је Центра изврности - Центра за неравнотежне процесе под руководством академика Зорана Петровића.

3.4. Активности у научним и научно-стручним друштвима

3.4.1. Организација научних скупова

Др Ненад Селаковић је био у локалном организационом комитету следећих међународних конференција:

22nd International Conference on Gas Discharges and Their Applications, од 2. до 7. септембра 2018. године у Новом Саду, Република Србија.

XX International Workshop on Low-Energy Positron and Positronium Physics, XXI International Symposium on Electron-Molecule Collisions and Swarms, V Workshop on Non-Equilibrium Processes POSMOL 2019, од 18 до 21 јула 2019. године у Београду, Република Србија.

3.5. Утицај научних резултата

Утицај научних резултата се огледа у броју цитата који су наведени у тачки 3.1.3. овог одељка, а значај резултата је описан у оквиру одељка 3.1. Пун списак радова и подаци о цитираности из Scopus базе су дати у прилогу.

3.6. Конкретан допринос кандидата у реализацији радова у научним центрима у земљи и иностранству

Кандидат је своју истраживачку и научну активност реализовао у Институту за физику у Лабораторији за гасну електронику под руководством академика Зорана Љ. Петровића (сада Лабораторија за неравнотежне процесе и примену плазме под руководством др Гордане Маловић). Његов допринос се огледа у експерименталним мерењима у којима је одређиван масени састав атмосферских плазми и посматран временско-просторни развој пражњења при варирању експерименталних параметара са посебним акцентом на утицај вариране влажности у протоку радног гаса. Дао је значајан допринос у објављеним радовима на којима је потписан као коаутор кроз експериментална мерења, обраду и анализу резултата, њиховом припремању и комуникацији са уредницима и рецензентима часописа.

4. Елементи за квантитативну оцену научног доприноса кандидата

Остварени М-бодови по категоријама публикација

Категорија	М-бодови по публикацији	Број публикација	Укупно М-бодова	Нормирани број М-бодова
M21a	10	1	10	10
M21	8	4	32	30.7
M23	3	1	3	3
M33	1	9	9	7.44
M34	0.5	21	10.5	10.13
M70	6	1	6	6

*Нормирање је урађено у складу са Прилогом 1 Правилника.

	Потребно	Остварено	Остварено (нормирано*)
Укупно	16	64.5	61.27
M10+M20+M31+M32+M33+M34+M41+M42	10	19.5	17.57
M11+M12+M21+M22+M23	6	45	43.7

*Нормирање је урађено у складу са Прилогом 1 Правилника.

5. СПИСАК ОБЈАВЉЕНИХ РАДОВА:

5.1 Радови у међународним часописима изузетних вредности (M21a):

1. D. Maletić, N. Puač, N. Selaković, S. Lazović, G. Malović, A. Djordjević and Z. Lj. Petrović
Time-resolved optical emission imaging of an atmospheric plasma jet for different electrode positions with a constant electrode gap
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5.2 Радови у врхунским међународним часописима (M21):

1. N. Puač, S. Živković, N. Selaković, M. Milutinović, J. Boljević, G. Malović, and Z. L. Petrović
Long and short term effects of plasma treatment on meristematic plant cells
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Characterisation of a multijet plasma device by means of mass spectrometric detection and iCCD imaging
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4. J. N. Stašić, **N. Selaković**, N. Puač, M. Miletić, G. Malović, Z. Lj. Petrović, D. N. Veljović, and V. Miletić
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IF: 2.453

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1. J. Čech, A. Brablec, M. Černák, N. Puač, N. Selaković, and Z. Lj. Petrović
Mass spectrometry of diffuse coplanar surface barrier discharge: influence of discharge frequency and oxygen content in N₂/O₂ mixture
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ISSN: 1434-6060 (print) 1434-6079 (web)
DOI: doi.org/10.1140/epjd/e2016-70607-5
Citiran 8 puta
IF: 1.393

5.4 Саопштење са међународног скупа штампано у целини M33

1. S. Živković, S. Lazović, D. Maletić, G. Malović, M. Miletić, S. Mojsilović, N. Puač, **N. Selaković**, Z. Petrović, M. Milutinović, J. Boljević, D. Bugarski, D. Vuković
Plasma needle as a source of atmospheric pressure non-equilibrium plasma and a tool for biomedical applications
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Slovenian Society for Vacuum Technique (DVTS Društvo za vakuumsko tehniko Slovenije),
Teslova 30, SI-1000 Ljubljana, Slovenia
pp. 1 - 4, Bohinjska Bistrica, Slovenia, 19. - 23. Jan, 2014
2. N. Puač, G. Malović, Z. Petrović, **Nenad Selaković**
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IPCS (International Plasma Chemistry Society) Summer School Montreal, Canada
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7. **N. Selaković**, N. Puač, M. Miletić, I. Živanović, I. Dakić, G. Malović, D. Vuković, Z. Lj. Petrović
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8. Z. Lj. Petrović, N. Puač, **N. Selaković**, J. Čech, A. Brablec, M. Černák
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Low temperature plasma needle reduces the survival of cancer cells
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5.5 Саопштење са међународног скупа штампано у изводу М34

1. S. Živković, G. Malović, Z. Lj. Petrović, N. Puač, **N. Selaković**, M. Milutinović, J. Boljević
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2. G. Malović, Z. Petrović, N. Puač, S. Živković, N. Selaković, M. Milutinović, J. Boljević
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Time resolved optical spectroscopy of high frequency atmospheric pressure plasma jets
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7. Z. Lj. Petrović, N. Škoro, S. Živković, M. Milutinović, O. Jovanović, N. Selaković, N. Puač,
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5.6 Одбрањена докторска дисертација (M70)

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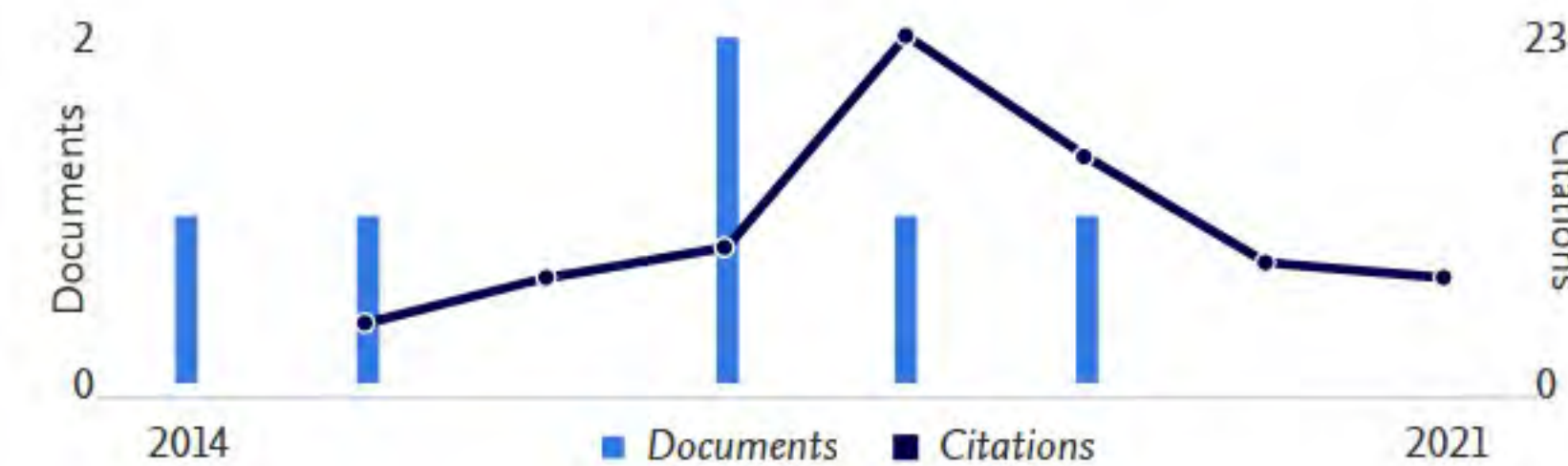
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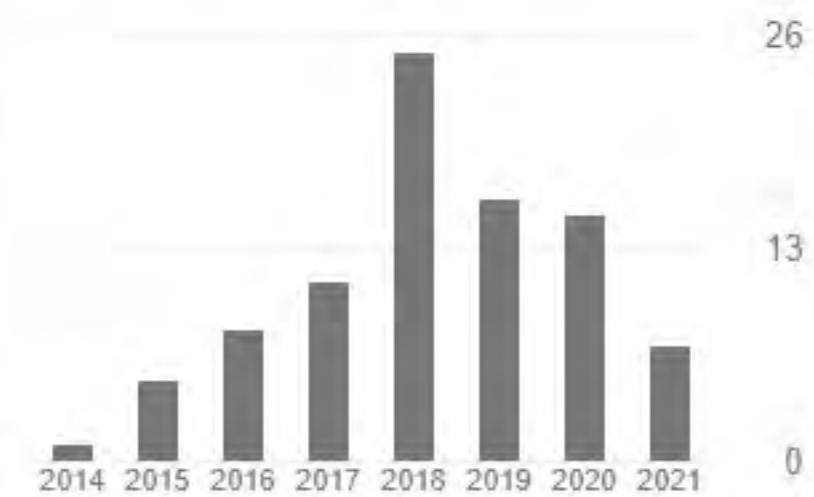
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<input type="checkbox"/> Electrical and optical characterization of an atmospheric pressure, uniform, large-area processing, dielectric barrier discharge A Zeniou, N Puač, N Škoro, N Selaković, P Dimitrakellis, E Gogolides, ... Journal of Physics D: Applied Physics 50 (13), 135204	9	2017
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На основу члана 29 Закона о општем управном поступку («Службени гласник РС» број 18/2016 и 95/2018), и члана 149 Статута Универзитета у Београду - Физичког факултета, по захтеву **НЕНАДА СЕЛАКОВИЋА**, мастер физичара, издаје се следеће

У В Е Р Е Њ Е

НЕНАД СЕЛАКОВИЋ, мастер физичар, дана 19. фебруара 2021. године, одбранио је докторску дисертацију под називом

"МАСЕНА СПЕКТРОМЕТРИЈА ПЛАЗМЕНОГ МЛАЗА И ПРИМЕНЕ ЕЛЕКТРИЧНИХ ПРАЖЊЕЊА НА АТМОСФЕРСКОМ ПРИТИСКУ У БИОМЕДИЦИНИ" (Mass spectrometry of plasma jet and application of electrical discharges operating at atmospheric pressure in biomedicine)

пред Комисијом Универзитета у Београду - Физичког факултета и тиме испунио све услове за промоцију у **ДОКТОРА НАУКА – ФИЗИЧКЕ НАУКЕ**.

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Дисертација под називом "МАСЕНА СПЕКТРОМЕТРИЈА ПЛАЗМЕНОГ МЛАЗА И ПРИМЕНЕ ЕЛЕКТРИЧНИХ ПРАЖЊЕЊА НА АТМОСФЕРСКОМ ПРИТИСКУ У БИОМЕДИЦИНИ" (Mass spectrometry of plasma jet and application of electrical discharges operating at atmospheric pressure in biomedicine) пријављена дана **19. новембра 2018. године**. На седници Наставно-научног већа Факултета одржаној дана **21. новембра 2018. године** одређена је Комисија за оцену испуњености услова и оправданост предложене теме за израду докторске дисертације у саставу:

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**Masena spektrometrija plazmenog mlaza i
primene električnih pražnjenja na
atmosferskom pritisku u biomedicini**

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**Mass spectrometry of plasma jet and
application of electrical discharges operating
at atmospheric pressure in biomedicine**

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Nenad Selaković

Naslov disertacije: „Masena spektrometrija plazmenog mlaza i primene električnih pražnjenja na atmosferskom pritisku u biomedicini“

Sažetak:

Proučavanje DBD plazmenih mlazova je u poslednjih par decenija u žiži interesovanja velikog broja naučnika. Ovi izvori električnog pražnjenja na atmosferskom pritisku imaju veliki potencijal u širokom spektru primena. Istovremeno, savremena tehnologija je omogućila razvoj velikog broja moćnih dijagnostičkih tehnika koje pružaju sofisticiranu analizu fizičkih fenomena ovih pražnjenja. Fizički fenomen koji karakteriše plazmene mlazove je pojava „pulsnog strimera na atmosferskom pritisku“ (eng. pulsed atmospheric pressure streamer - PAPS), brzog jonizacionog fronta koji ljudsko oko ne može da detektuje, ali je njegovo posmatranje omogućeno korišćenjem brzih ICCD kamera. „PAPS“ koji su, takođe, u literaturi poznati kao „plazmeni meci“, se šire izvan međuelektrodnog prostora u okolnu atmosferu. Da bi se ovaj tip izvora plazme integrisao u biomedicinske primene i da bi se dobijeni efekti bolje razumeli, neophodno je izvršiti analizu ponašanja i sastava električnog pražnjenja.

U ovoj doktorskoj disertaciji biće predstavljeni rezultati karakterizacije tri različita izvora atmosferskih plazmi (plazmenog mlaza, mnogostrukog plazmenog mlaza i dielektričnog koplanarnog površinskog pražnjenja). Prvi izvor koji treba istražiti, DBD plazmeni mlaz sa dve providne elektrode, izvor je električnog pražnjenja na atmosferskom pritisku za koji je izabrana konfiguracija elektroda u kojoj PAPS (pulsni strimer koji u obliku kuglice izleće iz staklene cevčice u okolinu) ima najduži domet. Drugi izvor je DBD mnogostruki plazmeni mlaz, u konfiguraciji sa jednom elektrodom i metom kao uzemljenom elektrodom, koji može da radi u dva različita režima (spojeni i nespojeni režim). Poslednji izvor za koji je izvršena analiza je dielektrično koplanarno površinsko barijerno pražnjenje (eng. DCSBD), posebna vrsta DBD izvora u kojem je elektroda integrisana u dielektrični materijal gde je ulogu uzemljene elektrode imala meta.

ICCD kamera je korišćena za vremensku evoluciju prostiranja plazmenih mlazeva, dok je maseni spektrometar korišćen za analizu sva tri izvora gde su detektovani maseni spektri neutrala i pozitivnih i negativnih jona. Pored ove dve metode, izvršena je i električna karakterizacija plazmenih mlazova, kojom je obezbeđeno merenje i kontrola snage koja je predata plazmi kao i određivanje njene impedanse. Takođe, korišćen je i merač vlažnosti u protoku radnog gasa za praćenje i kontrolu vlažnosti unutar plazmenog mlaza.

Istražujući propagaciju PAPS-a u plazmenom mlazu, utvrđeno je da vlažnost unutar protoka radnog gasa igra važnu ulogu u njegovom formiranju pod određenim eksperimentalnim uslovima. Veštački dodata vlažnost u protok vraća oblik PAPS-a nakon prosušavanja protočnog sistema radnog gasa, ali to značajno utiče na njegov domet kao i na promenu masenog sastava pozitivnih jona. Takođe je izvršeno sinhronizovano merenje vremenske evolucije prostiranja plazme, pozitivnih jona i strujno-naponskih signala. Ovo merenje je dalo uvid u sastav PAPS-a tokom njegovog kretanja od plazmenog mlaza do masenog spektrometra.

Primene masene spektrometrije i ICCD snimanja mnogostrukog plazmenog mlaza su pored određivanja sastava i prostiranja plazme ka meti, dale uvid o kompleksnoj međusobnoj interakciji između različitih jonizacionih kanala mlaznica i kako njihovo spajanje/razdvajanje utiče na maseni sastav pozitivnih i negativnih jona kao i na propagaciju pražnjenja.

Kod DCSBD-a, urađena je masena spektrometrija za šest različitih smeša azota i kiseonika sa variranom pobudnom frekvencijom. Pokazano je da reaktivne vrste azota i kiseonika koje prate N_x , O_3 i NO_x imaju najveću proizvodnju na najnižoj frekvenciji.

Za potrebe biomedicinskih primena korišćen je izvor električnog pražnjenja na atmosferskom pritisku, plazma igla, za koji je pre ove studije već urađena detaljna analiza. Plazma iglom su tretirane MRSA

bakterije, biljni ćelije - kalusi *Daucus carota*, kancerogene ćelije HeLa, A549 i nekancerogene ćelije BEAS-2B kao i 3D model (višećelijski tumorski sferoid) kancerogenih ćelija.

MRSA bakterije su bile zasađene u Petri posudama i u mikrotitar pločicama. Nakon tretmana određivane su zone inhibicije kao i preživljavanje bakterija analizom MTT testa. Pokazano je da za najduže vreme tretmana i najveću primenjenu snagu plazma igla daje najefikasnije tretmane.

Kalusi su bili zasađeni u mikrotitar pločice i nakon izlaganja tretmanu plazma iglom posmatrani su kratkoročni (nakon 24 h) i dugoročni (nakon dve nedelje) efekti na fiziologiju tretiranog uzorka. Rezultati su pokazali da dolazi do povećanja aktivnosti enzima unutar biljnih ćelija koje su deo odbrambenog mehanizma pri pojavi kiseoničnih vrsta proizvedenih u plazmi usled tretmana. Ovi efekti utiču na metaboličke procese u ćeliji.

Kancerogene i nekancerogene ćelije su bile pripremljene u mikrotitar pločice i bile su izložene tretmanu plazma iglom za različite eksperimentalne parametre. Iz analiza nakon tretmana MTT testom je pokazana smanjena vijabilnost kancerogenih ćelija u većoj meri u odnosu na nekancerogene ćelije (posebno kod HeLa ćelija). Iz analize ćelijskog ciklusa prikazano je da plazma utiče na značajnu redukciju kancerogenih ćelije dok se nekancerogene ćelije pored doživljenog stresa usled tretmana plazmom brže i bolje oporavljaju. 3D model (višećelijski tumorski sferoid) kancerogenih ćelija nakon tretmana nije prikazao veće promene i moramo nastaviti dalja istraživanja.

Plazma igla se pokazala kao veoma efikasan i prilagodljiv izvor plazme u biomedicinskim primenama. Ključne reči: plazmeni mlaz, PAPS, DBD, masena spektrometrija, ICCD, električno pražnjenje na atmosferi, sterilizacija bakterija, tretman kancerogenih ćelija, tretman kalusa

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Abstract:

The study of DBD plasma jets has been the focus of interest for a large number of scientists in the last few decades. These sources of electrical discharge at atmospheric pressure have a great potential in a wide range of applications. At the same time, modern technology has enabled development of a large number of powerful diagnostic techniques that provide a sophisticated analysis of physical phenomena of these discharges. The physical phenomenon that characterizes plasma jets is the appearance of a "pulsed atmospheric pressure streamer" (PAPS), a fast ionization front that cannot be detected by a human eye, but its observation is enabled by the use of high-speed ICCD cameras. The "PAPS" that are, also, known in the literature as "plasma bullets", extend beyond the electrode space into the surrounding atmosphere. In order for this type of plasma source to be integrated into biomedical applications and for the obtained effects to be better understood, it is necessary to perform analysis of the electrical discharge's behavior and composition.

In this doctoral dissertation, results of characterisation of three different atmospheric pressure plasma sources (plasma jet, multijet plasma device and dielectric coplanar surface barrier discharge). will be presented. The first source to be investigated, the DBD plasma jet with two transparent electrodes, is a source of electrical discharge at atmospheric pressure for which an electrode configuration is selected in which the PAPS (ball-shaped pulse streamer ejects from a glass tube into the environment) has the longest range. The second source was the DBD multijet plasma device, in a configuration with one electrode and a target as the ground electrode. The source can operate in two different modes (coupled and uncoupled mode). The last source for which analysis was performed was dielectric coplanar surface barrier discharge (DCSBD), a special type of DBD source in which electrode was immersed in dielectric material where the role of the grounded electrode was played by the target.

ICCD camera was used for the temporal evolution of plasma jet propagation, while a mass spectrometer was used for mass analysis of all three sources for detection of the mass spectrum of neutrals and positive and negative ions. In addition, to these two methods, electrical characterization of the plasma jets was performed, which provided measurement and control of the power delivered to the plasma and allowed us to determine its impedance. Also, a dewpoint transmitter was used to monitor and control the humidity in the flow system of the working gas of the plasma jet.

When investigating the propagation of PAPS in a plasma jet, it was found that the humidity within the working gas flow plays an important role in its formation under certain experimental conditions. Artificially added humidity to the flow restores the form of PAPS after drying the gas flow system, but it significantly affects its range as well as the change in the mass composition of positive ions. In addition, a synchronized measurement of the time evolution of plasma propagation, positive ions and current-voltage signals was performed. This measurement gave an insight into the mass composition of PAPS during its movement from the plasma jet to the mass spectrometer.

Mass spectrometry and ICCD recording of multiple plasma jets, in addition to determining the mass composition and propagation of plasma to the target, provided an insight into the complex interaction between different nozzle ionization channels and how their coupling/uncoupling affects the mass composition of the positive and negative ions and discharge propagation.

Mass spectrometry for six different mixtures of nitrogen and oxygen with varying excitation frequencies was investigated in DCSBD. It has been shown that reactive nitrogen and oxygen species of interest associated with N_x , O_3 and NO_x have the highest production at the lowest frequency.

For the needs of biomedical applications, a source of electrical discharge at atmospheric pressure, a plasma needle, was used, for which a detailed analysis had already been performed before this study.

Plasma needle was used to treat MRSA bacteria, plant cells - calluses of *Daucus carota*, cancer cells HeLa, A549 and non-cancer cells BEAS-2B as well as 3D model (multicellular tumor spheroid) of cancer cells.

MRSA bacteria were seeded in Petri dishes and in microtiter plates. After treatment, inhibition zone areas measurement was performed as well as bacterial survival determined by MTT assay. It has been shown that for the longest treatment time and the highest applied power, plasma needle provides the most effective treatments.

Calluses were seeded in microtiter plates and after exposing to plasma needle treatment short-term (after 24 h) and long-term (after two weeks) effects on treated sample physiology were observed. The results showed that there is an increase in the activity of enzymes within plant cells that are part of the defense mechanism in the appearance of oxygen species produced in plasma due to treatment. These effects affect the metabolic processes in the cell.

Cancerous and non-cancerous cells were prepared in microtiter plates and subjected to plasma needle treatment for various experimental parameters. The analysis after the MTT test showed a reduced viability of cancer cells to a greater extent as compared to non-cancer cells (especially in HeLa). From the analysis of the cell cycle, it has been shown that plasma affects a significant reduction in the number of cancerous cells. Non-cancerous cells, in addition to the experienced stress due to plasma treatment, recover faster and better. 3D model (multicellular tumor spheroid) of cancerous cells after the treatment did not show significant result and further research is required.

The plasma needle has proven to be a very efficient and adaptable plasma source in biomedical applications.

Keywords: plasma jet, PAPS, DBD, mass spectrometry, ICCD fast imaging, electrical discharge at atmospheric pressure, sterilization of bacteria, cancer cell treatment, callus treatment

Scientific field: Physics

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UVOD – MOTIVACIJA I ORGANIZACIJA TEZE

Da bi se određeni izvor niskotemperaturne plazme na atmosferskom pritisku adekvatno primenio u medicini i biologiji neophodno je uraditi detaljnu analizu samog izvora. Poznavanje svih parametara koji mogu da utiču na dobijeni efekat tretmana od izuzetne je važnosti. Poznavanje masenog sastava plazme kao i proučavanje propagacije pražnjenja je od vitalnog značaja za dalje razumevanje i analizu tretiranih uzoraka na kojima je izvršena određena promena.

Fokus ove disertacije je na dijagnostici masenog spektra dva različita tipa plazmenih mlazova kao i masene spektrometrije posebnog tipa dielektričnog barijernog pražnjenja, uticaju vlažnosti na propagaciju električnog pražnjenja koje radi u režimu tzv. „plazma metka“ kao i primenu plazma igle (izvora plazme za koji je već urađena detaljna analiza) u biologiji i plazma medicini.

Ova disertacija bi trebalo da da odgovore na još uvek nekompletno razjašnjene fenomene opažene kod impulsnog strimera, na promenu samog masenog spektra neutrala, pozitivnih i negativnih jona usled menjanja određenih parametara kao što su: primenjeni napon, konfiguracija elektroda, protok radnog gasa i njihova interakcija sa uzorkom (metom), kao i uticaj vlažnosti na formiranje samog strimera. Do sada u literaturi nisu publikovani sinhronizovani vremenski razloženi ICCD snimci sa vremenskom evolucijom jona za tip električnog pražnjenja koji je korišćen u disertaciji, što će doprineti boljoj slici formiranja brzih pulsnih paketa, plazma strimera. Takođe, korišćenje plazma igle na atmosferskom pritisku u tretiranju kancerogenih ćelija kao i njihovih 3D modela, u tretmanima biljnih matičnih ćelija – kalusa kao i u tretmanima MRSA bakterija biće prikazani i dobijeni rezultati bi trebalo da budu dobra polazna tačka za primene u medicini i biologiji i približavanju *in vivo* tretmanu plazmom. Takođe ovi eksperimentalni rezultati mogu da se poređenjem sa teorijskim opisima plazme pokažu kao dobra osnova za validaciju teorije ali i razumevanje fizičkih fenomena koji karakterišu ovu vrstu neravnotežne plazme.

Ova doktorska disertacija se može podeliti na dve odvojene celine:

1. Masena spektrometrija tri različita izvora plazme na atmosferskom pritisku: DBD plazmeni mlaz, DBD mnogostruki plazmeni mlaz i dielektrično koplanarno površinsko pražnjenje,
2. Primena plazma igle u tretmanima sledećih uzoraka biološkog porekla: biljne ćelije – kalusi *Daucus carota*, MRSA bakterije i različiti tipovi kancerogenih ćelija.

Struktura teze podeljena je u 8 glava:

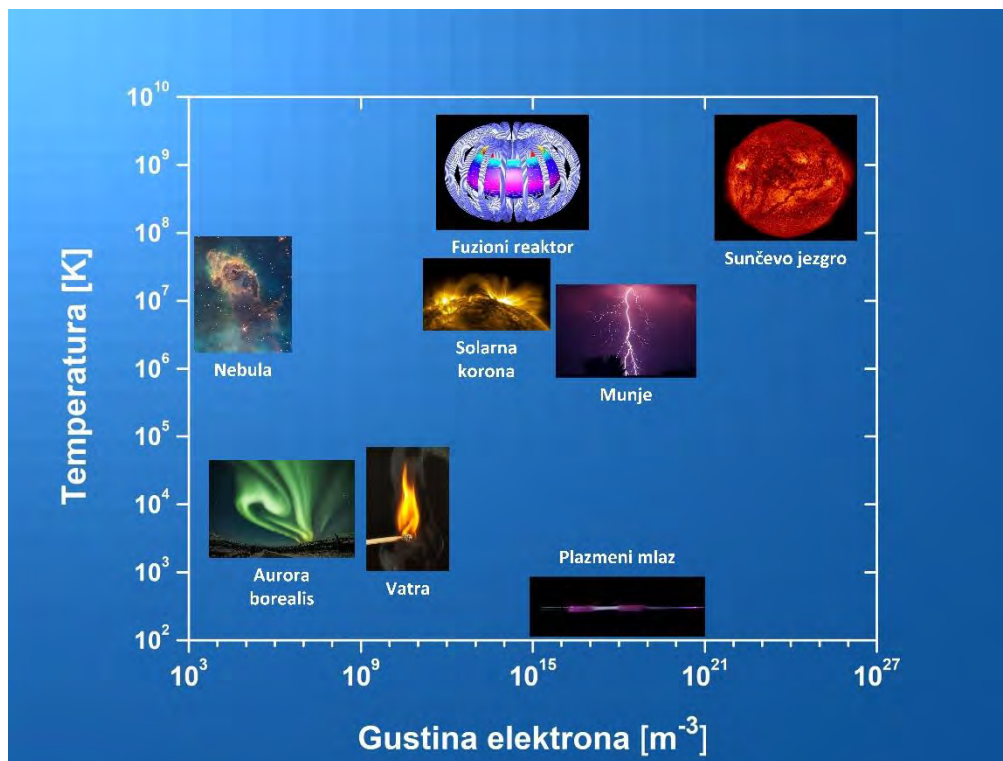
- Prva glava posvećena je neravnotežnim plazmama, opisu mehanizama koji dovode do proboja i do održavanja radiofrekventnih pražnjenja na atmosferskom pritisku. Dat je pregled najzastupljenijih dijagnostičkih metoda kao i onih koje su korišćene u disertaciji. Na kraju glave opisane su i primene električnih pražnjenja u biomedicini,
- U drugoj glavi je detaljno opisana eksperimentalna postavka plazmenog mlaza kao i glavna dijagnostička metoda, masena spektrometrija. Pored nje opisane su i druge dijagnostičke metode, električna karakterizacija i snimanje brzom ICCD kamerom, koje upotpunjuju analizu i daju širu sliku plazme kao dopunu glavne dijagnostičke metode. Takođe opisana je i problematika koja se javlja prilikom merenja kompleksnim instrumentom kao što je maseni spektrometar,
- U trećoj glavi su prikazani rezultati dijagnostike DBD plazmenog mlaza. Urađena je vremenska evolucija PAPS-a i uticaj vodene pare na njegovo formiranje. Vremenski razloženi snimci intenziteta emisije plazmenog mlaza su dobijeni korišćenjem ICCD kamere, masenim spektrometrom je urađena analiza sastava pražnjenja dok je tokom celog eksperimenta korišćen merač vlažnosti u protoku radnog gasa.
- Četvrta glava posvećena je masenoj spektrometriji DBD plazmenog mlaza. Prikazana je vremenska evolucija PAPS-a sinhronizovanog sa vremenski razloženim pozitivnim jonima i električnim signalima. Ova snimanja su urađena korišćenjem masenog spektrometra, ICCD kamere i naponskih sonde,

- Peta glava opisuje vremenski razloženo snimanje ICCD kamerom i masenu spektrometriju mnogostrukog plazmenog mlaza,
- U šestoj glavi prikazani su rezultati analize masenog spektra radikala DCSBD-a, posebnog tipa DBD izvora, koji za radni gas koristi 6 različitih smeša azota i kiseonika,
- Sedma glava disertacije je posvećena plazma igli i primenama u biomedicini. Opisan je tretman MRSA bakterija plazma iglom. Uz pomoć MTT testa provereno je preživljavanje bakterija nakon tretmana i određene su zone inhibicije. Takođe su tretirane i biljne matične ćelije – kalusi pomoću plazma igle. Analiziran je odgovor biološkog sistema na oksidativni stres koji električno pražnjenje unosi u metabolizam ćelije. Poslednja primena bila je vezana za tretman kancerogenih ćelija. Ispitivana je vijabilnost ćelija usled tretmana plazmom kao i ćelijski ciklus i tretman 3D modela kancerogenih ćelija čija je uloga da oponaša kancerogeno tkivo,
- Osmo glava daje sveobuhvatni zaključak tj rezime svih zaključaka iz prethodnih glava i nakon nje sledi literatura.

1 NERAVNOTEŽNA PLAZMA NA ATMOSFERSKOM PRITISKU

Plazma je skup slobodnih naelektrisanih čestica koje se kreću u proizvoljnim smerovima i koja je u proseku i u najvećem delu, električno neutralna (Lieberman, 2005). Preciznija definicija plazme bi glasila: „Plazma je kvazineutralni gas naelektrisanih i neutralnih čestica koji iskazuje kolektivno ponašanje“ (Chen, 2006).

Otprilike oko 99% vidljive materije u svemiru je sačinjeno od plazme. Na primer, zvezde su vrsta plazme čije je jezgro veoma toplo i gusto (Chabert *et al.*, 2011). S druge strane, hladna plazma se može nalaziti u prostoru između zvezda. Iste razlike važe i za veštački proizvedene plazme. Postoje vruća i gusta plazma koja se koristi za nuklearnu fuziju i hladna, slabo jonizovana plazma, tzv. niskotemperaturna plazma, koja se koristi u raznim industrijskim i biomedicinskim primenama (Slika 1.1). Na Zemlji se plazma javlja pri pojavi munja ili polarne svetlosti (*lat. aurora borealis*), eksperimentalno u laboratorijskim uslovima, ali i u svakodnevnom životu (neonske cevi za osvetljavanje, aparati za zavarivanje, ekrani plazma televizora, i dr.).



Slika 1.1 Različite vrste plazmi u zavisnosti od temperature i gustine elektrona.

Najčešće se plazma proizvodi u vakuumskoj komori ispunjenoj gasom na određenom niskom pritisku, zagrevanjem gasa do tačke gde je kinetička energija dobrog dela njegovih konstituenata veća od njihove energije jonizacije. To inicira jonizaciju atoma gasa i molekula, tako da u moru velikog broja elektrona postoji i veliki broj jona. Stvorena slobodna naelektrisanja čine plazmu električno provodljivom, zbog čega je uticaj elektromagnetnog polja značajan.

U plazmi nije neophodno da dođe do jonizacije svih čestica; uobičajeni uslov u hemiji plazme je da gasovi budu samo delimično jonizovani. Stepenn jonizacije (tj. odnos gustine glavnih naelektrisanih vrsta prema neutralnom gasu) u konvencionalnim plazma-hemijskim sistemima kreće se u opsegu 10^{-7} – 10^{-4} . Kada je stepenn jonizacije blizu jedinice, takva plazma se naziva potpuno jonizovanom plazmom. Potpuno

jonizovane plazme su uobičajene za termonuklearne plazma sisteme: tokamak, stelaratori, plazma pinches, plazma fokusi itd.. Kada je stepen jonizacije nizak, plazma se može nazvati slabo jonizovanom plazmom, o kojoj će i biti reči u ovoj diseraciji.

Plazma se karakteriše osobinama kao što su sastav, temperatura, stepen jonizacije, gustina, dimenzije, itd. Za svaki sistem, potrebno je kvalitativno poznavati sastav, tj. sve vrste konstituenata. Ako posmatramo plazmu gasa vrste A, u njoj se mogu javiti neutrali kao što su molekuli (A_2), atomi u osnovnom stanju (A), kao i atomi u različitim pobuđenim stanjima (ekscitovani atomi) (A^*), ali i naelektrisane vrste kao jednostruko pozitivni joni u osnovnom stanju (A^+) i pobuđenim stanjima (A^{+*}), kao i dvostuko (A^{2+}) i višestruko (A^{n+}) naelektrisani pozitivni joni, zatim negativni joni (A^-) i elektroni.

Za poznavanje kvantitativnog sastava plazme potrebno je znati gustine pojedinih konstituenata, kao i broj čestica iste vrste (α) u jedinici zapremine n_α . Tada je ukupan broj čestica u jedinici zapremine $n = \sum n_\alpha$, a zapremina na raspolaganju jednoj čestici n_α^{-1} . Ako se pretpostavi da se čestica nalazi u kocki, onda dužina njene ivice $d_\alpha = n_\alpha^{-1/3}$ predstavlja srednje rastojanje između dve čestice vrste α , dok je rastojanje između bilo koje dve čestice $d = n^{-1/3}$.

Kao i kod bilo kog gasa, temperatura u plazmi određena je prosečnim energijama čestica plazme (neutralne i naelektrisane) i njihovih relevantnih stepena slobode (translacionih, rotacionih, vibracionih i onih povezanih sa elektronskim pobuđivanjem). Dakle, plazme, kao višekomponentni sistemi, mogu da pokazuju više temperatura. U električnim pražnjenjima elektroni dobijaju energiju od električnog polja tokom svog srednjeg slobodnog puta i tokom sledećeg sudara sa teškom česticom gube samo mali deo te energije (jer su elektroni mnogo lakši od teških čestica). Zbog toga je temperatura elektrona u slabo jonizovanoj plazmi mnogo viša od temperature teških čestica jona i neutrala. Ukoliko postoji jaka sprega između naelektrisanih čestica (viši i visok stepen jonizacije) onda dolazi do preraspodele energije sa elektrona na jone pa onda sudarima i na neutrale.

Zbog složenog sastava i velike razlike u masi između elektrona i teških čestica (jona i neutrala) mogu se definisati različite temperature komponenti plazme: T_e (temperatura elektrona), T_j (temperatura jona) i T_g (temperatura gasa). Ukoliko su energije svih čestica jednake, plazma je u termodinamičkoj ravnoteži i u tom slučaju govorimo o ravnotežnoj (termalnoj) plazmi. Ukoliko je temperatura elektrona (T_e) znatno veća od temperature jona i neutrala ($T_e \gg T_j \approx T_g$) plazma je neravnotežna. Primene termalnih i neravnotežnih plazmi razlikuju se u velikoj meri. Termalne plazme su agresivnije i prenose više energije na tretiranu površinu, a neravnotežne plazme deluju blaže i pogodne su za primenu na osetljivim materijalima. One na površinu šalju značajan ali ne i veliki broj, čestica velike kinetičke energije i hemijskog potencijala ali su u stanju da utiču samo na nekoliko slojeva pri površini, nikako ne na unutrašnjost materijala koji se tretira. U laboratorijskim uslovima mogu se proizvesti neravnotežne plazme na niskom i atmosferskom pritisku.

Hemijski aktivna plazma je višekomponentni sistem visoko reaktivan zbog velikih koncentracija naelektrisanih čestica (elektroni, negativni i pozitivni joni), pobuđenih atoma i molekula (glavni doprinos imaju elektronska i vibraciona pobuda), aktivnih atoma, radikala i UV fotona. Svaka komponenta hemijski aktivne plazme igra svoju specifičnu ulogu u plazma-hemijskoj kinetici. Kao primer mogu se navesti elektroni, koji obično prvo primaju energiju iz električnog polja, a zatim je koriste da proizvedu nove elektrone i disosuju molekule čime se dobijaju hemijski aktivne čestice. Promena parametara elektronskog gasa (gustina, temperatura, funkcija raspodele energije elektrona) često omogućavaju kontrolu i optimizaciju plazma-hemijskih procesa. Joni su naelektrisane teške čestice, koje su sposobne da daju značajan doprinos plazma-hemijskoj kinetici bilo zbog visoke energije (kao u slučaju raspršivanja i reaktivnog nagrizanja jonima) ili zbog svoje sposobnosti da potisnu aktivacione barijere hemijskih reakcija (Fridman, 2008).

1.1 Radiofrekventno pražnjenje na atmosferskom pritisku

1.1.1 Osnovni pojmovi

Radio spektar je deo elektromagnetnog spektra sa frekvencijama od 30 Hz do 300 GHz. Elektromagnetni talasi u ovom frekvencijskom opsegu, tzv. radio talasi, široko se koriste u modernoj tehnologiji. Kada su u pitanju radiofrekventna električna pražnjenja na atmosferskom pritisku, u širem smislu njihovog definisanja, mogu biti klasifikovani prema navedenom opsegu frekvencija koji služe za njihovo generisanje. Na primer, veliki broj DBD izvora pražnjenja na atmosferskom pritisku generiše se frekvencijom reda veličine u kilohercima i u užem smislu oni mogu biti klasifikovani kao radiofrekventna (RF) pražnjenja na nižim radio frekvencijama. U literaturi je uobičajeno da se pri korišćenju izraza „RF plazma“ podrazumeva izvor koji je generisan pri pobudnoj frekvenciji od 13.56 MHz. Ova frekvencija je 1947. godine ustanovljena od strane Internacionalne Telekomunikacione Unije (eng. International Telecommunication Union - ITU) za korišćenje u industriji, nauci i medicini sa ciljem sprečavanja ometanja komunikacionih sistema. Ono što je jedna od glavnih razlika između RF pražnjenja na niskoj i visokoj frekvenciji je korišćenje kola za prilagođenje (eng. matching box). Bez njega generisanje RF električnog pražnjenja na visokoj frekvenciji praktično nije moguće jer je neophodno da se ostvari sprega u predaji snage generisane u električnom kolu ka pražnjenju. Kod električnih pražnjenja sa niskom frekvencijom kolo za prilagođenje nije neophodno. Fizički međjutim razlika između pražnjenja na niskim i na višim radio frekvencijama je u poređenju perioda pražnjenja sa vremenom preleta čestica između dve elektrode. Kod sporijih polja pražnjenja se mogu opisati kvazi stacionarnom aproksimacijom što je nemoguće na višim poljima.

Glavna podela radiofrekventnih električnih pražnjenja na atmosferskom pritisku jeste na kapacitivno i induktivno spregnute izvore.

Kod kapacitivno spregnutih izvora deo RF snage koja se unosi u plazmu odbija se nazad u napajanje u vidu reflektovane snage. Maksimalno rasipanje (disipaciju) snage u plazmi daje spoljni RF izvor kada je impedansa plazme pražnjenja Z jednaka impedansi z spoljnjeg izvora energije (teorema o prenosu maksimalne snage). Impedansa plazme se određuje samostalno, kako spoljašnjim parametrima plazme, tako i unutrašnjim osnovnim svojstvima molekula radnog gasa. Stoga je neophodno korišćenje kola za prilagođenje impedanse između RF elektrode i izvora napajanja. Ako se ne upotrebi kolo za prilagođenje može doći do značajne refleksije snage sa plazme i do teškog oštećenja RF izvora.

Tipični primeri kapacitivno spregnutih električnih pražnjenja na atmosferskom pritisku su različite vrste plazmenih mlazova, mikroplazma, plazma igla, dielektrično barijerno pražnjenje i drugi (Weltmann *et al.*, 2010; Kunhardt *et al.* 2005).

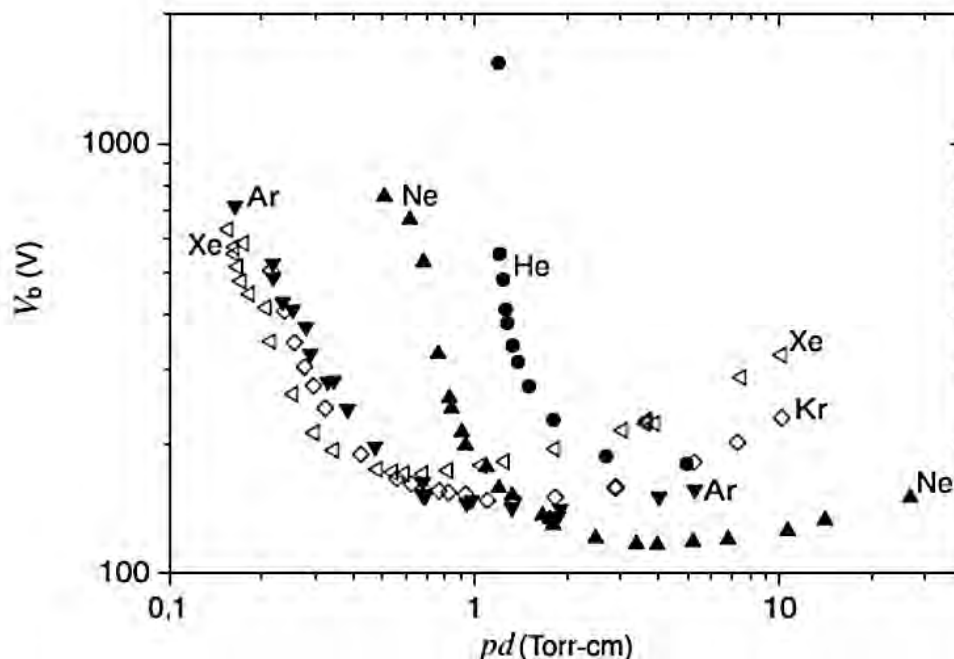
Ukoliko se radiofrekventna (RF) plazma održava pomoću RF elektromagnetnog polja koje zrači iz RF zavojnice onda se govori o induktivno spregnutoj plazmi (ICP) ili transformatorski vezanoj plazmi (TCP) na osnovu razlike u rasporedu trenutne zavojnice. Primer induktivno spregnute plazme na atmosferskom pritisku je plazmena baklja (eng. plasma torch) (Owano i Kruger, 1993).

1.1.2 Uslovi proboja

Kada su u pitanju neravnotežne plazme na atmosferskom pritisku postizanje proboja nije jednostavan zadatak. Do uslova za proboj u gasu došao je nemački fizičar Fridrih Pašen koji se bavio električnim pražnjenjima. On je probojni napon za jednosmerna polja opisao sledećom jednačinom:

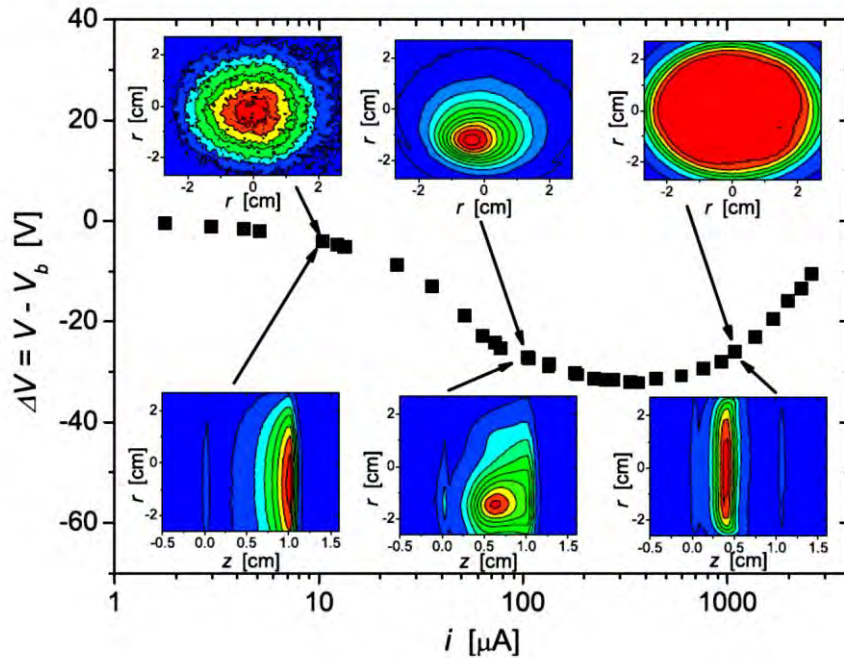
$$V_b = \frac{Bpd}{\ln Apd - \ln[\ln[1 + \frac{1}{\gamma_{se}}]]}$$

Vidimo da je probojni napon V_b zavisan od proizvoda pd (pritisak p , razmak između elektroda d). Za velike vrednosti pd , V_b se suštinski linearno povećava sa pd . Za mali pd postoji granična vrednost $pd = A^{-1} \ln(1 + \frac{1}{\gamma_{se}})$ ispod koje ne može doći do proboja. Napon proboja je minimalni V_{min} pri nekoj vrednosti $pd = (pd)_{min}$. Tipično minimum je na pd približno jednak 1 Torr cm. Kriva $V_b(pd)$ naziva se Paschenovom krivom i predstavlja funkciju od gasa, a slabije funkciju od stanja površine i materijala elektrode. Tipične krive proboja za plan-paralelne elektrode prikazane su na Slici 1.2 (Marić et al., 2014; Phelps i Petrović, 1999). Kako bi se na atmosferskom pritisku približili uslovima minimuma Pašenove krive i omogućili proboj, pribegava se primeni gasova poput helijuma ili argona koji imaju minimum Pašenove krive na manjim vrednostima napona tj. probojni napon je manji nego u slučaju vazduha.



Slika 1.2 Pašenova kriva za proboj u različitim inertnim gasovima (Marić et al., 2014)

Marić, Petrović i saradnici su u svojim merenjima prikazali formiranje i održavanje različitih načina pražnjenja - difuzno pražnjenje niske struje (Taunzend), oscilatorni režim na prelazu iz Taunzend pražnjenja u normalno tinjavo (*eng.* normal glow), suženo normalno tinjavo i abnormalno tinjavo pražnjenje. Slika 1.3 ilustruje odnos između karakteristika napona i struje i strukture pražnjenja za tri različita režima. To je urađeno ICCD snimanjem radialne (Slika 1.3 (gore)) i aksijalne strukture (Slika 1.3 (dole)) neravnotežnog DC pražnjenja na niskom pritisku.



Slika 1.3 Taunzendove oblasti pražnjenja. Volt-amperske karakteristike pražnjenja na niskom pritisku sa prostornom strukturom naznačenih najkarakterističnijih režima pražnjenja. (Marić *et al.*, 2009)

Kada sa jednosmernog predjemo na radiofrekvencna polja moramo prvo odrediti kako se period polja odnosi prema vremenu preleta izmedju elektroda elektrona i jona. Na nižim poljima i joni i elektroni imaju vreme da se prilagode promenama polja i da se proboj odvija po Paschenovom zakonu skaliranom na sinusoidnu promenu primenjenog napona. Kod povećanja frekvencije oscilacija doćice se na negde oko 1 MHz i pritiscima oko 0.1-1 Torr do tačke u kojoj joni prestaju da prate promene polja a elektroni u tome uspevaju. Joni su tada uhvaćeni u potencijalnom minimumu u centru pražnjenja i tu ostaju dok oni koji dospeju do prielektrodnih oblasti budu zahvaćeni visokim poljem i ubrzavaju se ka trenutnoj elektrodi. Tada opada probojni napon jer su smanjeni gubici naelektrisanih čestica. Joni svojim poljem drže zarobljene i elektrone niskih energija, dok elektroni u prielektrodnim oblastima bivaju ubrzani ka plazmi i proizvode novu jonizaciju. (Kakuta *et al.*, 1993).

Na još višim frekvencijama počinju uslovi u kojima ni elektroni ne uspevaju da stignu do elektroda tokom perioda i sve veći broj ostaje zarobljen u centru a gubici i probojni napon se još vise smanjuju. Na kraju na jako visokim frekvencijama dolazi se do mikrotalasnih učestanosti i tu su veoma mali gubici ali na kraju pražnjenje se ne može održati.

Fiziku radiofrekvencnog proboja prikazali su Marija Puač, Zoran Petrović i saradnici (Petrović *et al.*, 2017; Savić *et al.*, 2011; Puač *et al.*; 2018). U tim uslovima dolazi do pojave krivih probojnih napona sa dvostrukim vrednostima gde u dve veoma različite radne tačke dolazi do proboja na osnovu različitih fizičkih procesa. Pri RF probouju nije neophodno imati proizvodnju sekundarnih elektrona na katodi jer povratnu spregu obezbedjuje promena pravca kretanja elektrona. I pored toga takozvani gama procesi mogu da veoma utiču na proboj i radne uslove.

Kod visokih pritisaka dolazi do novog vida proboja. On naravno započinje Townsendovom klasičnom lavinom u kojoj se elektroni umnožavaju eksponencijalno. Međutim, kada se stvori dovoljno pozitivno i negativno naelektrisanje plazma oblak se formira koji obriše polje u unutrašnjosti a u visokom polju na frontu oblaka elektroni se ubrzavaju i umnožavaju (ili vrše ekscitaciju koja proizvodi fotone visoke energije koji su u stanju da jonizuju postojeće molekule. Ovakvi oblaci naelektrisanja su u stanju da se šire odnosno da se prividno kreću kroz gas čak i van domena spoljašnjeg električnog polja (Maletić *et al.*, 2015; Puač *et al.*, 2012).

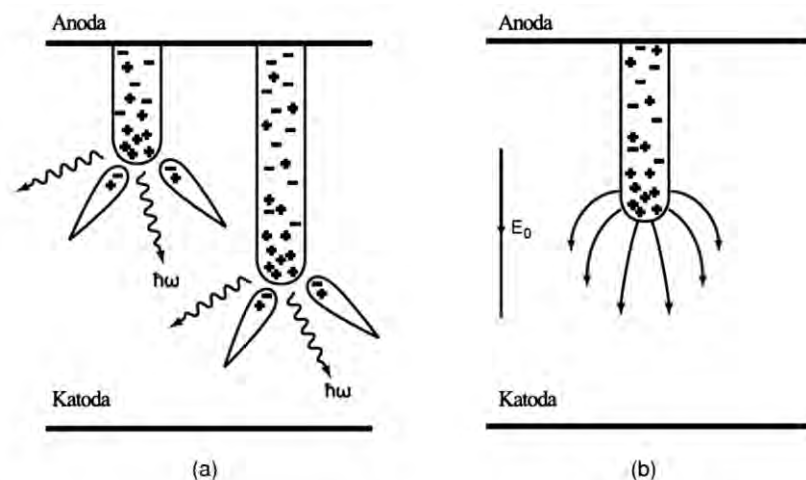
1.1.3 Jonizacioni talasi i mehanizam strimera

Stvaranje i propagacija jonizacionih talasa spada u električne fenomene koji se dešavaju u gasovima, tečnostima i čvrstoj materiji. Svetlosni jonizacioni frontovi ovih talasa kreću se brzinama $10^5 - 10^9 \text{ cm} \cdot \text{s}^{-1}$. Jonizacioni talasi predstavljaju najvažnije elemente u pre-probojnim procesima, i mogu se javiti u raznim vrstama impulsnih električnih pražnjenja, poput korona, zapreminskih i površinskih DBD-a i munja. Slični fenomeni, povezani sa brзом propagacijom svetlosnih paketa plazme, primećeni su u „sprajt-ovima“ (eng. sprite), pražnjenjima u gornjim slojevima atmosfere i kod plazmenih mlazeva na atmosferskom pritisku. Jonizacioni talasi mogu biti različite prostorne strukture, npr., ukoliko se kreću u gasovima duž površine dielektrika karakteriše ih gotovo ravan oblik dok se kod strimera pojavljuju u sfernom obliku.

Pri propagaciji jonizacionih talasa, nastaju joni i elektroni usled jonizacije atoma i molekula medijuma elektronskim udarom u lokalizovanim oblastima sa povećanim električnim poljem - talasnim frontovima. Vrednost pojačanog električnog polja talasnog fronta određuje se gustinom zapreminskog električnog naelektrisanja koje formira sam talas i može u velikoj meri premašiti primenjeno električno polje. Osobina jonizacionih talasa da proizvode snažno električno polje u svojim frontovima, koji mogu da jonizuju sredinu (brzina jonizacije snažno zavisi od vrednosti električnog polja), omogućava njihov prodor u područja slabijeg električnog polja. Dakle, električni proboj kod rastojanja sa neujednačenom raspodelom primenjenog električnog polja, npr. šiljak-ravan (eng. Point to Plain) ili žica-ravan (eng. Wire to Plain), postaje moguć. Stvarajući se u području jakog primenjenog električnog polja u blizini šiljka ili žičanih elektroda, stvaraju se varnični proboji koji se kreću ka suprotnoj elektrodi, formirajući provodne kanale.

Mehanizam varničnog proboja zasnovan je na konceptu strimera. Strimer (eng. streamer) se može opisati kao tanak jonizacioni kanal koji se brzo prostire u prostoru između dve elektrode prateći trag primarne elektronske lavine. Ova lavina stvara i fotone, koji pokreću brojne sekundarne lavine u blizini primarne lavine. Elektroni sekundarnih lavina privučeni jakim električnim poljem u pozitivno naelektrisan trag primarne lavine, stvaraju strimer koji se brzo prostire između dve elektrode. Oblast strimera u kojoj se odvijaju procesi jonizacije, elektronske lavine i emisija zračenja naziva se glava strimera (eng. streamer head). Tanak jonizacioni kanal koji povezuje glavu strimera i visokonaponsku elektrodu naziva se strimerski kanal (eng. streamer channel).

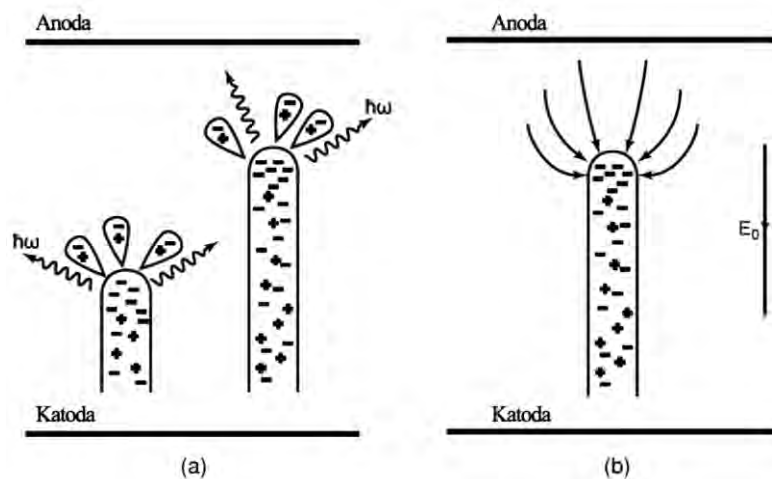
Prema smeru propagacije, strimeri mogu biti pozitivni (usmereni ka katodi) i negativni (usmereni ka anodi). Mehanizam pozitivnog strimera, gde je smer drifta elektrona, od katode do anode, suprotan smeru propagacije strimera, prikazan je na Slici 1.4 Primarna lavina elektrona ispred fronta strimera nastaje usled jonizacije srednjih vrsta energijskim fotonima emitovanim iz područja jakog električnog polja - glave strimera. Ovi elektroni, krećući se prema glavi, generišu lavine koje pojačavaju pozitivno prostorno naelektrisanje, a time i električno polje ispred kanala evolucionog fronta plazme.



Slika 1.4 Prikaz katodnog (pozitivnog) strimera (a) prostiranje strimera i (b) električno polje blizu glave strimera (Raizer, 1991)

Generisanje lavina rezultuje produženjem kanala. Osim fotojonizacije, lavinski elektroni mogu da se proizvedu i od spoljnih izvora jonizacije, ili u elektronegativnim medijumima, odvajanjem elektrona od negativnih jona u području jakog polja neposredno ispred fronta strimera. U slučaju ponavljajućih strimera šireći se istim putem, lavinski elektroni se mogu napajati prethodnim strimerima. Za strimere koji se šire duž dielektričnih površina, lavinski elektroni mogu da se proizvedu i fotoemisijom.

Mehanizam negativnog strimera (Slik 1.5), gde je drift elektrona u smeru širenja strimera, sličnog je karaktera, mada u ovom slučaju nije potrebno formiranje semenskih elektrona, lavine ispred glave strimera mogu pokrenuti elektroni koji dolaze u pravcu kretanja strimera polazeći sa prednjeg dela plazme.



Slika 1.5 Prikaz anodnog (negativnog) strimera (a) prostiranje strimera i (b) električno polje blizu glave strimera (Raizer, 1991.)

Uslov za formiranje strimera, odnosno tranziciju lavine u strimer, poznat je kao Mikov kriterijum. (Meek, 1940) Formiranje strimera zahteva da je električno polje prostornog naelektrisanja u lavini E_a približno jednako spoljašnjem električnom polju E_0 :

$$E_a = \frac{e}{4\pi\epsilon_0 r_A^2} \exp\left[\alpha\left(\frac{E_0}{p}\right) \cdot x\right] \approx E_0$$

gde je r_A poluprečnik sfere u kojoj je skoncentrisano naelektrisanje, tj. poluprečnik glave lavine a x je put koji lavina pređe . Uzimajući da je radijus glave lavine $r_A \approx \frac{1}{\alpha}$, kriterijum za formiranje strimera u prostoru između elektroda na rastojanju d može se predstaviti kao zahtev da parametar pojačanja lavine αd pređe kritičnu vrednost:

$$\alpha \left(\frac{E_0}{p} \right) \cdot d = \ln \frac{4\pi\epsilon_0 E_0}{e\alpha^2} \approx 20$$

$$N_e = \exp(\alpha d) \approx 3 \cdot 10^8$$

Ovaj fundamentalan i važan kriterijum za formiranje strimera poznat je kao Mikov uslov za proboj ($\alpha d \geq 20$), a proizvod $\alpha d = M$ naziva se Mikovim brojem.

Minimalno električno polje koje je potrebno da bi se formirao strimer iznosi 26 kV/cm za vazduh. U slučaju neuniformnosti polja ono može značajno uticati na uslove proboja i prelazak iz lavine u strimer. Ukoliko se napon primenjuje neravnomerno dolazi do intenzivne multiplikacije elektrona u blizini elektrode i do pojave strimera. Kada nastane plazma kanal on raste zbog jakog električnog polja same glave strimera. U slučaju veoma dugačkih neuniformnih sistema srednje električno polje za proboj bi iznosilo 2 kV/cm - 5 kV/cm. Prag za proboj kod neuniformnih električnih polja može da zavisi od polariteta, pa je npr. prag u slučaju negativno naelektrisanje šipke oko dva puta veći nego za pozitivno naelektrisanu šipku (Fridman, 2008).

1.1.4 Fizički i hemijski procesi kod atmosferskih pražnjenja

Razvoj proboja u gasovima indukuje električna gasna pražnjenja i samim time i proizvodnju velikog broja atomskih/molekulskih, jonskih i ekscitovanih vrsta koje dovode do fizičkih i hemijskih reakcija, a koje možda nije uvek moguće ostvariti na bilo koji drugi uobičajen način. Neki od najvažnijih mehanizama predstavljeni su u Tabeli 1. U plazmi može doći do ogromnog broja hemijskih procesa tako da navedene reakcije ne treba smatrati jednim mogućim. Prikazane reakcije mogu da igraju ulogu i u prostiranju i stabilnosti plazme, ali u svakom slučaju svaka reakcija makar i one koje se retko dešavaju mogu da ostvare uticaj na žive i nežive mete i da budu osnova za neku željenu primenu.

Reakcije kao što su elektronska jonizacija, elektron-jon rekombinacija, disocijativna jonizacija, sekundarna elektronska emisija, jonska rekombinacija i fotojonizacija igraju ključnu ulogu kod proboja i održavanja električnog pražnjenja.

Jedna od reakcija koja je navedena u Tabeli 1 a kojom se opisuje fenomen PAPS-a je fotojonizacija. Da bi se ona obezbedila, talasna dužina fotona obično treba da bude manja od 100 nm, što spada u ultraljubičasto zračenje. Doprinos procesa fotojonizacije obično nije veoma značajan zbog niskih koncentracija fotona visoke energije u većini tipova pražnjenja. Međutim, ponekad fotojonizacija igra veoma bitnu ulogu - uglavnom brzim snabdevanjem elektrona koji su zaslužni za naknadnu elektronsku jonizaciju (Fridman, 2008; Bošnjaković *et al.*, 2016).

U ovim reakcijama, posebno kada su u pitanju električna pražnjenja na atmosferskom pritisku može se generisati veliki broj reaktivnih vrsta. Reaktivne kiseoničke vrste (*eng.* reactive oxygen species - ROS) i reaktivne azotne vrste (*eng.* reactive nitrogen species – RNS, zajedno se ROS i RNS označavaju i sa RONS) se generišu u atmosferskim plazmama koje se koriste u primenama namenjenim za biomedicinske svrhe. Ove vrste takođe imaju glavnu ulogu u onome što se ponekad naziva „redoksom“ ili oksidaciono-redukcionom biologijom. Oksidaciono-redukciona biohemija je osnova za celokupnu aerobnu biologiju. ROS i RNS su možda najpoznatiji kao agensi povezani sa bolestima, umešani u dijabetes, rak, bolesti srca i pluća, autoimune bolesti i mnoštvo drugih bolesti, uključujući starenje i razne zarazne bolesti. Takođe je poznato da ove vrste igraju aktivnu ulogu u imunološkom sistemu životinja i

biljaka i ključni su signalni molekuli, među mnogim drugim važnim ulogama (Graves et al., 2012). Da zaključimo, žive ćelije se moraju braniti od neželjenog dejstva prevelike količine RONS ali ne mogu ih potpuno eliminisati jer oni imaju važnu biološku funkciju.

Tabela 1. Fizički i hemijski procesi u plazmi

Elektronske i Atomske/Molekulske reakcije	
Reakcije	Opis
$e^-_{brzi} + A_{spori} \rightarrow e^-_{sporiji} + A_{brzi}$	Elastični sudar
$e^- + A \rightarrow e^- + A^*$	Ekscitacija elektronima
$e^- + A \rightarrow e^- + A^+ + e^-$	Elektronska jonizacija
$e^- + A^+ \rightarrow A$	Elektron-jon rekombinacija
$e^- + A \rightarrow A^-$	Elektronski atačment
$e^- + A_2 \rightarrow A^- + A$	Disocijativni atačment
$e^- + A_2 \rightarrow A^+ + A + e$	Disocijativna jonizacija
$A^* ili A^+ + B \rightarrow A + e^-$	Sekundarna elektronska emisija
$e^- + A_2 \rightarrow A_2 + 2e^-$	Detačment
Atomske, Molekulske i fotonske reakcije	
Reakcije	Opis
$M^* + A_2 \rightarrow M + 2A$	Peningova disocijacija
$M^* + A_2 \rightarrow M + A_2^+ + 2A$	Peningova jonizacija
$M^\pm + A \rightarrow M + A^\pm$	Prenos naelektrisanja (Charge transfer)
$M^- + A^+ \rightarrow MA$	Jonska rekombinacija
$h\nu + A \rightarrow e^- + A^+ ili A^*$	Fotojonizacija i ekscitacija

Neki od najvažnijih ROS bitnih za postizanja različitih efekata u biološkim sistemima su: O_2^- , OH, CO_3^- , H_2O_2 , O_3 , CO. Kod RNS to bi bile sledeće vrste: NO, NO_2 , NO_3 , NO^+ , NO^- , HNO_2 i dr.

1.2 Dijagnostika radiofrekventnih pražnjenja na atmosferskom pritisku

Od samog početka eksperimentalnih izučavanja električnih pražnjenja pa sve do sada nastao je veliki broj dijagnostičkih metoda. Ove metode služe da pruže informacije o karakteristikama plazme. Plazme koje se koriste u proizvodnji integriranih kola, tretmanima uzoraka biološkog porekla, prečišćavanju i aktivaciji vode spadaju u široku kategoriju niskotemperaturnih plazmi. Tipične karakteristike ovih plazmi uključuju električne osobine, gustinu elektrona, temperaturu elektrona i radnog gasa, hemijski sastav, pritisak gasa od atmosferskog do vakuuma i pobudne frekvencije od jednosmerne struje do mikrotalasne i dr.. Ovi parametri obuhvataju relativno širok spektar eksperimentalnih uslova i čine izazov pri izboru najboljih alata za dijagnostiku (Hebner et al., 2000).

U ovom uvodnom poglavlju biće opisane metode koje su korišćene u doktoratu:

- Električna merenja,
- ICCD,
- Masena spektrometrija.

kao i najčešće korišćene metode koje pored masene spektrometrije služe za određivanje fizičko-hemijskih osobina i sastava električnog pražnjenja na atmosferskom pritisku:

- Optička spektroskopija,
- Laserski indukovana fluorescencija.

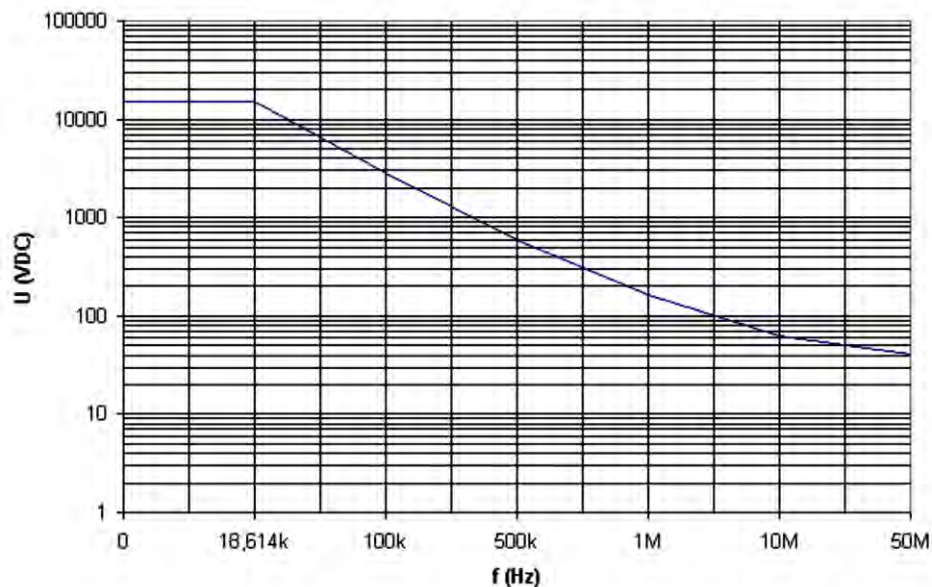
1.3.1 Električna merenja

Merenje struje i napona korišćenjem strujnih i naponskih sondi predstavlja elementarnu i jednu od najčešćih metoda u dijagnostici plazme. Računanje snage, impendanse i kapacitivnosti iz dobijenih vrednosti struje i napona je važno za razumevanje ponašanja plazme i olakšava njeno kontrolisanje i obezbeđivanje stabilnih i reproduktivnih uslova prilikom tretmana.



Slika 1.6 Visokonaponska sonda Agilent N2771B.
(https://prc.keysight.com/Content/PDF_Files/N2771-92001.pdf)

Merenje napona se kod izvora plazmi na nižim frekvencijama postiže korišćenjem naponskih sondi ili visokonaponskih sondi (opseg napona kV) koje se povezuju na merni uređaj (osciloskop). Struja koja prolazi kroz ove sonde je zanemarljiva s obzirom na vrednost njihove impendanse (1 Mohm Agilent N2771B, Slika 1.6). Vrh visokonaponske sonde fizički se povezuje sa izlaznim vodom električnog kola koji je povezan na napajanu elektrodu plazma izvora i šalje trenutnu vrednost napona mernom uređaju koji na ekranu prikazuje oblik i vrednost signala. Slika 1.7 prikazuje karakteristiku visokonaponske sonde korišćene u disertaciji i njena ograničenja pri korišćenju gde mora da se obrati pažnja koji napon je dozvoljen da se meri pri određenoj pobudnoj frekvenciji.



Slika 1.7 Kriva dozvoljenog napona za datu frekvenciju.
https://prc.keysight.com/Content/PDF_Files/N2771-92001.pdf

Što se tiče merenja struje postoje dva načina. Prvi je pomoću komercijalne strujne sonde koja radi po principu transformatora gde struju koja je prošla kroz njega pretvara u kalibrisan naponski signal (Slika 1.8) i vrednost struje je moguće očitati na osciloskopu. Drugi način bi bio korišćenjem naponske sonde i otpornika za dobijanje vrednosti struje. Pad napona na otporniku mora biti dovoljno velik da bi sonda mogla da meri vrednost signala. Drugi način se ne može koristiti na višim frekvencijama gde se provodnici ponašaju kao transmisioni vodovi i mora se uzeti u razmatranje kompletna impedansa sa reaktivnim delom.

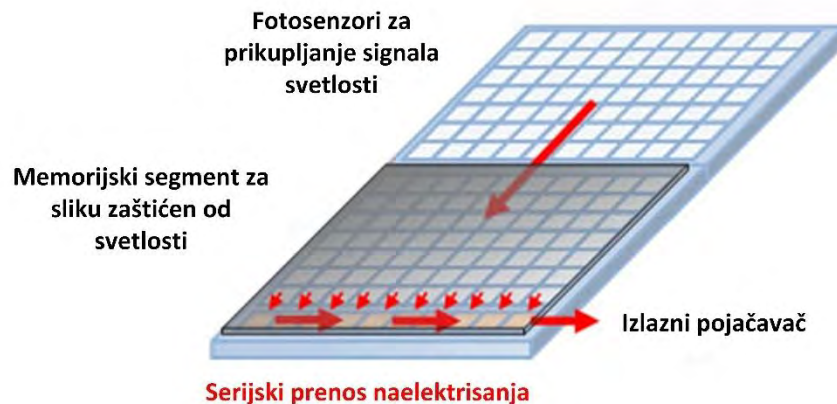


Slika 1.8 Komercijalna strujna sonda
https://www.keysight.com/upload/cmc_upload/ck/1A/images/AC-DC-Current-Probe.jpg

1.3.2 ICCD kamera

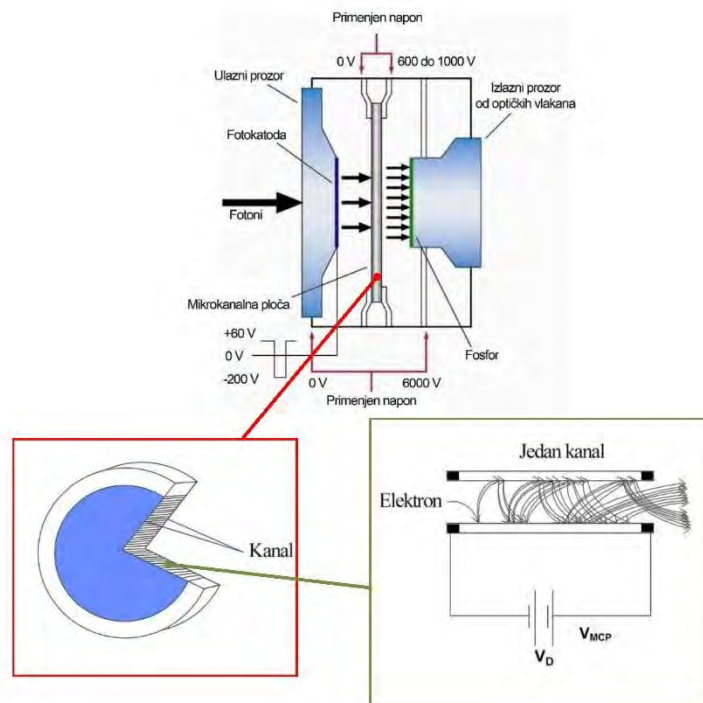
ICCD (*eng. Intensified Charge-Coupled Device*) kamere u dijagnostici plazme igraju veliku ulogu jer zbog svojih tehničkih mogućnosti mogu da se koriste za posmatranje vrlo brzih procesa u električnim pražnjenjima kao što su njeno formiranje ili vremenska evolucija prostornih profila. Takođe, mogu se povezati sa optičkim spektrometrom za snimanje spektralno razloženih podataka iz kojih se mogu dobiti podaci, kao npr. temperatura ili prilikom LIF merenja gde se ICCD kamera koristi za snimanje selektivne vrednosti intenziteta talasne dužine korišćenjem spektroskopskih filtera.

Krajem 60-ih godina prošlog veka kompanija Bell Lab prva je predstavila kameru sa CCD senzorom sačinjenog od integrisanog kola koje sadrži niz povezanih, tj spregnutih, kondenzatora. CCD senzor (Slika 1.9) ima sposobnost brojanja upadnih fotona. Podaci o broju fotona koji su upadaju na jedan piksel senzora mogu biti memorisani na čipu i oni se mogu obrađivati (integracija na čipu, horizontalno i vertikalno sabiranje, i dr). Veliki napredak u CCD tehnologiji čini kompanija Andor sa predstavljanjem ICCD kamere, 1995. godine. Oni su prvi ponudili potpuno integrisani ICCD koji je uključivao digitalni generator kašnjenja (*eng.* Digital Delay Generator - DDG) visokih performansi, visokonaponski „*eng.* gating“ uređaj i kameru.



Slika 1.9 CCD senzor (izvor: https://www.vision-doctor.com/images/stories/kamera/grundlagen/ccd/en_Frame_Transfer_Sensor.jpg)

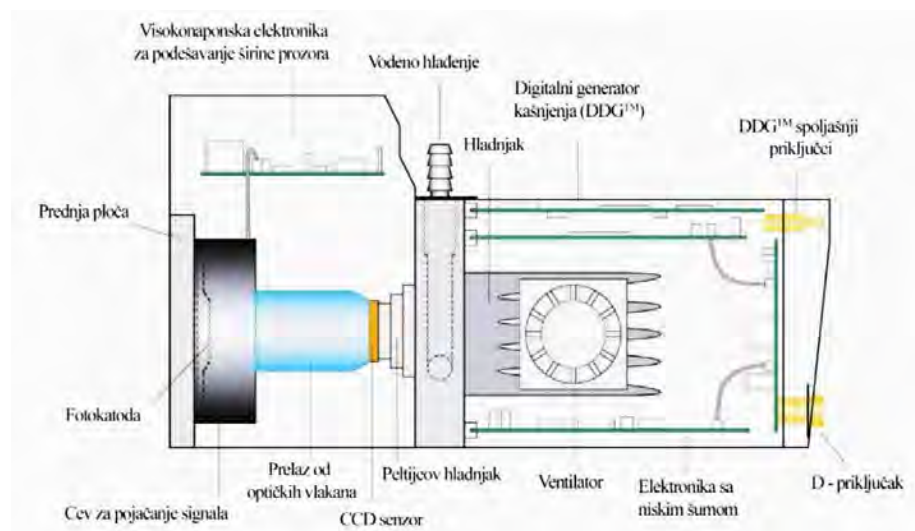
Glavna razlika i prednost ICCD kamere nad standardnim CCD-om jesu optička svojstva okidača (*eng.* shutter). Ovo je ostvareno uređajem „pojačavač intenziteta slike“ (*eng.* Image Intensifier) koji može da se koristi kao vrlo brzi optički prekidač (*eng.* shutter), hvatajući optički signal u milijarditim delovima sekunde. Kod ovog tipa kamera se usled korišćenja pojačanja povećava i grejanje čipa što može povećati šum slike, međutim, uz pomoć sistema hlađenja vrednost šuma se značajno smanjuje. Takođe imaju dodatnu mogućnost da mogu postići vrlo brzo „gate“ vreme. Povezivanje i pojačavanje se dešava u cevi za pojačavanje intenziteta slike.



Slika 1.10 Shema cevi pojačavača intenziteta slike i mikrokanalne ploče.
(izvor: Andor uputstvo)

Cev pojačavača intenziteta slike je evakuisana cev koja se sastoji od fotokatode, mikrokanalne ploče (MP) i fosfornog ekrana (Slika 1.10), a njihova svojstva određuju performanse uređaja.

Princip funkcionisanja cevi pojačavača intenziteta slike je sledeći: Fotokatoda je obložena tankim slojem na unutrašnjoj površini ulaznog prozora i snima upadne fotone. Kada foton udari u fotokatodu, emituje se fotoelektron koji se zatim električnim poljem privlači prema MP. MP je tanki disk (debljine oko 1 mm) koji ima oblik saća i sačinjen je od staklenih kanala debljine 6-10 μm , gde je svaki sa otpornim premazom.



Slika 1.11 Shematski prikaz ICCD kamere kompanije Andor. (izvor: Andor uputstvo)

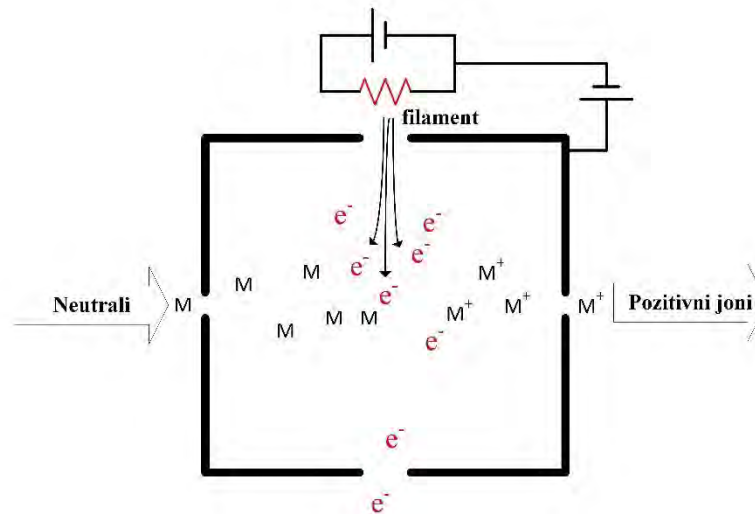
Visok potencijal se primenjuje na MP (500-1000 V) omogućavajući fotoelektronu da ubrza jedan od kanala na disku. Kada fotoelektron ima dovoljno energije, on izbacuje sekundarne elektrone iz zidova kanala. Ovi elektroni zauzvrat trpe ubrzanje što rezultira oblakom elektrona koji izlaze iz MP. Dobici veći od 10.000 mogu se lako postići. Stepenn umnožavanja elektrona zavisi od pojačanog napona primenjenog na MP koji se može kontrolisati u kameri. Nivo pojačanja korisnik reguliše podešavanjem pojačanja (*eng.* Gain). U ovoj disertaciji je izabrano pojačanje od 250 da bi se dobile dovoljno svetle fotografije pražnjenja.

Po izlasku iz MP, elektroni se usmeravaju i ubrzavaju ka sloju fosfora taloženom na kvarcnom prozoru. Upadni elektroni se zatim transformišu u fotone. Oni se mrežom optičkih vlakana prenose u CCD ćeliju koja ponovo transformiše fotone u elektrone. CCD se sastoji od 1024x1024 piksela. Vreme ekspozicije postavlja korisnik koji će odrediti trajanje tokom kojeg će se fotokatoda napajati, a koja ovde ima ulogu okidača. U zavisnosti od razlike potencijala primenjene na njega (-200 ili 50V), propuštaće ili neće propuštati elektrone koji se potom mogu usmeriti na MP. Na Slici 1.11 prikazan je shema poprečnog preseka ICCD kamere.

1.3.3 Masena spektrometrija

Masena spektrometrija predstavlja dijagnostičku metodu koja se koristi za identifikaciju hemijskog sastava gasa a koja se bazira na odnosu mase i naelektrisanja uzorkovanih čestica (Sparkman, 2000; Hoffman *et al.* 2007). 1912. godine prošlog veka nobelovac Džozef Dž. Tomson konstruisao je prvi maseni spektrometar. U to vreme, naučnici koji su se bavili masenom spektrometrijom koristili su samostalno proizvedene uređaje koji su omogućavali analizu samo nekoliko masenih spektara dnevno. Narednih decenija maseni spektrometri su značajno uznapredovali što je omogućilo odličnu analitičku dijagnostiku. U današnje vreme moguće je nabaviti komercijalne masene spektrometre manjih dimenzija koji nude visoku osetljivost, granicu detekcije i brzinu.

Osnovni princip masene spektrometrije je generisanje jona iz uzorka, razdvajajući ih prema odnosu mase i naelektrisanja m/z i detektujući ih kvantitativnom metodom. Dakle, sam uređaj čine (najmanje) tri glavna odeljka: jonizaciona komora, maseni analizator i detektor.

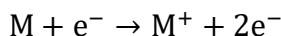


Slika 1.12 Shematski prikaz jonizacione komore

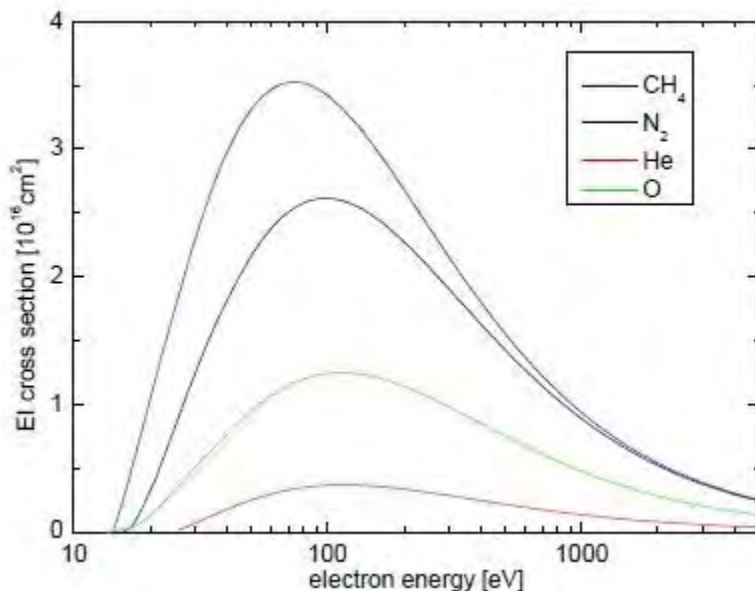
S obzirom na to da maseni analizator može da radi samo sa naelektrisanim česticama, a uzorak se npr. sastoji od neutralnog gasa, potrebno je postojanje izvora koji generiše jone (jonizaciona komora) iz uzorkovanih atoma i molekula. U slučaju merenja jona iz uzorka izvor jona nije potreban. Postoje različite metode jonizacije, elektronska jonizacija, hemijska jonizacija, termalna jonizacija ili jonizacija

vezivanjem elektrona (elektronski atačment). Najčešće se koristi elektronska udarna jonizacija (eng. electron ionization EI). U osnovi, elektroni sa visokom energijom u sudarima interaguju sa neutralnim vrstama unutar jonizacione komore kako bi se kreirali joni. Mora se uzeti u obzir da uzorak gasa treba da bude jako razređen da bi se obezbedio dug slobodan put jonima. U suprotnom vreme života jona bilo bi prekratko za naknadnu analizu. Pritisak je obično u opsegu od 10^{-4} Pa za elektronsku udarnu jonizaciju.

Izvor jona ili jonizaciona komora sadrži filament (Slika 1.12), koji emituje elektrone termalnom emisijom. Ovi elektroni su ubrzani primenjenim naponom do željene energije elektrona (EE) i fokusirani su u jonizacionu zapreminu ispunjenu neutralnim gasom. Energija elektrona je obično 70 eV. Kada npr. neki od elektrona koji poseduje željenu energiju pogodi neutralne čestice koje se nalaze u jonizacionoj komori, deo EE se prenosi na neutrane, što može dovesti do izbacivanja jednog elektrona iz orbite neutralnog molekula:

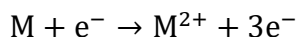


Ova vrednost EE je izabrana zato što preseki za elektronsku jonizaciju σ_{EI} (mera za verovatnoću jonizacije) za skoro sve gasove imaju svoj maksimum blizu ove energije. Ovo se može videti na Slici 1.13 gde su prikazani preseki za različite gasove u funkciji energije elektrona EE (NIST data podataka). Za jonizaciju atoma ili molekula potrebna je određena minimalna energija (~ 7 eV– 20 eV). Ispod ove jonizacione energije ili praga energije, presek je nula i ne dolazi do stvaranja jona.



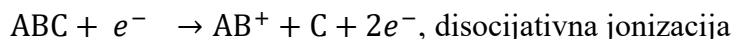
Slika 1.13 Presek elektronske jonizacije za različite gasove u funkciji energije elektrona (NIST-ova baza podataka)

U zavisnosti od energije elektrona, moguće je da se ne samo jedan, nego dva elektrona izbace tokom elektronske jonizacije:



Tokom tumačenja masenog spektra, potrebno je uzeti u obzir da se dvostruko naelektrisane vrste M^{2+} pojavljuju u odnosu masa/naelektrisanje od $M/2$, čak i kada se njihova masa ne razlikuje od vrste M^+ .

Pored jonizacije, nekoliko drugih procesa se mogu dogoditi prilikom masene analize kada je u pitanju uzorkovanje molekula, poput disocijativne jonizacije i disocijativnog preuređenja (ili fragmentacije).



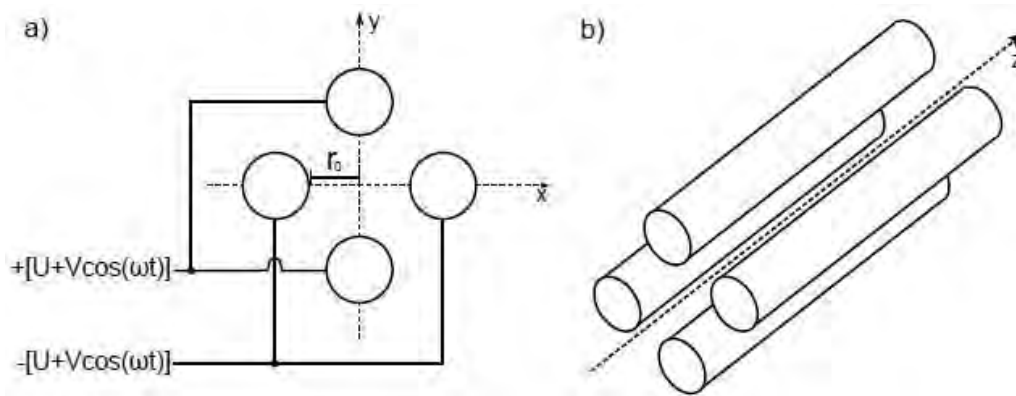
- $A^+ + BC + 2e^-$, disocijativna jonizacija
- $AC^+ + B + 2e^-$, disocijativno preuređenje
- $B^+ + AC + 2e^-$, disocijativno preuređenje.

U masenoj spektrometriji, fragmentacija predstavlja disocijaciju molekula pri prolasku kroz jonizacionu komoru masenog spektrometra usled bombardovanja elektronima energije veće od praga za disocijaciju molekula. Fragmenti molekula sačinjavaju jedinstveni skup signala (patern) u masenom spektru. Ove reakcije su dobro dokumentovane tokom decenija i dobijeni paterni fragmentacije su korisni za određivanje molarne mase kao i strukturnih informacija nepoznatog molekula (McLafferty, 1993; Chhabil, 2007). Naravno, bila bi velika prednost imati masenu analizu bez disocijacije neutralnih molekula kao što se postiže primenom transfera protona na molekule mete.

Joni koji napuštaju jonizacionu komoru odbijaju se ekstrakcionom elektrodom u maseni analizator. Analizator mase je odgovoran za razdvajanje jona prema odnosu m/z . Klasifikacija masenih spektrometara može biti određena prema tipu masenog analizatora. Postoji nekoliko različitih poznatih tipova masenih analizatora, koji koriste različite principe kao što su: vreme preleta, električni ili magnetni sektor polja, linearni kvadrupol ili zamka za jone. Uprkos različitim tehnikama razdvajanja jona, svima je zajedničko to što koriste (statičko i/ili dinamičko) električno i/ili magnetno polje za razdvajanje jona.

Linearni kvadrupolni maseni spektrometar, *eng. linear Quadrupole Mass Spectrometer (QMS)*, su obično odabrani za analizu plazme jer se ističu u poređenju sa drugim tehnikama zbog relativno niske cene, male mase, kompaktne konstrukcije i velike brzine skeniranja. Manji nedostatak QMS-a je relativno niska rezolucija (QMS: $M/\Delta M \approx 2000$, vreme preleta: $M/\Delta M \approx 20000$) i niske osetljivosti (QMS: 100 ppm, vreme preleta: 10 ppm).

Linearni kvadrupolni maseni spektrometar (QMS) predstavlja linearnu verziju Polove-zamke (Nobelova nagrada) koju su prvi opisali Pol i Steinvedel 1953 (Paul i Steinwedel, 1953). Kao što naziv govori kvadrupol se sastoji od četiri cilindrične ili hiperboličke elektrode postavljene u kvadratnoj konfiguraciji gde su podešene da budu striktno paralelne jedna prema drugoj (Slika 1.14).



Slika 1.14 Presek, električna veza a), i shematski prikaz b) cilindričnog masenog analizatora

Svaki suprotni par elektroda povezan je sa istim električnim potencijalom, koji se preklapa DC komponentom U i AC komponentom $V\cos(\omega t)$. Dobijeni rezultat ukupnog potencijala Φ_0 je u tom slučaju: $\Phi_0 = U + V\cos(\omega t)$. Kada čestica koja ima naelektrisanje z uđe u kvadrupol, konfiguracija u x - y -ravni koja putuje u z -smeru, Kulonova sila deluje na jone po sledećem principu:

$$m \frac{d^2x}{dt^2} = -q \frac{d\Phi(x,y)}{dx}$$

$$m \frac{d^2y}{dt^2} = -q \frac{d\Phi(x,y)}{dy}$$

$$m \frac{d^2 z}{dt^2} = 0$$

Idealizovana distribucija potencijala $\Phi(x,y)$ unutar kvadrupola daje $\Phi(x,y) = \Phi_0 \frac{x^2-y^2}{r_0^2}$, gde r_0 predstavlja polovinu rastojanja između dve naspramne elektrode. Dalje kretanje jona prikazano je setom diferencijalnih jednačina:

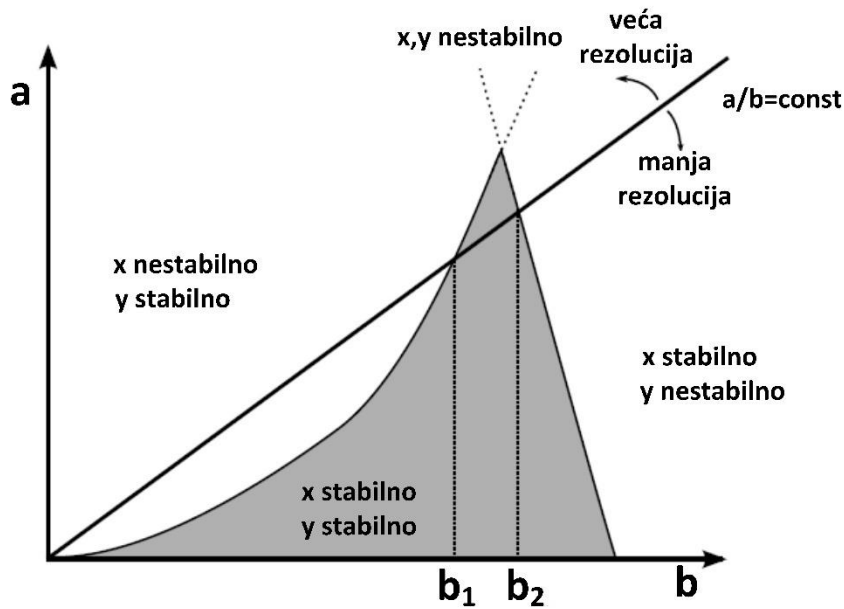
$$\frac{d^2 x}{dt^2} + \frac{2q}{mr_0^2} [U + V \cos(\omega t)] x = 0$$

$$\frac{d^2 y}{dt^2} - \frac{2q}{mr_0^2} [U + V \cos(\omega t)] y = 0$$

$$m \frac{d^2 z}{dt^2} = 0$$

Unutar kvadrupola, jon počinje da osciluje u x - y -ravni. Rezonatan jon će proći kvadrupol prelazeći ga stabilnom trajektorijom, što znači da se neće sudariti ni sa jednom elektrodom. Ovo je slučaj kada x i y nikada ne prelaze r_0 . Ako uvedemo bezdimenzione parametre:

$$a_x = -a_y = \frac{8q}{mr_0^2 \omega^2} U, \quad b_x = -b_y = \frac{4q}{mr_0^2 \omega^2} U, \quad \tau = \frac{\omega t}{2},$$



Slika 1.15 Prvi Mathieu-ov region stabilnosti u a-b-ravni.

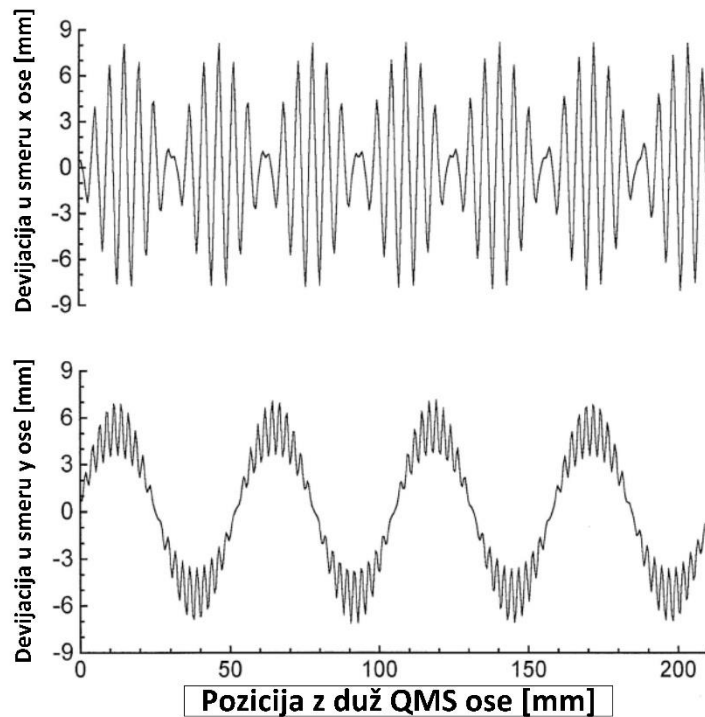
jednačine kretanja se mogu transformisati u Mathieu-ove diferencijalne jednačine:

$$\frac{d^2 x}{d\tau^2} + [a_x + 2b_x \cos(2\tau)] x = 0$$

$$\frac{d^2 y}{d\tau^2} - [a_y + 2b_y \cos(2\tau)] y = 0$$

Mathieu-ova diferencijalna jednačina ima dve vrste rešenja: 1) stabilno rešenje, u kom su x i y

ograničeni za $\tau \rightarrow \infty$ i 2) i nestabilno rešenje, u kom se amplitude u x -smeru i y -smeru eksponencijalno povećavaju za $\tau \rightarrow \infty$. Samim iscertavanjem parametra a naspram parametra b , dobija se dijagram stabilnosti. Takozvana prva regija Mathieu-ove stabilnosti prikazana je na Slici 1.15. Ovaj dijagram prikazuje različite regione za stabilnost x -trajektorija i y -trajektorija. Međutim, samo za sivi trougao-stabilnosti postoje stabilne trajektorije x i y . Za svaku tačku unutar ovog trougla, set (a, b) postoji za x - i y -koordinate jona koje su manje od r_0 . Na Slici 1.16 je prikazana simulacija x -putanje i y -putanje za jon unutar kvadrupola. Tu se može uvideti da se kretanje jona sastoji od makro i mikro oscilacija, ali nikada ne prelazi polovinu elektrode (ovde: $r_0=8.33$ mm). Za kvadrupol sa zadatim r_0 i fiksnom frekvencijom ω , preostali radni parametri su naponi U i V . Odnos $a/b=2U/V$ je nezavisan u odnosu na m/z i ostaje postojan tokom rada MS-a. a/b je odabran na takav način da predstavlja da ab -linija preseca trougao stabilnosti najmanje u jednoj tački (vidi Sliku 1.20). Interval $[b_1, b_2]$ predstavlja stabilnu oblast za koju joni mogu da prođu kvadrupol.



Slika 1.16 Simulacija x -trajektorije i y -trajektorije jona koji prolazi kroz kvadrupol (Blaum *et al.*, 1998)

$$m_1 = \frac{4V}{r_0^2 \omega^2} b_1^{-1} \quad m_2 = \frac{4V}{r_0^2 \omega^2} b_2^{-1}$$

Skaliranjem odnosa $\frac{a}{b}$ (tj. U u odnosu na V) iznad, $\frac{a}{b}$ -linija postaje strmija i samim tim se postiže veća rezolucija. Obično je rezolucija za određeni m/z izabrana na osnovu toga da vrste sa $(m/z) \pm 0,5$ mogu da prođu kroz maseni filter.

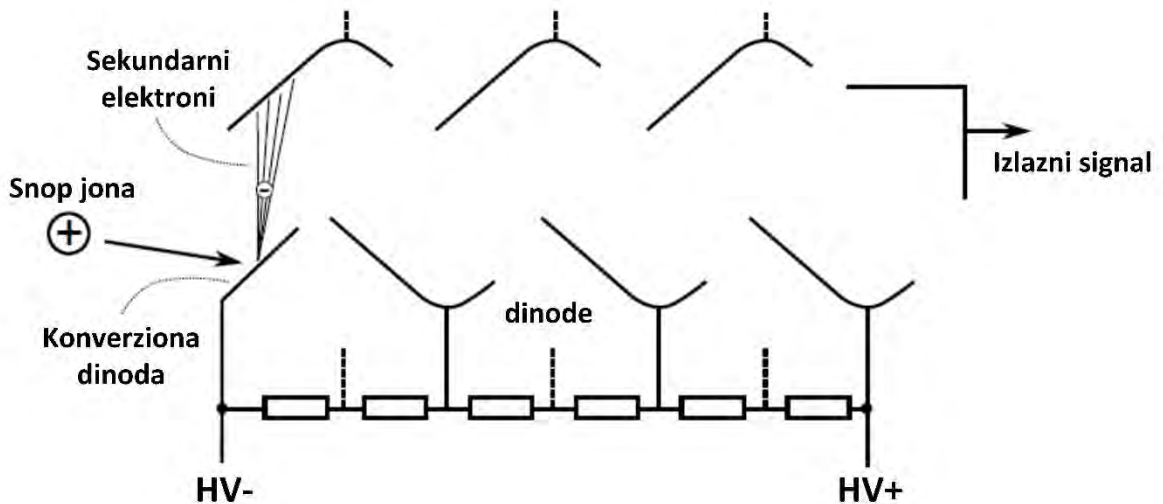
Svaka m/z vrednost ima svoj trougao stabilnosti. U tom slučaju kada se menjaju U i V , dok se $\frac{a}{b} = \frac{2U}{V}$ odnos održava konstantnim, trougao stabilnosti se pomera duž ab -linije. Na taj način, može se izvesti skeniranje masa.

Jako je važno sprečiti sudar jona sa pozadinskim gasom zbog pravilanog rada masenog analizatora. Ovo zahteva dug srednji slobodni put jona i samim tim dobar vakuum ($p < 10^{-3}$ Pa). Nakon razdvajanja

jona m/z odnosom, jone je potrebno transformisati u signal koji se može proceniti. Taj proces se izvršava pomoću detektora jona.

Najjednostavniji detektor za jone je Faradejeva čaša (Faradejev kavez). Faradejeva čaša se sastoji od, kao što i samo ime opisuje, metalne čaše koja sakuplja sve jone koji napuštaju maseni filter. Struja koja teče dalje od Faradejeve čaše transformiše se otpornikom visoke impendance u napon. Ovaj napon je proporcionalan struji udarajućeg jona i koristi se za dalju analizu. Osetljivost Faradejeve čaše je vremenski konstantna i ne zavisi od mase jona. Međutim, osetljivost je relativno mala, a vreme odziva je sporo.

Detektor koji je najčešće korišćen u masnoj spektrometriji je sekundarni elektronski multiplikator (SEM). Skica SEM-a prikazana je na Slici 1.17 SEM sadrži konverziju dinodu koja je na visokom potencijalu čija se polarnost suprotstavlja naelektrisanju jona koji izlaze iz masenog filtera. Iz tog razloga se joni ubrzavaju prema ovoj konvertovanoj dinodi i udaraju u nju. Taj proces izaziva emisiju nekoliko sekundarnih elektrona koji se ponovo ubrzavaju prema sledećoj dinodi. Sledeći multiplikator elektrona koji se sastoji od nekoliko dinoda pojačava sekundarnu elektronsku struju u kaskadnom procesu sa dobitkom od nekoliko redova veličina. U zavisnosti od primenjenog visokog napona i broja dinoda, SEM može dostići faktor pojačanja od 10^6 - 10^8 . Pojačana elektronska struja se prikuplja elektrodom i zatim pretvara u naponski signal koji je proporcionalan broju udarajućih jona. Prednost SEM-a u odnosu na Faradejevu čašu je visoki faktor pojačanja i brzo vreme odziva.



Slika 1.17 Shematski prikaz sekundarnog elektronskog multiplikatora SEM.

Međutim, životni vek trajanja SEM-a je ograničen svega na nekoliko godina. Uticaj jona na dinode dovodi do kontaminacije njihove površine a samim tim i do opadajućeg pojačanja tokom godina. Dakle, pojačanje SEM-a zavisi od mase jona. Različiti joni koji su iste energije, imaju zbog različite mase takođe i različitu brzinu. Na taj način teži joni, zbog svojih manjih brzina, proizvode manje sekundarnih elektrona u odnosu na lakše jone. Iz tog razloga teški joni su diskriminisani od strane lakših.

1.3.4 Optička spektroskopija

Optička spektroskopija predstavlja neinvazivnu dijagnostičku metodu pomoću koje se detektuje intenzitet svetlosti emitovan iz plazme na posebnoj talasnoj dužini. Pomoću ove metode mogu se odrediti kvalitativni i kvantitativni sastav plazme, temperatura gasa, energija, jačina električnog polja, koncentracija elektrona. Dobijena talasna dužina atomske (molekulske) spektralne linije u emisionom spektru daje identitet elementa dok intenzitet emitovane svetlosti može biti proporcionalan broju pobuđenih atoma (molekula) elementa na određenoj talasnoj dužini. Optička spektroskopija se može

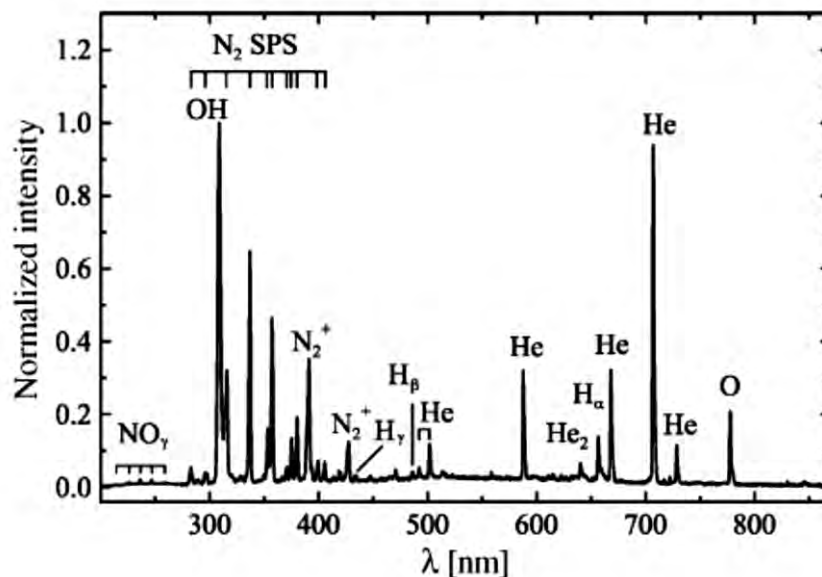
podeliti na dve vrste: emisionu i apsorpcionu spektroskopiju. Kod emisione se detektuje emisija zračenja iz plazme dok se kod apsorpcione posmatra slabljenje intenziteta zračenja koje prolazi kroz plazmu.

Optička emisiona spektroskopija se može podeliti i na pasivnu i aktivnu spektroskopiju. Metode pasivne spektroskopije predstavljaju prosto posmatranje zračenja emitovanog iz plazme i u njih spadaju:

- Doplerovo pomeranje,
- Doplerovo širenje,
- Štarkov efekat,
- Štarkovo širenje,
- Odnosi spektralnih linija,
- Zemanov efekat.

Kod metoda aktivne spektroskopije se pristupa stimulisanju atoma plazme, na neki način, i posmatranju dobijenog rezultata (emisija zračenja, apsorpcija stimulisnog zračenja i dr.). U ovaj tip metoda spadaju sledeće spektroskopske tehnike:

- Apsorpciona spektroskopija,
- Spektroskopija emisije snopa (*eng.* beam emission spectroscopy),
- Laserski indukovana fluorescencija (LIF),
- Dvo-fotonska apsorpciona laserski indukovana fluorescencija (*eng.* Two-photon absorption laser induced fluorescence TALIF).



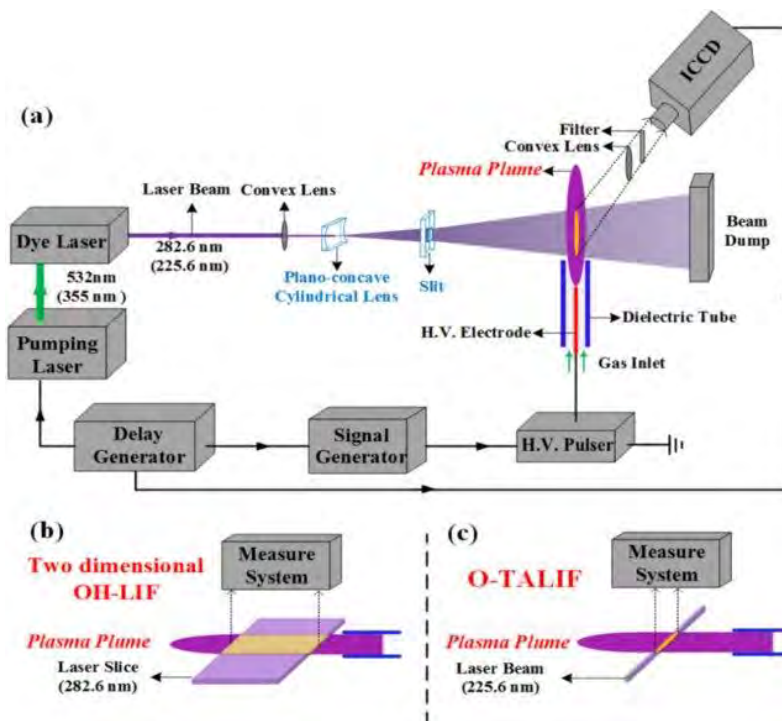
Slika 1.18 Primer emisionog spektra dobijenog DBD plazmenog mlaza. (Sretenović et al. 2012)

Na Slici 1.18 prikazan je tipičan primer kvalitativne metode gde je dobijen pregled spektra emisije zračenja iz DBD plazmenog mlaza (Sretenović *et al.*, 2012). Informacije koje su dobijene iz spektra prikazuju reaktivne vrste koje su poželjne ili neophodne za primenu ovog tipa pražnjenja u primenama u medicini. Takođe, u optičkoj spektroskopiji je primenom širenja spektralnih linija moguće odrediti lokalno električno polje u električnom pražnjenju na atmosferskom pritisku. (Sretenović *et al.*, 2017)

1.3.5 Laserski indukovana fluorescencija

Laserski indukovana fluorescencija (LIF) je spektroskopska metoda u kojoj se atom ili molekul pobuđuje na viši energijski nivo apsorpcijom laserskog snopa koji je praćen spontanom emisijom svetlosti (Kinsey, 1977; Solarz i Paisner, 1986). Prvi put su eksperiment LIF uradili su Zare i saradnici 1968. godine (Tango, 1968; Zare, 2012).

LIF se koristi za proučavanje strukture molekula, otkrivanje izabranih čestica plazme i vizuelizaciju protoka. Talasna dužina se često bira presek prema onoj vrsti koja ima najveći presek. Ekscitovane atomske ili molekulske vrste će se nakon određenog vremena, obično reda od nekoliko nanosekundi do mikrosekundi, deekscitovati i emitovati svetlost na talasnoj dužini većoj od talasne dužine pobude. Ova fluorescentna svetlost se obično snima fotomultiplikatorom (FM) ili filtriranim fotodiodama.

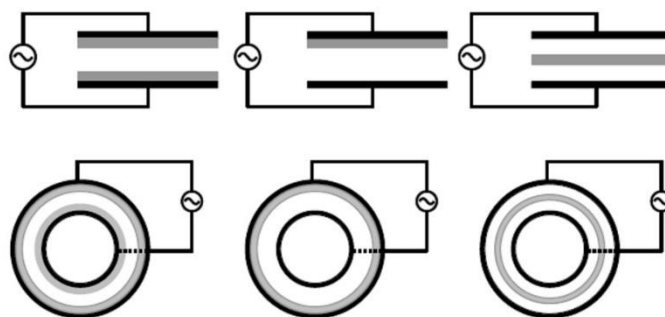


Slika 1.19 Tipičan primer dijagnostike plazmenog mlaza LIF metodom. (Yue *et al.*, 2017)

Na Slici 1.19 prikazan je shematski prikaz eksperimentalne postavke Yue i saradnika pri LIF metodi gde su za različite tipove plazmenih mlazova merili koncentraciju OH i O radikala (TALIF).

1.3 DBD plazmeni mlaz

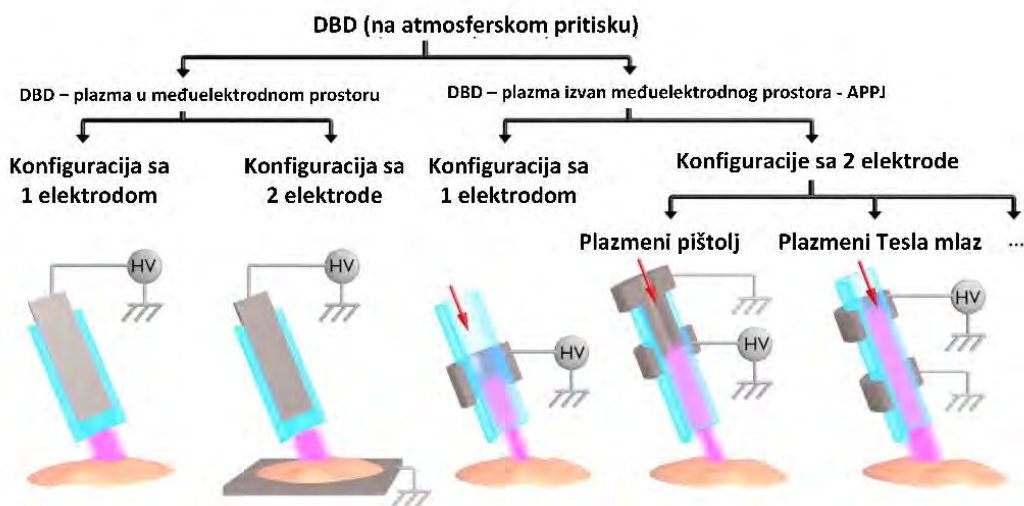
Jedan od najjednostavnijih načina za dobijanje električnog pražnjenja na atmosferskom pritisku jeste korišćenje konfiguracije dielektričnog barijernog pražnjenja (*eng.* dielectric barrier discharge - DBD) kod kojeg se plazma formira između dve elektrode od kojih je bar jedna zaštićena slojem dielektrika (Slika 1.20). Formirana plazma između tih elektroda u najčešćem slučaju se prostire u filamentarnom modu, međutim, pod određenim eksperimentalnim uslovima moguće je ostvariti homogeno difuzni mod pražnjenja. Pokazalo se da je ovaj tip konfiguracije pražnjenja prilično ograničen kada su u pitanju primene u biomedicini gde je potrebno imati lokalizovan tip tretmana.



Slika 1.20 Različite konfiguracije dielektričnog barijernog pražnjenja: crno – metalna elektroda, sivo – izolator. (diplomski rad, Selaković 2011.)

Veliki napredak je dobijen promjenom konfiguracije elektroda cilindrične geometrije gde se pražnjenje pod uticajem protoka radnog gasa prostire slobodno u okolni vazduh van elektrodne oblasti. U ovim izvorima temperatura gasa se kreće u opsegu od 25°C–200°C, gustina naelektrisanih čestica $10^{11} \text{ cm}^{-1} - 10^{12} \text{ cm}^{-1}$, a prisutna je i velika koncentracija hemijski aktivnih čestica formiranih u plazmi, reda 10 ppm–100 ppm. Plazmeni mlaz može biti optimizovan za primenu u različitim oblastima, a posebno privlači veliku pažnju u modifikaciji termoosetljivih materijala i mogućnostima primene u biomedicini (Shashurin *et al.*, 2008; Reuter *et al.*, 2012; Maletić *et al.*, 2015).

U zavisnosti od željene primene plazme, pogotovo za tretman biološkog materijala, konfiguracije izvora su različite, pri čemu je veoma važno najbolje moguće iskorišćenje reaktivnih čestica u plazmi. Plazmeni mlazovi se mogu razlikovati u pobudi, koja može biti radiofrekventna, sinusoidna ili impulsna, u različitoj postavci elektroda (Slika 1.21) (Judée *et al.*, 2019; Jiang *et al.*, 2009; Maletić *et al.*, 2017) i različitom primenjenom radnom gasu (najčešće se koriste argon i helijum, u određenim istraživanjima se u ova dva inertna gasa mogu dodati, u manjim procentima, neki od molekularnih gasova kao što su kiseonik ili azot radi variranja hemijskog sastava).



Slika 1.21 Različiti tipovi DBD izvora (levo) i DBD plazmenih mlazova (desno) prilikom tretmana biološkog tkiva. (Judée *et al.* 2019.)

Varijacije u postavci elektroda mogu biti najrazličitije (jedna, dve ili više elektroda različitih dimenzija) gde elektrode mogu biti izvan dielektrika (obmotane oko staklene cevčice) ili mogu biti uronjene u električno pražnjenje (centralna elektroda – npr. žica od volframa). Što se tiče materijala,

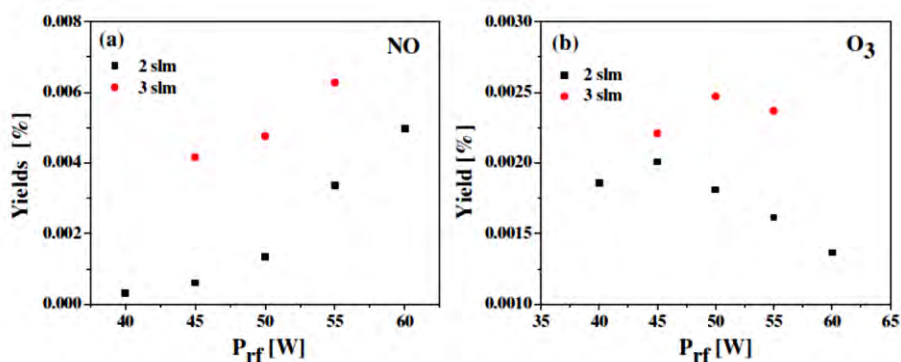
elektrode mogu biti sačinjene od različitog metala a u ovoj disertaciji ćemo koristiti elektrode sačinjene od PET folije koja je sa jedne strane obložena provodnim slojem indijum kalaj oksida (*eng.* indium tin oxide – ITO)(Puač *et al.*, 2012; Maletić *et al.*, 2015).

Obzirom na dimenzije atmosferskih plazmi primena mnogih dijagnostičkih tehnika razvijenih za plazme na nižim pritiscima je ili otežana ili čak nemoguća. Tu se na primer mogu nabrojati primene različitih sondi, posebno Lengmirovih (Langmuir). Čak je i laserska spektroskopija otežana posebno kada je potrebno izvršiti apsolutnu kalibraciju.

Do sada su korišćene različite dijagnostičke metode za karakterizaciju plazmenih mlazova. Najčešće korišćene metode za ispitivanje ovih vrsta izvora plazme su električna karakterizacija, ICCD snimanje, optička spektroskopija, masena spektrometrija, laserski indukovana fluorescencija (LIF), Šlirenova fotografija itd. Veliki broj istraživača primetio je da je deo plazme koja se slobodno širi u vazduhu okoline nije kontinualna kao što je vidi ljudsko oko. Upotreba ICCD kamere sa ekspozicijom reda veličine nanosekundne pokazala je da se ova vrsta plazme obično sastoji od diskretnih paketa (prvobitno nazvanog plazma metak) koji se kreću mnogo većom brzinom od brzine protoka radnog gasa (Lu *et al.*, 2006). Ovaj fenomen su prvi opisali Lou i Laroussi, gde su se pozvali na model širenja strimera zasnovan na fotojonizaciji.

Kasnije, (Robert *et al.*, 2012) je pokazano da elektroni imaju veći uticaj od fotojonizacije na formiranje jonizacionog fronta i od tada se za ovaj fenomen učvrstio naziv impulsni strimer na atmosferskom pritisku (*eng.*: Pulsed Atmospheric Pressure Streamer - PAPS). Takođe, pokazano je da dodavanje male količine molekularnog gasa azota smeši sa plemenitim gasom može dovesti do pojave niza do 8 PAPS u nizu u jednoj APPJ cevi (Park *et al.*, 2013). Najveći problem sa kojim se naučnici suočavaju kada su APPJ u pitanju je konzistentnost samog pražnjenja i osetljivost njegovih parametara čija mala promena može uticati na hemijski sastav koji bi mogao da utiče na performanse tokom primene.

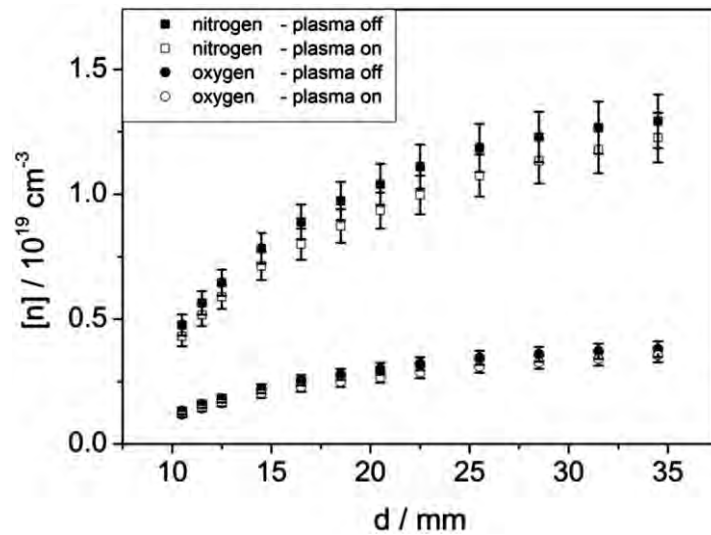
U poslednjoj deceniji masena spektrometrija, sofisticirana dijagnostička metoda, kojom se može odrediti sastav konstituenata plazme na atmosferskom pritisku doživljava svoju ekspanziju. Značajan broj radova je publikovan u proučavanju različitih tipova plazmenih mlazova u kojima se pokazalo da promene parametara električnog pražnjenja u velikoj meri utiču na promenu sastava plazme. Npr., u svom istraživanju, Maletić i saradnici su pokazali da u zavisnosti od primenjene snage na μ -plazmeni mlaz na atmosferskom pritisku i variranjem brzine protoka radnog gasa (2 slm i 3 slm helijuma) može dovesti do različite produkcije NO i O₃ (Maletić *et al.*, 2012). Ova dva radikala imaju vrlo bitne uloge u primenama plazmi. NO ima veliku ulogu u mehanizmima koji se dešavaju u biohemiji ćelija. Može se primetiti na Slici 1.22 povećanje priraštaja NO radikala sa povećanjem primenjene snage predate plazmi. Obrnuti trend se može primetiti za produkciju ozona.



Slika 1.22 Koncentracija (a) NO i (b) O₃ radikala za protoke gasa 2 slm i 3 slm helijuma (1% O₂) (Maletić *et al.* 2012)

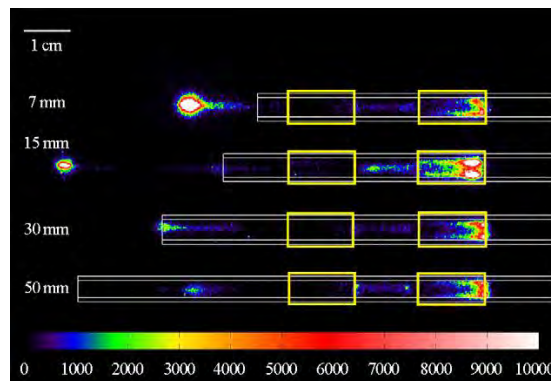
Dünnbier i saradnici, (Dünnbier *et al.*, 2013), su koristili masenu spektrometriju da bi proučili transport neutralnih čestica iz ambijentalnog vazduha u efluent argonskog plazmenog mlaza tokom

električnog pražnjenja. Dobijeni rezultat pokazao je smanjenje produkcije azota a povećanje produkcije kiseonika u slučaju kada je plazma bila uključena (Slika 1.23).



Slika 1.23 Gustine azota i kiseonika za različita rastojanja argonskog plazmenog mlaza kada je pražnjenje bilo uključeno i isključeno. (Dünnbier *et al.* 2013)

Deo ove doktorske disertacije predstavlja prirodan nastavak istraživanja dr Maletića koji je u svojoj disertaciji uradio opsežnu studiju proučavanjem PAPS-a za različite konfiguracije elektroda plazmenog mlaza koji je dizajniran u Laboratoriji za neravnotežne procese i primenu plazme Instituta za fiziku u Beogradu. Za proučavanje masene spektrometrije plazmenog mlaza odabrali smo konfiguraciju 15-15 mm za koju je u svom istraživanju Maletić *et al.* pokazano da PAPS ima najveći domet kao i najveću brzinu od 20 km s^{-1} (Slika 1.24). Po prvi put će biti prikazan uticaj vodene pare na formiranje PAPS-a kao i vremenski razloženo snimanje pozitivnih jona koji predstavljaju deo hemije pulsnog strimera koji se pojavljuje u uzlaznom delu pozitivnog dela poluperioda sinusoidnog signala korišćenog plazmenog mlaza. Takođe, biće prikazani maseni spektri mnogostrukog plazmenog mlaza koji može da radi u dva različita režima (spojeni i nespojeni) kao i dijagnostika masenim spektrometrom posebnog tipa dielektričnog barijernog pražnjenja - DCSBD-a gde je snimana produkcija reaktivnih vrsta radikala koji imaju značajan uticaj u primenama.



Slika 1.24 Vremenski razložene ICCD fotografije sa usrednjenih 160 snimaka za vreme trigera od $11.2 \mu\text{s}$ (Maletić *et al.* 2015)

1.4 Primene radiofrekventnih atmosferskih plazmi

Od početnih proučavanja plazme pa sve do danas naučnici su istraživali mogućnost primena električnih pražnjenja u različitim tehnološkim oblastima kao što su proizvodnja poluprovodničkih uređaja, integrisanih kola, optičkih uređaja, solarnih ćelija, itd (Poulsen, 1977; Economou, 2014; Janowiecki *et al.*, 1977; Samukawa *et al.*, 2012).

U poređenju sa plazmom na niskom pritisku, plazme na atmosferskom pritisku imaju značajnu prednost zbog činjenice da im nije potreban kompleksan i glomazan vakuumski sistem kao što su komora i vakuumska pumpa. Još jedna prednost u odnosu na izvore plazme na niskom pritisku jeste da se one mogu koristiti za tretman uzoraka koje ne trpe vakuum. Uglavnom su to uzorci biološkog porekla tako da je plazma na atmosferskom pritisku idealna za primene u biomedicini. One mogu raditi sa vrlo malom ulaznom snagom (od nekoliko vati), koja se može isporučiti portabilnim sistemom manjih dimenzija.

S obzirom na njen sastav koji čine elektroni, pozitivni i negativni joni, neutralni atomi i molekuli, plazma se odlikuje skupom različitih fizičkih osobina kao što su temperatura, različitim vrstama zračenja (npr. UV i termalno) i električnim poljima (Slika 1.25). Svi nabrojani konstituenti i osobine električnih pražnjenja mogu da utiču na modifikaciju tretiranog uzorka i neophodno je uraditi opsežna istraživanja kako bi se nad izvorom plazme uspostavila kontrola nad parametarima kojima je moguće dizajnirati željeni hemijski sastav, temperaturu plazme, zračenje i dr.



Slika 1.25 Kompozicija neravnotežne plazme na atmosferskom pritisku.

Poslednjih par decenija niskotemperaturne plazme na atmosferskom pritisku privukle su veliko interesovanje zbog širokog opsega mogućih primena u biomedicini. Zbog neravnotežnog svojstva ovih plazmi, elektroni u sudarima sa okolnim gasom u atmosferi stvaraju značajan broj reaktivnih vrsta, dok gas ostaje na sobnoj temperaturi. Plemeniti gasovi poput He i Ar uveliko se koriste za stvaranje plazmi na atmosferskom pritisku jer rezultuju stabilnim generisanjem tinjavog pražnjenja sa niskom temperaturom gasa. Pojedini izvori plazme imaju sposobnost da se prostiru u atmosferu, poput plazmenog mlaza, i interaguju sa N_2 i O_2 u vazduhu (Laroussi i Akan, 2007). Dodatak za smešu gasova u vidu molekularnog gasa kao što je O_2 često se dodaje plemenitim gasovima da bi se pojačalo

generisanje reaktivnih radikala. Električna pražnjenja na atmosferskom pritisku se mogu generisati primenom širokog opsega frekvencije, od jednosmerne do RF i mikrotalasa.

Neki od izvora plazme na atmosferskom pritisku koji se ispituju u biomedicinskim primenama su plazma igla, plazmena baklja, dielektrično barijerno pražnjenje - DBD, KinPen, razne vrste plazmenih mlazova i mikro plazmenog mlaza (Maletić *et al.*, 2012; Moon *et al.*, 2003; Pipa *et al.*, 2012; Fridman *et al.*, 2008; Goree *et al.*, 2006). Iako ovi izvori imaju različite konfiguracije elektroda, frekvencije pobude (jednosmerna, kHz, MHz, GHz), radni napon, zajednički cilj im je postizanje velike gustine reaktivnih vrsta uz održavanje niske temperature gasa i uzoraka (ispod 40°C). Tek tada se električno pražnjenje na atmosferskom pritisku može uspešno primeniti na živo tkivo. Mnogi očigledni efekti su empirijski utemeljeni bez detaljnog i potpunog razumevanja tačnih mehanizama interakcija plazma-ćelija.

Navedene izvore takođe karakteriše širok spektar dimenzija, od plazmi mikrometarske veličine koje su pogodne za vrlo precizne i lokalizovane tretmane, do pražnjenja većih dimenzija pogodnih za tretman uzoraka sa većom površinom. Sve navedene razlike u dizajniranju atmosferskih plazmi bitno utiču na njen sastav i produkciju RONS-a i zato se mora sa posebnom pažnjom uraditi odabir izvora u zavisnosti od primenjenog uzorka.

Prema tipu tretmana u biomedicini može se napraviti podela na 2 tipa direktnog i indirektnog tretmana:

1. Direktno pražnjenje predstavlja neposredno izlaganje uzorka električnom pražnjenju, a indirektno pražnjenje u biomedicini, predstavljao bi slučaj gde se plazmom tretira medijum bez biološkog uzorka. Kasnije se nakon tretmana uzorak dodaje u tzv. plazma aktiviran medijum - PAM. Ova podela sa sobom nosi i prednosti i mane. Neki uzorci su *in vitro* preosetljivi na direktno pražnjenje plazmom gde lako može doći do njihove kontaminacije i neophodno je koristiti indirektno pražnjenje, dok određeni uzorci bolje reaguju na direktno pražnjenje.
2. Direktno pražnjenje predstavlja izlaganje uzorka vidljivom delu električnog pražnjenja, a indirektno pražnjenje predstavlja slučaj gde plazma ostaje u oblasti elektrode i ne dodiruje uzorak. U tom slučaju hemijski reaktivne vrste ili difunduju ka uzorku ili se pod dejstvom protoka gasa usmeravaju ka njemu.

Plazma igla koju su razvili Stoffels i saradnici (Stoffels *et al.*, 2003) široko je prihvaćen uređaj koji se sastoji od oštre tanke elektrode (slične obliku konvencionalne igle) povezane na RF izvor i sa dielektričnom cevčicom koja je okružuje. Niskotemperaturna plazma se formira na vrhu elektrode gde je koncentrisano jako električno polje (Sakizama i Graves, 2009). Plazma igla je pogodna za lokalizovan tip tretmana. Plazma mlazovi spadaju u izvore plazmi kod kojih se plazma prostire izvan uređaja, i oni su pogodni za lokalizovane precizne primene. Prednost prostiranja plazme izvan međuelektrodnog prostora u okolni ambijent leži u činjenici da se zbog sudara čestica gasa iz atmosfere i električnog pražnjenja stvara bogata hemija pogodna za primene (Laroussi i Akan, 2007).

Prilikom tretmana medicinskih uređaja plazmom moguće je ukloniti gram-pozitivne i gram-negativne bakterije, gljivice, spore i viruse (Boudam *et al.*, 2006; Fridman *et al.*, 2006; Gadri *et al.*, 2000; Cheng *et al.*, 2006; Shimizu *et al.*, 2006). U ćelijskoj biologiji pražnjenja na atmosferskom pritisku mogu da indukuju ćelijsku smrt, proliferaciju i utiču na faze u ćelijskim ciklusima. Takođe, veoma su značajne primene u bolničkoj higijeni, tretmanu hroničnih rana, kožnih oboljenja (Fridman *et al.*, 2008; Fridman *et al.* 2006; Fridman *et al.* 2007; Reshetov *et al.* 2000; Shekhter *et al.* 2005), bolesti zuba (Sladek *et al.* 2004; Stoffels *et al.* 2006), za koagulaciju krvi, u kozmetici, u preciznoj hirurgiji (Stoffels *et al.* 2006; Priglinger *et al.* 2005; Stoffels *et al.*, 2007), za uklanjanje ožiljaka i precizno razdvajanje tkiva bez krvi, kao i druge primene (Laroussi *et al.* 2003; Laroussi *et al.* 2005; Kolb *et al.* 2006; Diplomski rad, Nenad Selaković, 2011).

U ovoj disertaciji biće korišćeno radiofrekventno pražnjenje na atmosferskom pritisku, tzv. plazma igla, koja predstavlja „poboljšanu verziju“ plazma igle koju su prvi napravili Stoffels i saradnici. Ova plazma igla razvijena je u Laboratoriji za neravnotežne procese i primenu plazme Instituta za fiziku u Beogradu. U dosadašnjim dostignućima u vezi sa primenom plazma igle glavni cilj bio je istraživanje temeljnih aspekata kod efekata plazme na živo tkivo poput rasta ćelija, povećanja, deobe i diferencijacije. Pre uvođenja u biomedicinske primene plazma igla je detaljno istražena u našoj laboratoriji korišćenjem naprednih dijagnostičkih tehnika kao što su masena spektrometrija za određivanje masenog sastava plazme, derivativne sonde za precizno merenje struje, napona i snage predate plazmi [Puač *et al.*, 2006, Malović *et al.*, 2010].

Veoma je važno okarakterisati plazmu pre bilo koje primene i odrediti optimalni opseg parametara. Fiksni parametri plazme omogućavaju poređenje odgovora različitih tipova ćelija nakon tretmana, koji variraju u zavisnosti od ćelijskih strukturnih razlika. Miletić i saradnici [Miletić *et al.*, 2013] pokazali su da je potencijal diferencijacije prema osteoblastima matičnih ćelija parodontalnih ligamenata izvedenih iz usta pacijenta značajno povećan nudeći potencijalno novo rešenje za lečenje parodontitisa zasnovano na regeneraciji tkiva matičnih ćelija. Takođe, u dosadašnjim istraživanjima plazma iglom je tretiran i dentin ljudskog zuba [Stasić *et al.* 2019] i posmatrani su efekti u promenama mikrostrukture dentina. Pokazano je da reaktivne vrste generisane u plazma igli utiču na promenu sastava ljudskog dentina menjajući mu strukturu što dovodi do povećanje površinske energije a samim tim i do veće hidrofilitnosti uzorka. Ovo je značajno za kliničko lečenje zuba gde je olakšano korišćenje stomatoloških materijala i izbegava se upotreba kiselina za nagrivanje dentina.

Matične ćelije nisu jedine ćelije koje imaju sposobnost diferencijacije. Biljne matične ćelije, kalusi, imaju istu sposobnost. U ovoj disertaciji kao jedan od tipova tretiranih bioloških uzoraka biće korišćeni biljni kalusi *Daucus carota*. Iskorak u odnosu na dosadašnja istraživanja biće u analizi proizvedenih reaktivnih vrsta u plazmi koji utiču na metabolički sistem biljnih ćelija. Takođe još jedan od sledećih dostignuća u primenama plazma igle biće tretman MRSA bakterija gde će se posmatrati efikasnost sterilizacije posmatranjem v veličine dobijenih inhibicionih zona koju plazma izaziva usled tretmana. I na kraju biće prikazani rezultati tretmana kancerogenih ćelija i efekti koje plazma izaziva kod ćelijskih linija koje su otporne na citostatike.

2 Eksperimentalne postavke i dijagnostičke metode korišćene za određivanje karakteristika pražnjenja DBD plazmenog mlaza

U ovom poglavlju će biti predstavljeni rezultati masene spektrometrije zračenja emitovanog iz plazmenog mlaza koji radi na atmosferskom pritisku a razvijen je u Laboratoriji za neravnotežne procese i primenu plazme Instituta za fiziku u Beogradu. Takođe će biti prikazani rezultati dobijeni snimanjem ICCD kamerom. U ovom eksperimentu biće proučavan i uticaj vlažnosti unutar protočnog sistema radnog gasa na karakteristike plazmenog mlaza.

2.1 Eksperimentalna postavka

2.1.1 Električno kolo izvora i protok gasa

Prvi izvor atmosferskog pražnjenja koji ćemo predstaviti u ovoj disertaciji je plazmeni mlaz sačinjen od Pyrex staklene cevčice unutrašnjeg prečnika 4 mm i spoljašnjeg prečnika 6 mm i dve cilindrične elektrode. Elektrode su napravljene od prozirne PET folije čija je jedna strana naparena provodnim slojem indijum-kalaj oksida (specifičnog otpora $R_s \leq 10\Omega$) i obmotane su oko staklene cevi. Neprovodna strana je orijentisana ka spolja a provodna strana elektrode je priljubljena uz staklenu cevčicu. Širina obe elektrode, razmak između njih kao i udaljenost od ivice staklene cevčice i prve elektrode iznosi 15 mm.

Prva elektroda (na slici 2.1 - El. 1) predstavlja napajanu elektrodu, dok je druga elektroda (na slici 2.1 - El. 2) uzemljena. Za napajanje električnog kola plazmenog mlaza kao i za trigerovanje korišćen je dvokanalni signal generator (RIGOL DG5102). Kanal A je korišćen za generisanje signala sinusoidnog oblika na frekvenciji od 80 kHz ($5.5 V_{\text{peak-peak}} - 7.5 V_{\text{peak-peak}}$) čija je amplituda povećana (do 1 kV) pomoću pojačavača napravljenog u Laboratoriji za neravnotežne procese i primenu plazme. Nakon pojačavača amplitudu dodatno povećavamo pomoću specijalno dizajniranog visokonaponskog transformatora i variramo vrednosti primenjenog napona na napajanoj elektrodi ($5.9 kV_{\text{peak-peak}} - 6.5 kV_{\text{peak-peak}}$). Kanal B je korišćen za generisanje signala četvrtastog oblika (TTL) na frekvenciji od 80 kHz i pomoću njega smo koristeći T-adapter trigerovali maseni spektrometar i ICCD kameru istovremeno i izvršili sinhronizaciju sa strujno-naponskim signalima. „Niska“ vrednost TTL signala je u oba slučaja podešena na 0 V, dok je za merenja uticaja vlažnosti na propagaciju PAPS-a „visoka“ vrednost podešena na 3.4 V, a za merenja vremenski razloženih jona na 4.7 V. Kod rezultata pri uticaju vlažnosti je triger bio pri početku uzlaznog dela signala napona, dok je u merenjima vremenski razloženih jona zbog sinhronizacije novih rezultata sa rezultatima dobijenim prvobitnim korišćenjem komparatora triger bio podešen u nuli nakon silaznog dela naponskog signala.

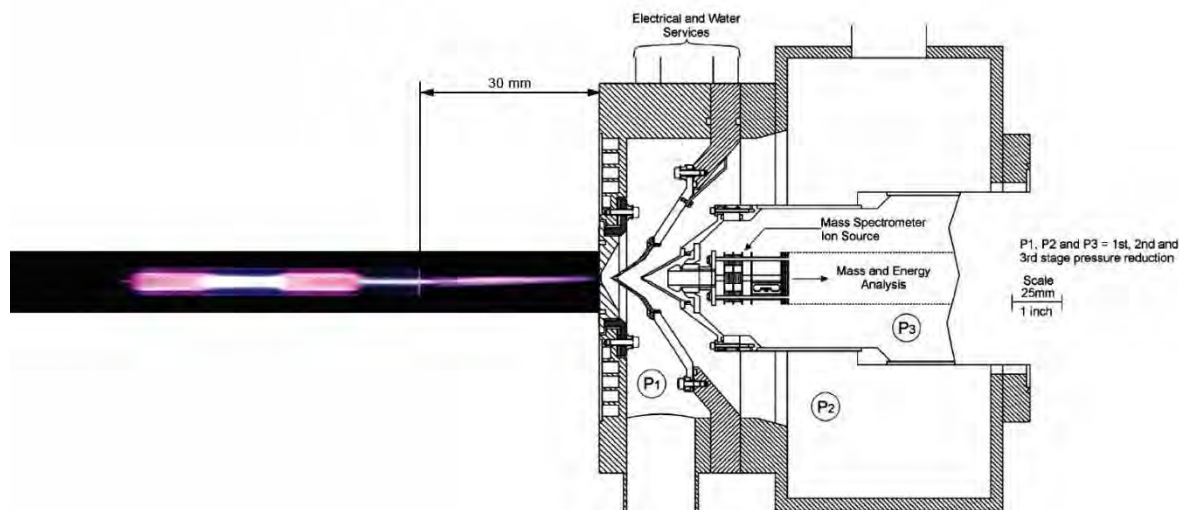
Kao radni gas koristili smo helijum (čistoće >99,999%) sa brzinom protoka od 3 slm i 4 slm regulisane pomoću protokomera (Bronkhorst EL-FLOW Select F-201AV), a takođe i mešavinu helijuma i malog procenta vodene pare. U svrhu mešanja gasova u eksperimentalnu postavku smo postavili dodatnu granu protočnog sistema (Slika 2.1 crveni kvadrat). Unutar ove grane postavljamo ispiralicu (staklena posuda na čijem je dnu tečnost, u ovom slučaju voda, a iznad koje se nalazi vodena para) pomoću koje dodajemo mali procenat vodene pare u helijum i kontrolišemo ga pomoću protokomera (Omega FMA 5400/5500).

Za električnu karakterizaciju, sa ciljem računanja snage predate plazmenom mlazu, korišćene su dve komercijalne sonde i osciloskop (Keysight MSO3202A). Visokonaponsku sondu (Slika 2.1 HV sonda – Agilent N2771A podele odnosa 1000:1) smo povezali na visokonaponski izlaz transformatora i korišćena je za dobijanje trenutnih signala napona. Za dobijanje trenutnih strujnih signala koristili smo

dewpoint transmitter) ispred staklene cevčice. Takođe smo želeli da istražimo uticaj vlage kada ona ima konstantnu vrednost i zbog toga nam je bilo potrebno da veštački navlažimo sistem koristeći ispiralicu.

2.1.2 Sistem za masenu spektrometriju

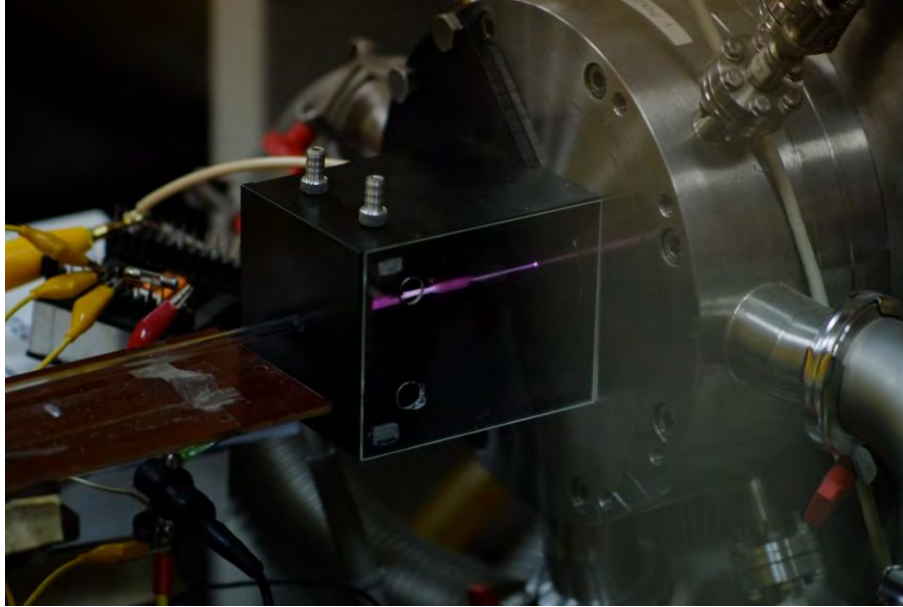
HIDEN HPR60, kvadrupolni maseni spektrometar koji radi na principu molekularnog snopa (MBMS), korišćen je za određivanje masenog spektra pozitivnih jona koji potiču iz plazmenog mlaza. Slika 2.2 prikazuje ispitivani položaj izvora plazme u odnosu na otvor za uzorkovanje MBMS-a. HPR60 MBMS ima tri stepena sa tri različita pritiska P_1 , P_2 i P_3 ispred četvorostrukog detektora. Za dobijanje masenih spektara pozitivnih jona korišćen je poseban režim za skeniranje, tzv. „masena spektrometrija sekundarnih jona +“ (eng. secondary ions mass spectrometry – SIMS+). U ovom SIMS+ režimu, jonizaciona komora spektrometra nije aktivna, što znači da svi pozitivni joni koje ćemo prikazati potiču iz pražnjenja. Vrednosti pritiska u tri faze tokom eksperimenta bile su: $P_1=3.0 \cdot 10^{-1}$ Torr, $P_2=2.9 \cdot 10^{-6}$ Torr i $P_3=3.8 \cdot 10^{-7}$ Torr. Maseni spektar pozitivnih jona prikazanih u disertaciji predstavlja srednju vrednost tri uzastopna ciklusa snimljena za isti skup parametara.



Slika 2.2 Eksperimentalna postavka za masenu spektrometriju (plazmeni mlaz postavljen je normalno na otvor za uzorkovanje; na slici je prikazan poprečni presek HPR60 masenog spektrometra)

Za razliku od DBD izvora, izvora plazme koji pokrivaju veću površinu, plazmeni mlazovi po svojoj prirodi imaju različitu geometriju, čije je pražnjenje koncentrisano u jednom kanalu - mlazu. Pre samog početka merenja, kada je u pitanju masena spektrometrija plazmenog mlaza na atmosferskom pritisku, potrebno je precizno podesiti mlaz na inlet (otvor) za uzorkovanje. Za postavku plamenog mlaza i njegovo precizno podešavanje korišćen je xyz stalak konstruisan i napravljen u radionici Instituta za fiziku. Za tu potrebu, primenjivan je poseban režim skeniranja, eng. MID scan, koji omogućava praćenje odbroja signala pozitivnih jona tokom vremena (vremensku evoluciju). Za odbroj signala odabrani su neki od najzastupljenijih pozitivnih jona čije je prisustvo očekivano, kao što su, na primer, molekulske vrste azota i kiseonika. Problem koji se tada javlja jeste gubitak signala usled treperenja mlaza plazme na otvoru masenog spektrometra usled osetljivosti pražnjenja na turbulencije koje se javljaju prilikom nepredviđenih strujanja vazduha u laboratoriji. Kako bi se sprečio gubitak signala zbog vazdušnih

strujanja, kao i zbog relativno malih vrednosti otvora za uzorkovanje (od 0.1 mm ili 0.3 mm), ispred spektrometra smo ugradili komoru dužine 10 cm, širine 8 cm i visine 8 cm. Zidove komore smo obojili u crnu mat boju (zbog smanjenja uticaja refleksije emisije svetlosti prilikom optičkih merenja) i pričvrstili je na prednji deo masenog spektrometra oko otvora za uzorkovanje. Sa jedne strane komore postavili smo prozor od pleksiglasa kroz koji je moguće posmatranje i snimanje pražnjenja. Prozor sadrži dva otvora čiji položaj ne utiče na nesmetano prostiranje mlaza plazme. Glavna uloga ovih otvora je da obezbedi dovoljnu količinu vazduha za atmosfersko pražnjenje (bez rupa unutrašnjost komore bi se vrlo brzo ispunila helijumom i došlo bi do pojave električnog luka između elektroda izvan cevčice plazma mlaza). Slika 2.3 prikazuje eksperiment u kome je APPJ postavljen normalno na otvor za uzorkovanje masenog spektrometra.



Slika 2.3 Komora plazmenog mlaza sa prozorom koji propušta dovoljnu količinu vazduha neophodnu za atmosfersko pražnjenje

2.2 Električna karakterizacija plazmenog mlaza

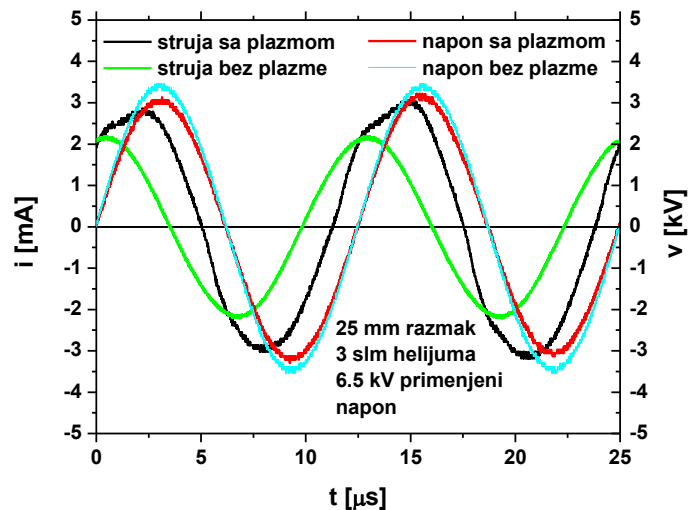
2.2.1 Uvod

U ovom poglavlju biće prikazana električna karakterizacija plazmenog mlaza u eksperimentalnoj postavci gde je staklena cevčica izvora plazme postavljena normalno na otvor za uzorkovanje masenog spektrometra. Maseni spektrometar korišćen u eksperimentu je uređaj koji je sačinjen u velikoj meri od nerđajućeg čelika tako da električno pražnjenje koje stupa u kontakt sa njim može biti perturbovano ne samo zbog sudara čestica sa zidom inleta masenog spektrometra već i zbog uticaja električnog polja koje se formira u okolini oblasti elektroda plazmenog mlaza usled primene visokog napona. Cilj ovog merenja jeste utvrđivanje električnih parametara plazmenog mlaza kao i uticaj prisustva masenog spektrometra na električnu karakteristiku za dva korišćena rastojanja pri kojima može da se posmatra vremenska evolucija PAPS-a.

2.2.2 Strujno naponske karakteristike i srednje snage plazmenog mlaza

Na Slici 2.4 prikazana su dva perioda uporednih strujno-naponskih signala sa plazmom i bez nje izmereni naponskim sondama u funkciji vremena. Za slučaj kad nema pražnjenja, fazna razlika između struje i napona iznosi približno 90° , kada je impendansa sistema reda veličine $M\Omega$ što odgovara

kapacitetu vrednosti reda pF. S druge strane, kada je pražnjenje formirano, strujni signal je veći, malo deformisan i skoro u fazi sa naponskim. U isto vreme naponski signal se smanjuje, pošto je u pitanju radni napon koji je znatno manji od napona proboja.



Slika 2.4 Primer strujno-naponski signala plazmenog mlaza koji se nalazi na rastojanju od 25 mm u odnosu na maseni spektrometar.

Snimljena je zavisnost korena srednje kvadratne vrednosti napona od korena srednje kvadratne vrednosti struje ($U_{rms} = f(I_{rms})$). Snaga predata plazmi je izvedena iz korena srednjih kvadratnih vrednosti struje i napona koji su računati po formulama:

$$f_{RMS} = \sqrt{\frac{1}{T_2 - T_1} \int_{T_1}^{T_2} [f(t)]^2 dt}$$

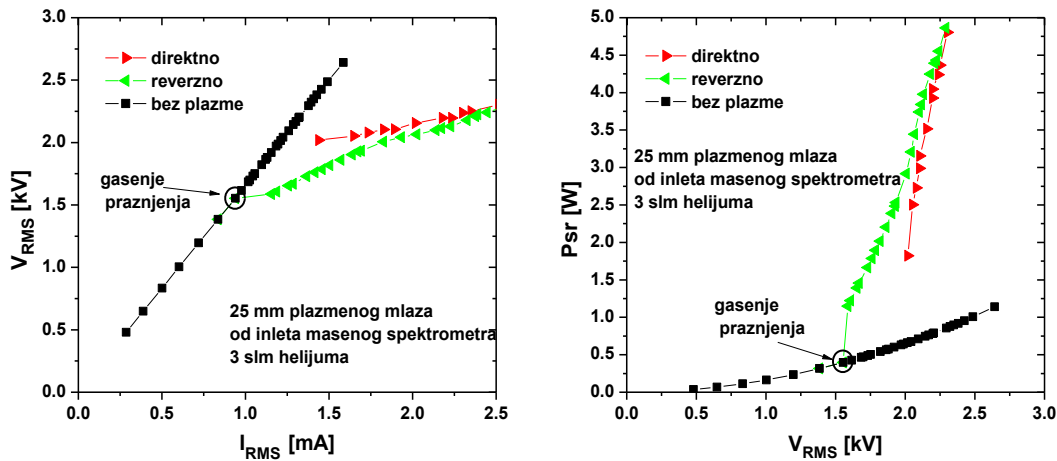
$$U_{RMS} = \sqrt{\frac{1}{nT} \int_0^{nT} [U(t)]^2 dt}$$

$$I_{RMS} = \sqrt{\frac{1}{nT} \int_0^{nT} [I(t)]^2 dt}$$

$$P_{sr} = \frac{1}{nT} \int_0^{nT} UI dt$$

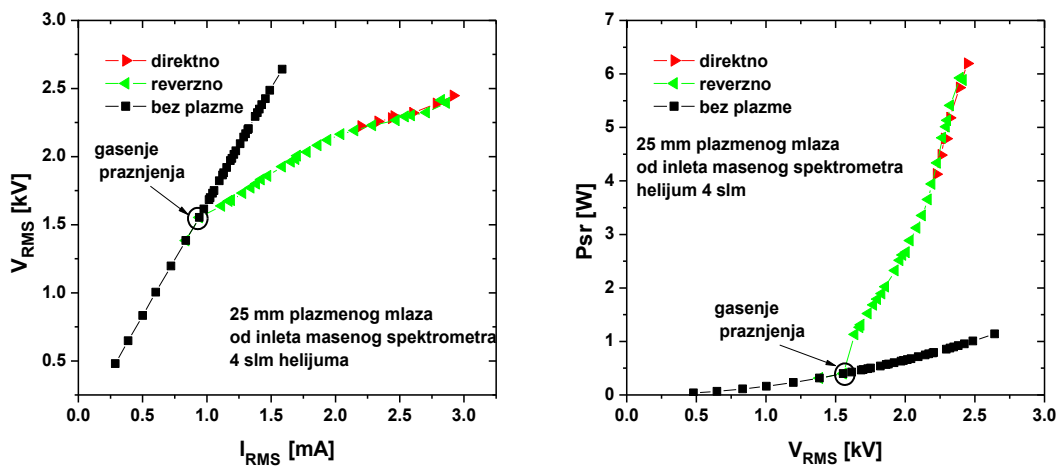
Na Slici 2.5 mogu se videti strujno-naponska karakteristika plazmenog mlaza (levo) kao i vrednosti snage predate plazmi (desno) u eksperimentalnoj postavci gde je rastojanje između izvora plazme i masenog spektrometra iznosilo 25 mm a brzina protoka radnog gasa helijuma bila 4 slm. Crna linija na grafiku strujno-naponske karakteristike prikazuje električnu karakteristiku sistema plazma mlaza kada nema električnog pražnjenja i ima osobinu izrazito linearne funkcije sa izmerenom impedansom od

1.66 M Ω . Pri ovom rastojanju, ako posmatramo crvenu i zelenu liniju na istom grafiku, možemo primetiti promenu nagiba prilikom formiranja pražnjenja što se može predstaviti kao uvođenje paralelne nelinearne impedanse u električnom kolu te se impedansa sistema smanjuje na vrednost od 1.51 M Ω . Konkretno, do proboja je došlo pri vrednostima od 2 kV V_{RMS} i 1.5 mA I_{RMS} (početak crvene krive označene sa „direktno“). Napon je povećavan do određene „bezbedne“ tačke pri kojoj ne može doći do pregrevanja pojačavača korišćenog u električnom kolu (2.5 kV V_{RMS} i 2.5 mA I_{RMS}). Zelena kriva (označena sa „reverzno“) prikazuje postepeno smanjenje primenjenog napona i može se primetiti da se plazma održava i pri nižim vrednostima struje i napona od tačke u kojoj je došlo do proboja. Nelinearna impedansa sistema u ovom slučaju iznosi 1.56 M Ω . Na graficima je označena tačka u kojoj se pražnjenje gasi. Ukupna snaga plazmenog mlaza za ove eksperimentalne uslove ide do vrednosti od 5 W.



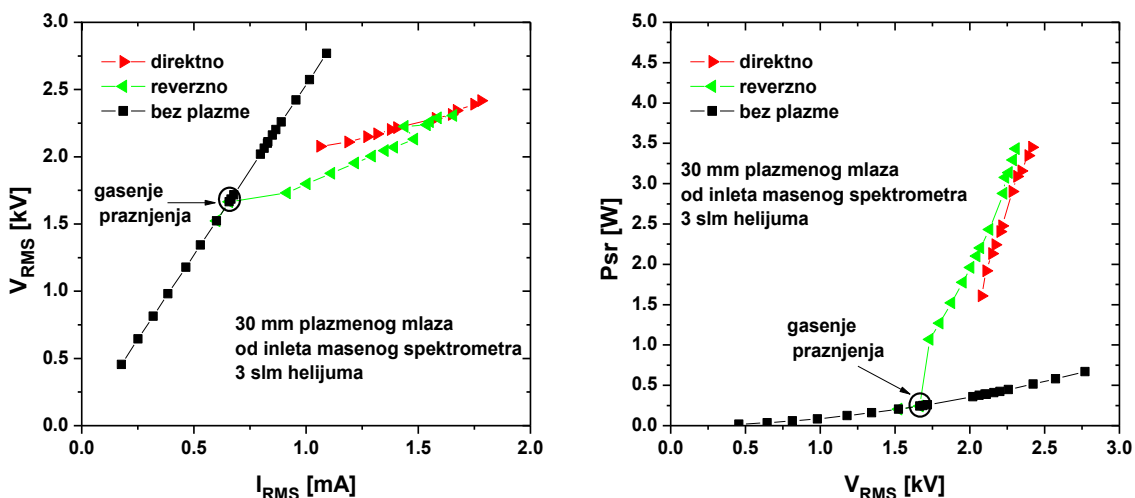
Slika 2.5 Snaga plazmenog mlaza u funkciji od napona (levo) i strujno-naponska karakteristika (desno) plazmenog mlaza na rastojanju od 25 mm od inleta masenog spektrometra, protok helijuma 3 slm.

Pri identičnom rastojanju od 25 mm a pri višem protoku radnog gasa od 4 slm električna karakteristika se ne menja značajnije (Slika 2.6 levo) te su i impedanse električnog kola sa formiranim pražnjenjem približnih vrednosti (1.05 M Ω za 3 slm naspram 1.04 M Ω za 4 slm). Povećanje brzine protoka helijuma ne utiče ni na promene ukupne snage plazmenog mlaza (Slika 2.6 desno) koja se kreće od 1 W - 5 W unutar opsega napona od 1.5 kV - 2.5 kV.



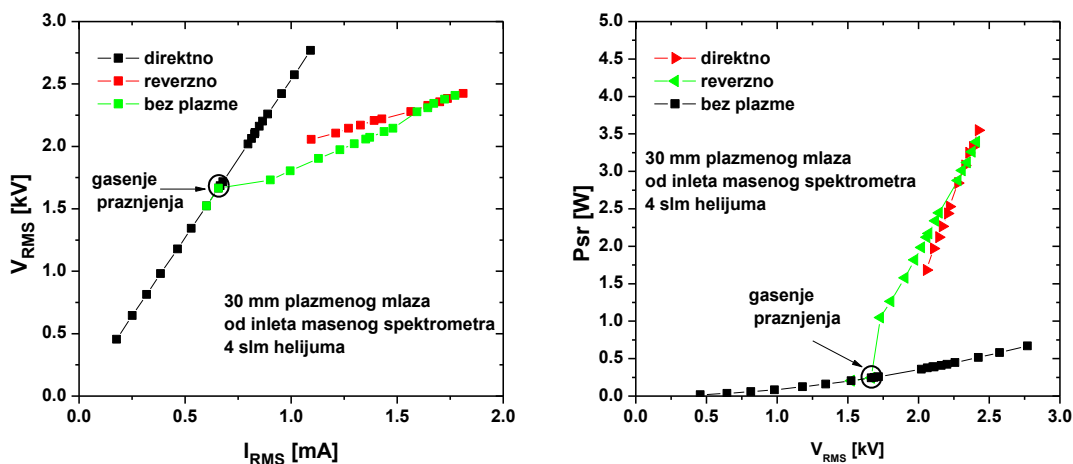
Slika 2.6 Snaga plazmenog mlaza u funkciji od napona (desno) i strujno-naponska karakteristika (levo) plazmenog mlaza na rastojanju od 25 mm od inleta masenog spektrometra, protok helijuma 4 slm.

Kada je u pitanju povećanje rastojanja plazmenog mlaza u odnosu na maseni spektrometar sa 25 mm na 30 mm (Slika 2.7) dolazi do promena i u strujno-naponskoj karakteristici i u snazi plazmenog mlaza. Krive su zadržale vrednosti svojih nagiba, međutim opsezi struje, napona kao i snage plazmenog mlaza su se smanjile tokom pražnjenja a primetno je i manji opseg struje i kod režima bez pražnjenja. Impendansa sistema bez pražnjenja iznosi $2.53 \text{ M}\Omega$ dok vrednosti impedansi za crvenu i zelenu krivu iznose $1.51 \text{ M}\Omega$ i $1.55 \text{ M}\Omega$, respektivno.

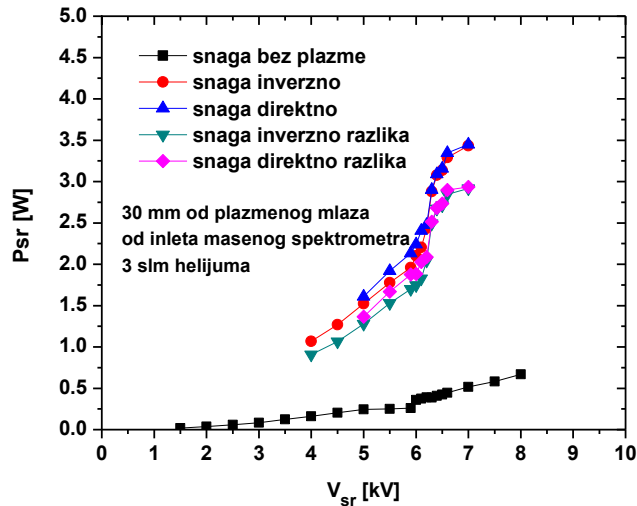


Slika 2.7 Snaga plazmenog mlaza u funkciji od napona (desno) i strujno-naponska karakteristika (levo) plazmenog mlaza na rastojanju od 30 mm od inleta masenog spektrometra, protok helijuma 3 slm.

Na slici 2.8, prikazane su strujno-naponske karakteristike za rastojanje od 30 mm, ali pri višem protoku radnog gasa od 4 slm. Impendansa sistema bez pražnjenja je ostala nepromenjena $2.53 \text{ M}\Omega$ dok su se vrednosti impedansi neznatno promenile s povećanjem protoka sa 3 slm na 4 slm ($1.50 \text{ M}\Omega$ i $1.48 \text{ M}\Omega$ za direktan i inverzan slučaj pri protoku 4 slm).



Slika 2.8 Snaga plazmenog mlaza u funkciji od napona (desno) i strujno-naponska karakteristika (levo) plazmenog mlaza na rastojanju od 30 mm od inleta masenog spektrometra, protok helijuma 4 slm.



2.9 Vrednosti snage plazmenog mlaza u funkciji od primenjenog napona na napajanoj elektrodi V_{sr} na rastojanju od 30 mm, protoku gasa 3 slm.

Prikazane snage plazmenog mlaza tokom električnog pražnjenja predstavljaju ukupnu vrednost snage električnog kola sa i bez električnog pražnjenja. Da bi se odredila realna vrednost snage predate električnom pražnjenju potrebno je oduzeti snagu izmerenu za vreme pražnjenja i snagu kada pražnjenje nije formirano (bez protoka helijuma). Na Slici 2.9. dat je ilustrovan primer izračunatih vrednosti realne snage predate plazmi i ona se kreće u opsegu od 0.7 W-2.8 W za razliku snage inverzno krive i 1.8 W-2.8 W za razliku snage direktne krive.

2.2.3 Zaključak

Samo određivanje struje, napona ili snage je specifično za svaku neravnotežnu plazmu. Tako je teško porediti dva veoma različita izvora jedino na osnovu merene snage, pa čak i kada bi bili sigurni koji deo snage je predat plazmi. Dodatne dijagnostike su potrebne da bi se na ravnopravnoj osnovi mogli porediti različiti izvori, pre svega merenje fluksa aktivnih čestica i njihove karakteristike. Električna merenja su međutim neophodna za verifikovanje optimalnog podešavanja i reproducibilnosti radnih uslova. Za isti uređaj se međutim, mogu porediti efikasnosti plazme na osnovu električnih merenja.

Urađena je električna karakterizacija i prikazane su strujno-naponske karakteristike plazma sistema sa i bez pražnjenja. Karakterizacija je vršena u eksperimentalnoj postavci sa masenim spektrometrom. Prikazane su vrednosti ukupne snage predate sistemu za dva različita rastojanja (25 mm i 30 mm) ruba cevčice plazma mlaza i masenog spektrometra i dva protoka radnog gasa (He 3 slm i 4 slm). Mogu se izvući sledeći zaključci:

- Pri snimanju električnih karakteristika plazmenog mlaza pri različitim rastojanjima dobijeni su sledeći opsezi parametara:
 - Struja: 0.9-2.98 mA
 - Napon: 1.14-2.5 kV
 - Snaga: 1.12-6.2 W
- Promena brzine protoka helijuma ne utiče na promenu impedanse električnog kola plazmenog mlaza kada se formira pražnjenje.
- Prisustvo masenog spektrometra u blizini izvora plazme utiče na promenu električnih karakteristika plazmenog mlaza; Povećanje rastojanja (sa 25 mm na 30 mm) dovodi do

povećanja impedanse električnog kola plazmenog mlaza u slučaju kada pražnjenje nije uključeno.

- U slučaju kada je pražnjenje prisutno ukupna impedansa sistema je jednaka bez obzira na primenjeni protok.

2.3 Snimanje ICCD kamerom

Intenziteti emitovanog zračenja koje potiče od plazmenog mlaza snimljeni su korišćenjem ICCD Andor iStar DH7341 (Slika 2.10). Kamera je korišćena za deo eksperimenta gde smo želeli da posmatramo vremensku evoluciju prostiranja pražnjenja pri različitim režimima rada plazmenih mlazova. Na kameri smo koristili dva fotografska objektiv u zavisnosti od potrebe eksperimenta: prvi fotografski objektiv bio je Nikon AF-Nikkor 105mm f -2.8, objektiv čiji široki otvor blende vrednosti f 2.8 omogućava maksimalno prikupljanje emitovane svetlosti iz pražnjenja pri zadatim okolnostima kao i fotografski objektiv Nikon 50mm f -1.4, model F-Nikkor kod kojeg je korišćen otvor blende vrednosti f 1.4. Korišćena je integracija na čipu ICCD kamere gde su pri vremenskoj ekspoziciji od 0.002 s, širini gejta od 25 ns intenziteti 160 fotografija sabirani u jednu. Takođe, za dobijanje što boljeg signala korišćeno je pojačanje (eng. *gain*) vrednosti 250 na čipu, pomoću kojeg se pored vrednosti signala povećava i šum na fotografiji, ali je on eliminisan snimanjem pri identičnim uslovima bez pražnjenja a zatim je vršeno oduzimanje intenziteta svetlosti fotografija sa i bez pražnjenja. Za ovaj model kamere se može reći da je brza jer omogućava ekspoziciju reda veličine ns.



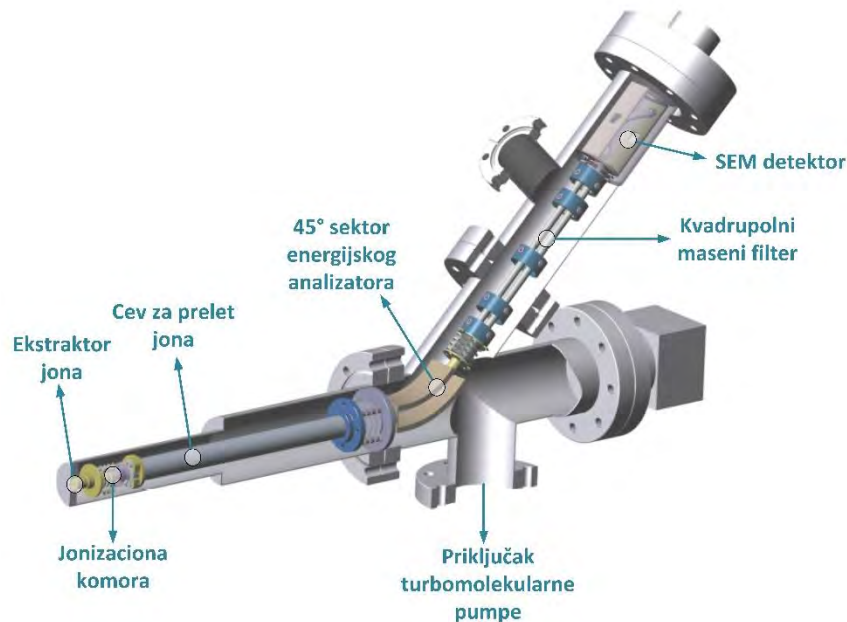
Slika 2.10 Fotografija korišćene ICCD kamere. (diplomski rad, N. Selaković 2011.)

Vremenske evolucije prostiranja pražnjenja omogućila nam je već pomenuta sekvencijalna akvizicija ICCD kamere (25 ns) dok smo koristili vremenski korak od 0.5 μ s da pokrijemo ceo period pulsa od 12.5 μ s (iznos jednog perioda za frekvenciju 80 kHz). Za praćenje vremenske evolucije plazme sinhronizovane sa strujno-naponskim signalima koristili smo generator TTL signala pomoću kojeg smo trigerovali kameru i signale snimane na osciloskopu. Detaljniji opis ove dijagnostičke metode plazmenog mlaza koji koristimo u ovoj disertaciji uradio je dr Maletić u okviru svoje doktorske studije (Maletić, doktorska disertacija, 2018).

2.4 Masena spektrometrija na atmosferskom pritisku

Plazma je kompleksan sistem sačinjen od smeše naelektrisanih i neutralnih čestica. Vrlo je važno, pre korišćenja plazme u primenama, da se obezbedi što više podataka o sastavu plazme, o tome kako se ponašanje njenih konstituenata menja za različite električne uslove, i o interakcijama koje se javljaju između konstituenata plazme i površine uzorka koji joj je izložen. Ovde ćemo istaći primenu maseno-

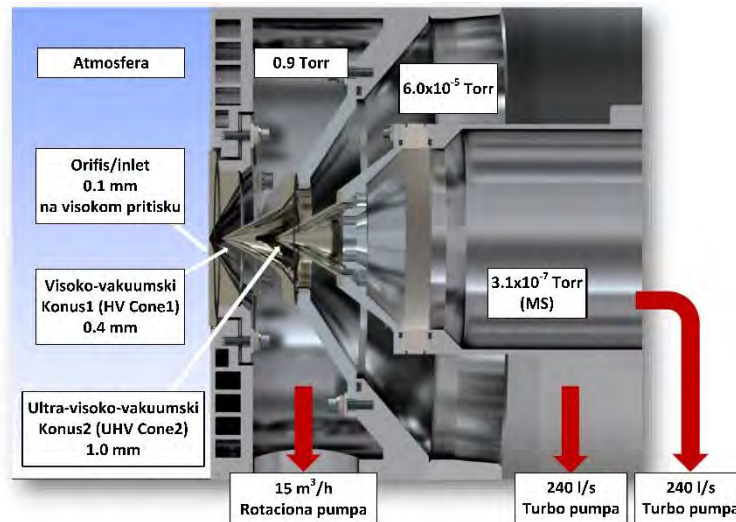
energijskog analizatora u dijagnostici plazme koja bi trebalo da se koristi u različitim vrstama primena. Biće opisani rezultati koji su dobijeni korišćenjem HIDDEN MBMS (*eng.* Molecular Beam Mass Spectrometer) maseno energijskog spektrometra, čiji analizator ima oblik kao što je prikazan na Slici 2.11.



Slika 2.11 Maseno-energijski analizator firme HIDDEN. (www.hiddenanalytical.com)

Glavni delovi analizatora su region za uzorkovanje jona (optika za ekstraktovanje jona), unutrašnji izvor jonizacije (jonizaciona komora koja se isključuje prilikom uzorkovanja jona iz plazme), cilindrični elektrostatički analizator energije, kvadrupolni filter mase i detektor jona (*eng.* Secondary Electron Multiplier SEM). Postoje različiti tipovi masenih spektrometara a za potrebe ove disertacije korišćen je model Hiden HPR60-EQP. Ovaj maseni spektrometar predstavlja potpuno integrisani sistem konfigurisan specijalno za merenje plazme na atmosferskom pritisku kao i reaktivnih procesa u primenama, uključujući analizu različitih tipova plazmenih mlazova, dielektričnih barijernih pražnjenja (DBD) i hemiju plamena (za merenja izvora na višim temperaturama potrebno je koristiti sistem za hlađenje).

Da bi uopšte gas iz atmosfere mogao da se uzorkuje neophodno je da postoji gradijent pritiska. HPR60 koristi tri faze smanjenja pritiska sa otvorom za uzorkovanje na svakoj međufazi kako bi se obezbedio brz prenos reaktivnih i neutralnih vrsta direktno na integrisani maseno-energijski analizator. Na Slici 2. 12 prikazan je poprečni presek sistema za uzorkovanje HPR60. On je sačinjen od inleta/orifisa na visokom pritisku, zatim visoko-vakuumski Konus 1 i ultra-visoko-vakuumskog Konusa 2 (komponente sistema za uzorkovanje su zamenjive i moguće je prema potrebi koristiti različite veličine njihovih otvora). Specijalan dizajn i konstrukcija sistema za uzorkovanje i pažljiv odabir veličine njihovih otvora u kombinaciji sa promenama pritiska obezbeđuje formiranje molekularnog snopa uzorkovanih čestica. Mehanička rotaciona pumpa obezbeđuje nizak vakuum vrednosti približno 1 Torr-a u prvoj međufazi smanjenja pritiska između orifisa na visokom pritisku i Konusa 1, nakon toga prva turbo-molekularna pumpa stvara visok vakuum između Konusa 1 i Konusa 2 gde je pritisak približan vrednosti 6×10^{-5} Torr-a i na kraju se uz pomoć druge turbo-molekularne pumpe obezbeđuje ultra-visok-vakuum reda veličine 10^{-7} Torr-a. Ultra-visok-vakuum obezbeđuje dug srednji slobodni put čestica uzorka koji je neophodan za rad maseno-energijskog analizatora.

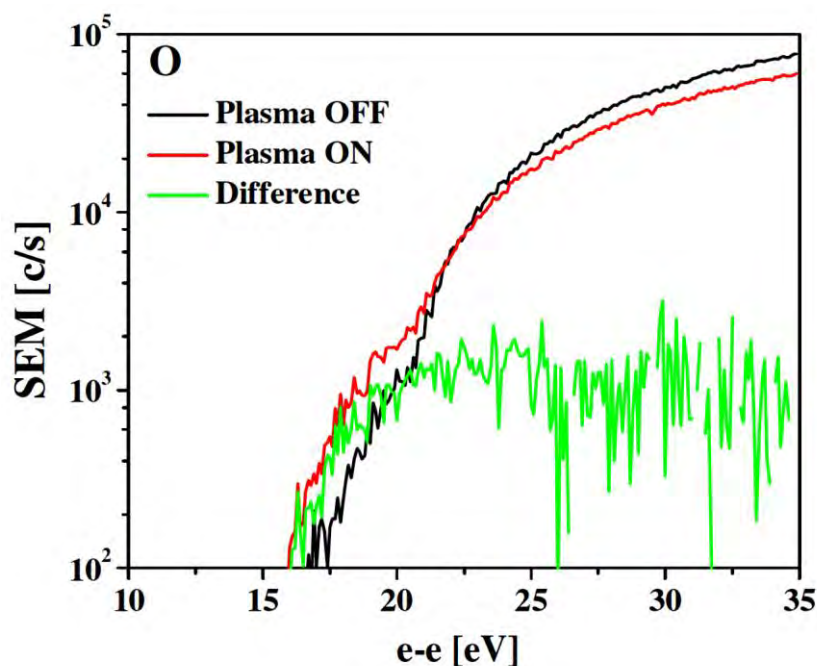


Slika 2.12 Poprečni presek sistema za uzorkovanje HIDEN HPR60 masenog spektrometra.
(www.hidenanalytical.com)

2.4.1 Merenje neutrala

Da bi se dobio spektar neutrala neophodno je da se atom ili molekul prevede u jon s obzirom na činjenicu da filter masa i detektor mogu da detektuju naelektrisane čestice prema odnosu m/z . Režim skeniranja neutrala kod HPR-60 MBMS-a naziva se RGA (*eng.* rezidual gas analyzer - RGA). U ovom režimu koristi se jonizaciona komora, izvor elektrona koji bombarduje neutralne čestice i prevodi ih u jone. Unutrašnja jonizaciona komora HPR60 masenog spektrometra sadrži dva filameta (iridijumska vlakna presvučena oksidom) koja se nalaze u radijalno simetričnom kavezu „*eng.* cage“. Sklop je usko povezan sa otvorom za uzorkovanje plazme za analizu visoke osjetljivosti neutrala i radikala. MASsoft softver omogućava preciznu kontrolu radnih parametara izvora jona, uključujući emisiju elektrona iz filameta, napon kaveza izvora i energiju elektrona za jonizaciju. Ključna karakteristika dizajna jonizacione komore je zadržavanje elektrona u blizini jonskog kaveza. Ovo omogućava visok stepen kontrole detaljnih merenja potencijala koji se koristi u analizi radikala. Takođe je dostupna opcija jonizacije sa elektronskim atačmentom tzv. „-ion RGA“ koja omogućava analizu negativnih jona proizvedenih u jonizacionoj komori za detekciju elektronegativnih vrsta. Ova tehnika daje dragocene informacije pri analizi radikala dobijenih iz elektronegativne plazme.

Identifikacija masenog spektra neutralnih vrsta se prvo vrši u odsustvu plazme, a zatim i sa njenim prisustvom. Fragmentacija merenog gasa i stvaranje novih vrsta u plazmi ili kroz njenu interakciju sa zidovima reaktora ili površinom uzorka lako se identifikuje. Da bi se poboljšala tačnost merenja, ponekad je preporučljivo da se uzorkovanje vrši dvostruko ili trostruko diferencijalnom geometrijom pumpanja koja uključuje korišćenje „*eng.* chopper-a“ kako bi se poboljšao odnos razlike između stvarnog signala plazme i pozadinskog signala koji potiče od gasa unutar masenog spektrometra (Singh *et al.*, 1999; Gonzalvo *et al.*, 2006). Podaci dobijeni o neutralima proizvedenim u plazmi mogu se značajno proširiti ukoliko se energija korišćenih elektrona u jonizacionoj komori masenog spektrometra varira. Tada je moguće proučavati promene u populacijama određenih jona generisanih u izvoru blizu praga energije jonizacije, jer se uzorkovani neutrali menjaju usled promena eksperimentalnih uslova plazme. Na Slici 2.13 prikazana je kriva za određivanje praga jonizacije za O_2^+ dobijena korišćenjem Hiden HPR60 od strane Maletić i saradnika pri ispitivanju električnog pražnjenja RF mikro-plazmenog mlaza u smeši helijuma i kiseonika (Maletić *et al.*, 2012).



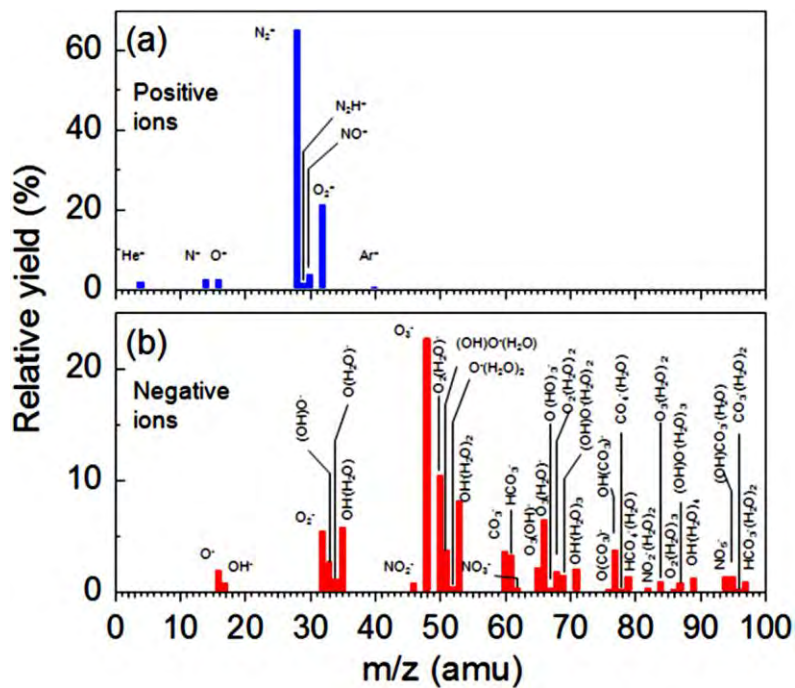
Slika 2.13 Zavisnost energije elektrona filameta na signal kiseonika. (Maletić *et al.* 2012.)

Budući da su mnogi gasovi koji se koriste u primenama, posebno za nagrizanje poluprovodnika i depoziciju DLC i oblaganje optičkih površina (*eng.* coating), elektronegativni (na primer, kiseonik, sumporni heksafluorid, metan i ugljen-dioksid), često je korisno koristiti izvor jonizacije komore masenog spektrometra za detekciju negativnih jona (Rees *et al.*, 1998; Stoffels *et al.* 1997; Teii *et al.* 2000). Čak i ako je isti dominantni negativni jon nastao od dva različita „roditeljska“ molekula, kao na primer u slučaju ugljen-dioksida i azot-oksida, gde oba daju O^- kao svoj dominantni negativni jon, varijacije u proizvodnji jona sa energijom elektrona su vrlo prepoznatljivi.

2.4.2 Merenje pozitivnih i negativnih jona

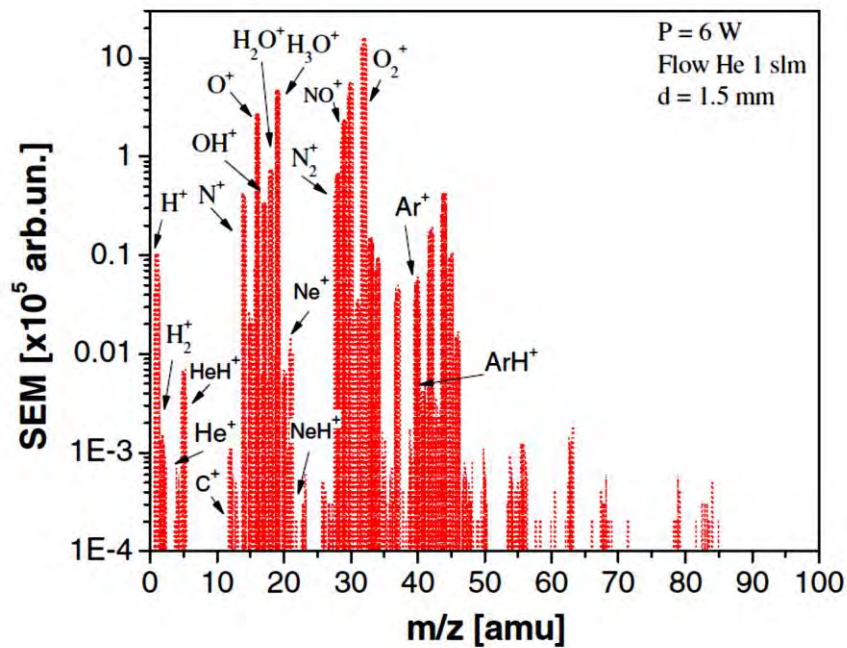
Za identifikaciju pozitivnih i negativnih jona proizvedenih u plazmi koristi se poseban režim rada tzv. masena spektrometrija sekundarnih jona (*eng.* Secondary Ion Mass Spectrometry – SIMS+/-). U SIMS+/- režimu izvor jonizacije komore nije uključen što znači da se detektuju joni koji su generisani isključivo u plazmi. U slučaju da maseni spektrometar nije u stanju da izmeri dovoljno dobar signal pozitivnih ili negativnih jona iz električnog pražnjenja moguće je napajati komponente sistema za uzorkovanje pozitivnim ili negativnim potencijalom. HPR60 maseni spektrometar takođe ima mogućnost da izmeri raspodele energije odabranih jona iz dobijenog masenog spektra. Često je korisno ako se merenja relativne zastupljenosti i energija vrše na jonima uzorkovanim u tački koja je direktno ekvivalentna onoj na podlozi koja se tretira. To se može postići ugrađivanjem otvora za uzorkovanje masenog spektrometra u površinu držača podloge ili u površinu druge elektrode. U ovoj disertaciji ćemo koristiti merenja distribucije energije pozitivnih jona za proveru pojave dodatnog pražnjenja prilikom uzorkovanja atmosferskih plazmi.

Na slici 2.14 prikazani su vremenski usrednjeni relativni prinosi masenih spektara pozitivnih i negativnih jona dobijenih u pražnjenju mikro-plazmenog mlaza (Beck *et al.*, 2009). Pokazano je da se u pozitivnom spektru jona pored He^+ , koji predstavlja jon koji potiče iz primarnog električnog pražnjenja (radni gas helijum), pojavljuju i druge atomske vrste kao što su N^+ , O^+ i Ar^+ . Od molekulskih vrsta dobijeni su N_2^+ , O_2^+ , NO^+ i N_2H^+ pozitivni joni. Dosta bogatija hemija dobijena je za maseni spektar negativnih jona gde pored kiseoničnih vrsta dominiraju dugoživeći klasteri vode.



Slika 2.14 Maseni spektri pozitivnih (a) i negativnih (b) jona (J-S Oh *et al.*, 2011)

Maseni spektar pozitivnih jona koji potiču od zračenja plazma igle koja će biti korišćena u ovoj disertaciji prikazan je na Slici 2.15 [Malović *et al.* 2010.]. Dok joni, pored činjenice da spadaju u reaktivne vrste, najverovatnije nisu efikasniji u tretmanima živih tkiva od odgovarajućih radikala, njihovo prisustvo u plazmi i u „sheath-površina“ interakciji omogućava njihov uticaj kao energijskih čestica.



Slika 2.15 Maseni spektar pozitivnih jona (Malović *et al.* 2010.)

U okviru ove disertacije biće prikazani maseni spektri DBD plazmenih mlazova. Biće određeni spektri pozitivnih i negativnih jona, neutrala kao i vremenski razložena snimanja pozitivnih jona. Pre nego što pređemo na te rezultate u narednim poglavljima će biti prikazana priprema HIDEN HPR60 masenog spektrometra sa ciljem dobijanja preciznih i reproducibilnih merenja.

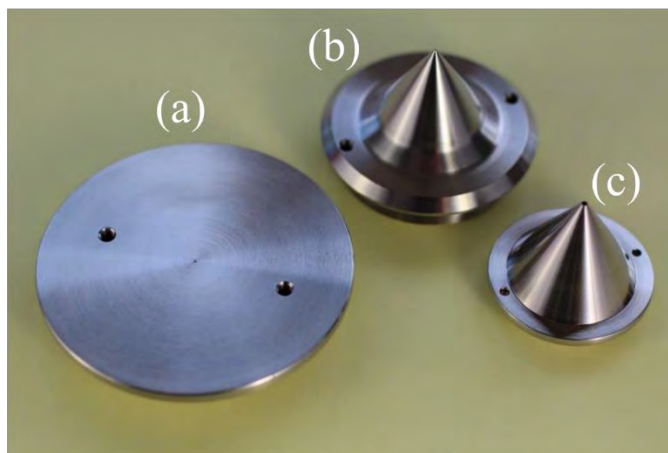
2.4 Problematika pripreme masenog spektrometra HPR60 - Merenje atmosfere i uticaj vode unutar masenog spektrometra

2.4.1 Uvod

Da bismo dobili što preciznije rezultate merene masenim spektrometrom, potrebno je detaljno analizirati sve parametre koji bi mogli da utiču na tačnost uređaja. Najverovatnije najveći problem kod ovog tipa masenog spektrometra predstavlja voda koja potiče od vlage prisutne u atmosferi koja se deponuje unutar uređaja. Stoga je potrebno pre svakog merenja obaviti pripremu i sačekati da se signal vode smanji (npr. tokom merenja atmosfere gde možemo da nadgledamo pad vodenog signala) tako da njegov uticaj na maseni spektar bude što zanemarljiviji. Visoka koncentracija molekula vode unutar masenog spektrometra u sudarima sa atomima i molekulama iz atmosfere značajno utiče na izmeren sastav atmosfere (da bi masenim spektrometrom usnimili neutrane iz atmosfere, koristimo integrisanu jonizacionu komoru pomoću koje dolazi do jonizacije neutralnih čestica koje zatim mogu lako da se detektuju). Ovo može dovesti do pojave određenih vrsta (npr. klastera vode) i do smanjenja drugih (atomske i molekulske vrste azota i kiseonika). U Tabeli 2.1 prikazane su tri različite kombinacije sistema za uzorkovanje koje su ispitane. Na Slici 2.16 prikazana je fotografija sva tri orifisa. Korišćenjem ovih kombinacija izvršena su MID scan merenja signala vode, kao i molekula azota i kiseonika iz atmosfere.

Tabela 2.1 Kombinacije sistema za uzorkovanje/orifisa

Orifisi	inlet	Konus 1	Konus 2
	[mm]	[mm]	[mm]
Kombinacija 1	0.3	0.3	1
Kombinacija 2	0.1	0.3	1
Kombinacija 3	0.1	0.4	1



Slika 2.16 Komponente sistema za uzorkovanje i formiranje molekularnog snopa: (a) inlet; (b) Konus 1; (c) Konus 2;

Specijalno dizajnirana geometrija sistema za uzorkovanje omogućava inleto i konusima idealno poravnavanje, koje omogućava formiranje molekularnog snopa bez uticaja sudara između uzorkovanih vrsta.

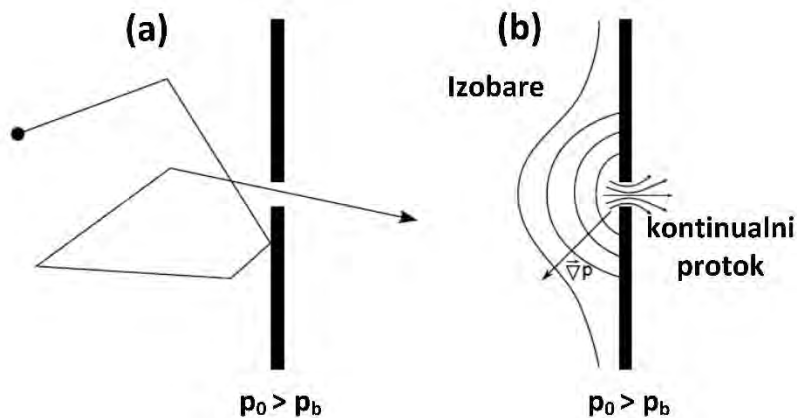
2.4.2 Molekularni snop

Molekularni snop (*eng.* molecular beam - MB) je usmereni snop atoma ili molekula u kojem ne dolazi do sudara između čestica (Benedikt *et al.*, 2012). On nastaje nakon što gas iz oblasti visokog pritiska započne širenje kroz mali otvor za uzorkovanje u oblast niskog pritiska. Postoje dva slučaja koja je potrebno razlikovati prema Knudsenovom broju. Knudsenov broj Kn je definisan kao odnos srednjeg slobodnog puta λ prema karakterističnoj dužini L :

$$Kn = \frac{\lambda}{L}$$

Kada je u pitanju maseni spektrometar koji je tema ove disertacije, λ predstavlja srednji slobodni put čestica u regionu visokog pritiska, a karakteristična dužina L je prečnik otvora kroz koji se gas širi u područje niskog pritiska.

U slučaju velikog srednjeg slobodnog puta u poređenju sa prečnikom otvora (tj. $Kn > 1$), dobija se *slobodan molekularni protok* kroz otvor. To znači da se u blizini otvora ne dešavaju sudari i da molekuli ne „osećaju“ otvor. Molekuli stohastički udaraju u otvor (Slika 2.17 (a)). Molekularni protok se često primenjuje za uzorkovanje plazme niskog pritiska, kao što je ilustrovano primerom Singh i sar. (Slika 2.17 (b)) (Singh *et al.* 1999)). Otvor za uzorkovanje, koji koriste Singh i saradnici, ima prečnik 0.9 mm, a srednji slobodni put je u opsegu od nekoliko milimetara



Slika 2.17 Prikaz kinetičke dinamike gasa za različite vrednosti Knudsovog broja za (a) srednji slobodni protok ($Kn > 1$) i (b) kontinualni protok ($Kn \ll 1$) (Dirk Ellerweg, doktorska disertacija)

Knudsenov broj je prema tome veći od jedan. Protok čestica iza otvora za uzorkovanje naziva se molekularnim snopom jer se iza otvora gotovo ne događa sudar čestica snopa sa drugim česticama snopa ili sa pozadinskim gasom.

Koncentracija n^{snop} molekularnog snopa na srednjoj liniji opada sa povećanjem udaljenosti:

$$n^{snop}(x) = \frac{1}{4} n_0 \left(\frac{r}{x}\right)^2$$

gde n_0 označava koncentraciju atoma i molekula na zidu bez otvora za uzorkovanje, r je poluprečnik otvora, a x (gde je $x \gg r$) je udaljenost od otvora. Koncentracija $n^{snop}(x)$ ne zavisi od karakteristika vrsta (npr. masa i adijabatski indeks) i određena je samo geometrijskim uslovima.

Treba naglasiti da je gustina snopa u ovom slučaju samo funkcija geometrije i nezavisna od svojstava gasa. Kada se uzorkuje smeša gasova, odnosi između pojedinih vrsta se ne menjaju. Relativni sastav snopa ostaje isti u molekularnom snopu u uslovima slobodnog molekularnog protoka i jednak je relativnom sastavu područja visokog pritiska ispred otvora. Kada se koristi veći početni pritisak i/ili veći otvor za uzorkovanje, Knudsenov broj postaje mnogo manji od jedan ($Kn \ll 1$), a dinamika gasa se određuje kontinualnim protokom. U kretanju čestica dominiraju sudari, a time i difuzija. Postoji gradijent pritiska prema otvoru za uzorkovanje, zbog čega molekuli gasa prolaze kroz otvor u područje niskog pritiska (Slika 2.17 (b)). Srednji slobodni put pri atmosferskom pritisku je oko $0.1 \mu\text{m}$, što je mnogo manje od tipičnih prečnika otvora za uzorkovanje od $20 \mu\text{m}$ do $500 \mu\text{m}$. Shodno tome, Knudsenov broj je mnogo manji od jedan, a uslovi kontinualnog protoka važe za uzorkovanje gasova na atmosferskom pritisku.

Za opisivanje ponašanja kontinualnog protoka kroz otvor može se koristiti jednačina Hugoniot (1887) (Pauly, 2000):

$$\frac{dA}{A} + \frac{dv}{v}(1 - M^2) = 0$$

gde A označava površinu preseka, v označava brzinu protoka, a $M = \frac{v}{c}$ je Mahov broj (c je brzina zvuka). U početnom stanju stagnacije (tj. regionu visokog pritiska), brzina molekula je manja od brzine zvuka ($v < c$, $M < 1$). U tom slučaju se brzina molekula povećava sa smanjenjem površine poprečnog preseka. Ako je gradijent pritiska dovoljno velik, molekuli dostižu brzinu zvuka ($M = 1$) na minimalnoj površini poprečnog preseka ($dA = 0$) tj. „grlu“ (otvor masenog spektrometra). Površina poprečnog preseka se ponovo povećava iza otvora, a za $M > 1$ povećava se i brzina protoka v . Tada se postižu supersonične brzine.

Jednačina energije:

$$h_1 + h_2 = \frac{1}{2}(v_2^2 - v_1^2) = c_p(T_1 - T_2)$$

otkriva da se entalpija h smanjuje kada raste brzina protoka v . Drugim rečima, molekuli se ubrzavaju prilikom zagrevanja gasa. To rezultuje promenom u temperaturi gasa koja je opisana desnom stranom jednačine. Koristeći jednačinu energije, može se izvesti maksimalna brzina čestica:

$$v_{max} = c \sqrt{\frac{2}{\gamma - 1} \left[\left(\frac{p_0}{p_b} \right)^{(\gamma-1)/\gamma} - 1 \right]}$$

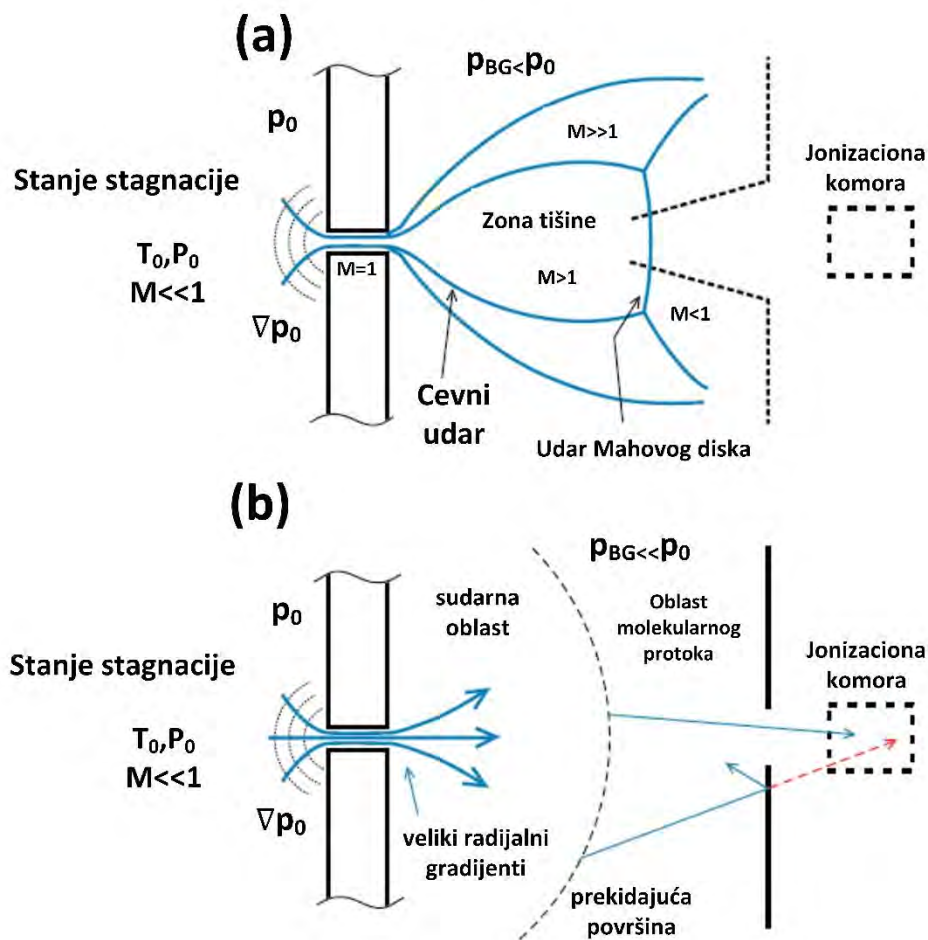
$\gamma = \frac{c_p}{c_v}$ je adijabatski indeks, p_0 i p_b su početni pritisak stagnacije i pozadinski pritisak. Umetanjem

$v = c$ dobija se kritični odnos pritiska $\frac{p_0}{p_b}$, potreban za postizanje brzine zvuka u grlu:

$$\frac{p_0}{p_b} \geq G = \left(\frac{\gamma + 1}{2} \right)^{\gamma/(\gamma-1)}$$

Odnos kritičnog pritiska G je manji od 2.1 za sve gasove. Ako je odnos pritiska niži od kritičnog odnosa G , (super)sonična brzina se nikada ne dobija, a izlazni pritisak na otvoru je pozadinski pritisak P_b . Tada se ne pojavljuje dalje širenje gasa. Međutim, ako je ispunjen uslov pritiska, brzina zvuka se postiže u grlu i izlazni pritisak je jednak $\frac{p_0}{G}$. Pošto je pritisak na izlazu veći od pritiska u početnom vakuuomskom delu, on se mora proširiti u područje nižeg pritiska. Struktura ovog proširenja *slobodnim-mlazom* (eng. free-jet) (Scoles, 1998) prikazana je na Slici 2.18 (a). Ova vrsta proširenja naziva se „slobodnim-mlazom“, jer se, za razliku od mlazeva rasprskivača (eng. nozzle-jets), ne koristi difuzni rasprskivač koji ograničava širenje.

Neprošireni gas se širi iza otvora, a pritisak u slobodnom mlazu opada sa povećanjem udaljenosti do otvora. Supersonični tok nije u stanju da „oseti“ granične uslove u smeru protoka, jer se informacije o granicama ne mogu širiti brzinom bržom od brzine zvuka. Shodno tome, slobodni-mlaz se neprekidno širi čak i kada njegov pritisak padne ispod pozadinskog pritiska P_b .



Slika 2.18 Shematski prikaz širenja slobodnog-mlaza za dva slučaja: (a) širenje u komoru sa značajnim pozadinskim pritiskom i formiranjem udarnih talasa i (b) širenje u komoru sa zanemarljivom pozadinskom gustinom i kontinualnim prelazom u režim molekularnog protoka (Benedikt *et al.*, 2012)

Tada se slobodni mlaz prekomerno širi i biva rekompresovan sistemom udarnih talasa kako bi se prilagodio graničnim uslovima. Udarni talasi su lokalizovane, izoentropijske zone velike gustine, pritiska, temperature i gradijenata brzine. Udarni talas na bočnim stranama slobodnog mlaza naziva se

"cevni udar", a na donjem kraju "udar Mahovog diska" (Slika 2.18 (a)). Zbog velikih gradijenata gustine unutar udarnog talasa, oni se mogu okarakterisati metodama rasejanja svetlosti (Bier i Schmidt, 1961; Crist et al. 1966). Debljina udarnog talasa je reda veličine srednjeg slobodnog puta i utvrđeno je da je pozicija Mahovog diska x_m (u jedinicama prečnika otvora d):

$$\frac{x_m}{d} = 0.67 \sqrt{\frac{P_0}{P_b}}$$

Supersonični tok unutar ovih udarnih talasa ne oseća nikakve spoljne uslove i stoga je izoentropijski i nezavisan od pozadinskog pritiska P_b . Iz tog razloga, jezgro regiona naziva se „zona tišine“. Za ekstrakciju molekularnog snopa potrebno je u slobodni-mlaz postaviti skimer u obliku konusa. Precizno postavljanje skimera je presudno. Molekularni snop se nužno izvlači iz protoka koji nije ometen udarnim talasima. To se može učiniti postavljanjem skimera suprotno od smera protoka ispred Mahovog diska, unutar zone tišine. U ovom slučaju, oblik skimera može biti čak i veoma važan kako bi se izbegli odvojeni udarni talasi ispred skimera. Neophodan je aerodinamični dizajn skimera. Sistem za uzorkovanje za maseni spektrometar HIDEN HPR60 dizajniran je prema ovom slučaju.

Ova razmatranja i izazovi mogu se izbeći ako se tokom širenja slobodnog-mlaza ne pojave udarni talasi. Kao što je prethodno rečeno, udarne zone se pojavljuju kada pritisak u ekspanziji padne ispod pozadinskog pritiska. Stoga se neće pojaviti zone udara ako se slobodni mlaz proširi u savršeni vakuum. Budući da se nikada ne može postići savršeni vakuum, pozadinski pritisak mora biti što niži za pomeranje Mahovog diska daleko od smera protoka. Na primer, ako se atmosferski gas uzorkuje u vakuumu od 10^{-2} Pa, pozicija Mahovog diska je $\frac{x_m}{d} = 2100$ ili $x_m = 21$ cm sa prečnikom otvora od 0.1 mm. Debljina Mahovog diska je nekoliko decimetara, pa se primetiti fin prelaz iz kontinualnog protoka u slobodni molekularni protok.

Važnost veoma dobrog pozadinskog pritiska može se opisati primerom komercijalnog masenog spektrometra HIDEN HPR-60 koji smo koristili u ovoj disertaciji. Gas se širi iz atmosfere u slab vakuum od npr. 2.8×10^{-1} Torr što odgovara vrednosti pritiska u paskalima od 37.33 Pa pozadinskog pritiska. Mahov disk se formira na rastojanju $x_m = 3.49$ mm od otvora za uzorkovanje ($\varnothing = 100$ μ m). To znači da skimer mora biti postavljen unutar prvih 3.4 mm neposredno iza otvora za uzimanje uzoraka. U suprotnom, udarni talasi će značajno iskriviti molekularni snop. Rastojanje između otvora inleta i Konusa 1 u našem slučaju je 3.15 mm što znači da se Mahov disk formira na zidovima Konusa 1 i ne remeti prostiranje formiranog molekularnog snopa.

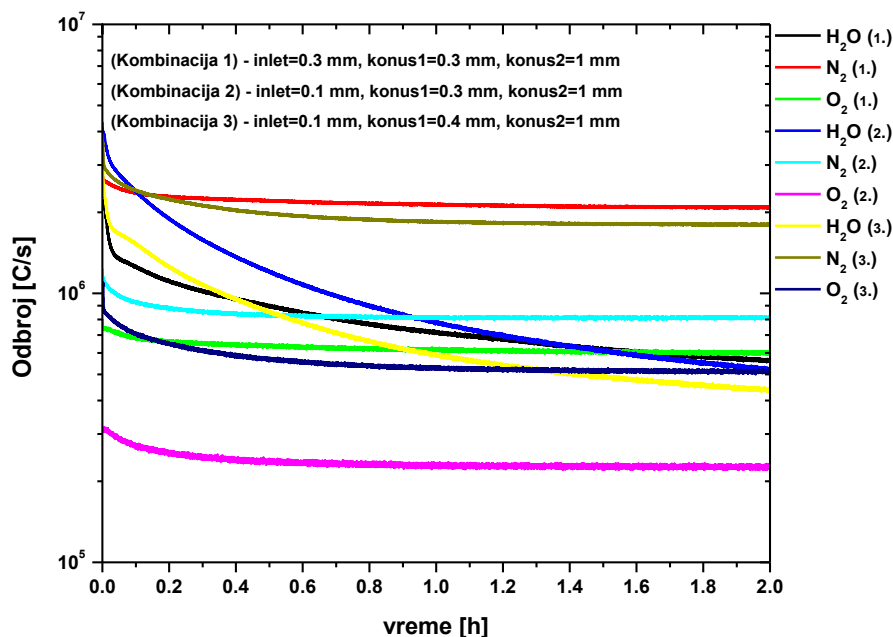
Kao aproksimacija, širenje slobodnog mlaza može se podeliti na dve oblasti, područje kontinualnog protoka iza otvora za uzorkovanje praćeno slobodnim molekularnim protokom (Scoles, 1988). Takozvana *prekidajuća površina* razdvaja obe oblasti (Slika 2.18 (b)). Pretpostavlja se da se nijedan sudar između čestica u molekularnom snopu ne dešava niz protok od prekidajuće površine. U stvarnosti, prekidajuća površina nije oštra granica već gladak prelazak u protok bez sudara. Lokacija x_q površine koja prestaje je približno (Scoles, 1988):

$$\frac{x_q}{d} \approx \left(\frac{M_\infty}{C_1} \right)^{1/(\gamma-1)}$$

gde je d prečnik otvora za uzorkovanje, M_∞ je konačni Mahov broj, a C_1 je 3.2, 3.6 ili 3.9 za adijabatski indeks γ od 5/3, 7/5, odnosno 9/7, respektivno. U slučaju atmosferskog vazduha uzorkovanog kroz otvor prečnika 100 μ m, pozicija prekidajuće površine je približno na $x_q = 2.1$ mm u smeru protoka od otvora za uzorkovanje.

2.4.3 Merenje sastava atmosfere

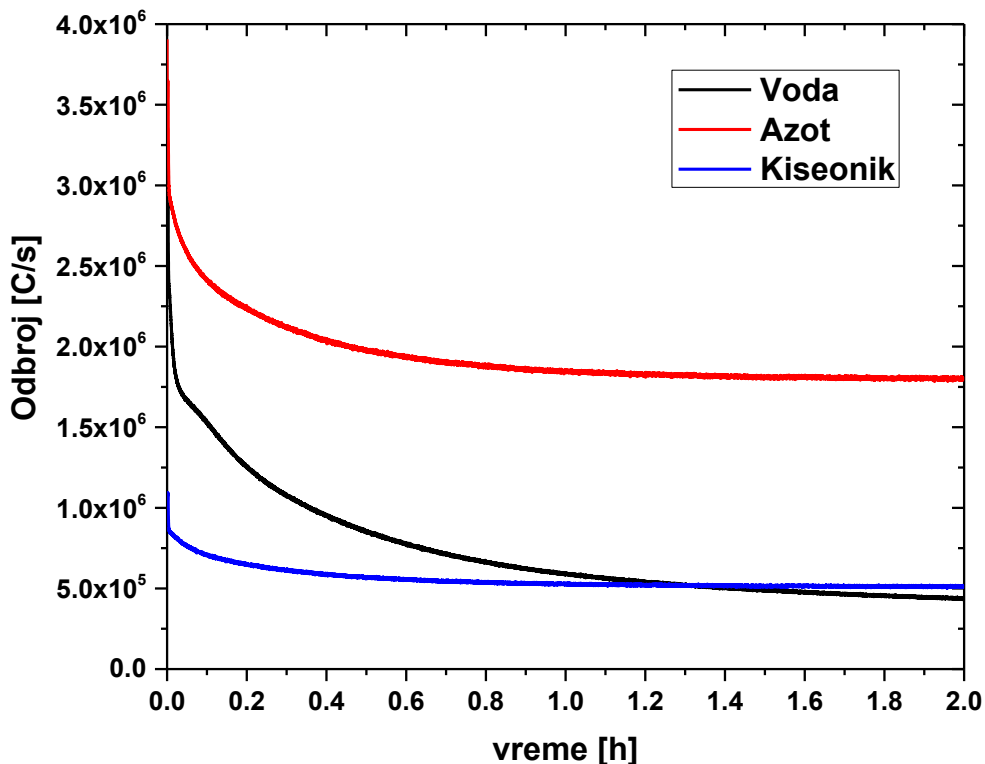
Slika 2.19 prikazuje signale molekula vode, azota i kiseonika merenih u funkciji od vremena za sve tri kombinacije sistema za uzorkovanje. Može se primetiti da prva i treća kombinacija imaju značajno veći signal azota i kiseonika u odnosu na drugu kombinaciju. Činjenica da korišćenje većih otvora (kod Kombinacije 1 je otvor inleta 3 puta veći od druge dve kombinacije, dok je u Kombinaciji 3 otvor Konusa 1 3 puta veći nego kod preostale dve kombinacije) pospešuje veći odbroj jeste zbog povećanja fluksa čestica koje bivaju uzorkovane. U svim slučajevima se tokom određenog vremena odbroj molekula vode smanjio, stabilizovao, i njegov pad je postao dovoljno spor da ne utiče značajno na odbroj azota i kiseonika. U nastavku ćemo se suočiti sa prednostima i nedostacima različitih podešavanja sistema uzorkovanja za masenu spektrometriju plazmenog mlaza.



Slika 2.19 Vremenska evolucija odbroja neutrala vode, kiseonika i azota za tri različite postavke sistema za uzorkovanje

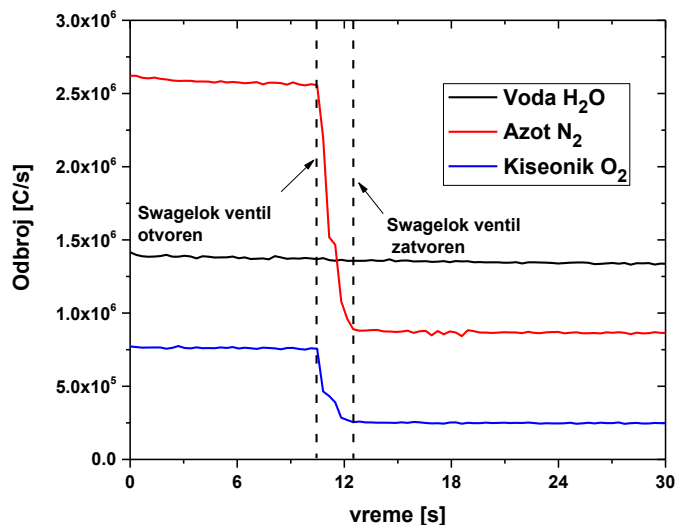
Pre nego što se počne sa detaljnom karakterizacijom nekog atmosferskog pražnjenja masenim spektrometrom najbolji način da se uradi njegova priprema jeste da se što preciznije izmeri maseni sastav atmosfere u laboratoriji u kojoj se uređaj nalazi. S obzirom na činjenicu da je sastav atmosfere prilično konstantan i dominiraju molekulske vrste azota i kiseonika, provera sastava atmosfere pomoću masenog spektrometra predstavlja dobru pripremu pred uzorkovanje plazme. Ukoliko se dobije maseni spektar atmosfere koji je uporediv sa literaturom može se zaključiti da maseni spektrometar zadovoljava uslove za prikupljanje preciznih rezultata merenja. Vlažnost deponovana u HPR60 masenom spektrometru može predstavljati veliki problem ukoliko se ne obrati pažnja na pripremu samog instrumenta pre merenja. Primećeno je da iako je maseni spektrometar tehnički spreman za merenje (maksimalni dozvoljeni pritisak u trećoj fazi: 4.5×10^{-6} Torr), ipak je bolje sačekati da vrednost odbroja signala vode padne na nižu vrednost gde će biti približno konstantna (imaće spor pad odbroja) pre početka merenja zato što previsok signal unutar masenog spektrometra i relativno brzo opadanje primetno utiču na promenu masenog spektra.

Prćenjem vremenske evolucije molekula vode, azota i kiseonika, na Slici 2.20 moēe se primetiti da se brzina odbroja signala vode brzo smanjuje u prvom satu nakon tehnički spremnog HPR60 za merenja (korišćena Kombinacija 3 sistema za uzorkovanje). U ovom periodu, pod uticajem pada odbroja signala vode, moēe se primetiti znaćajan pad signala molekula azota i kiseonika, a zatim njihova stabilizacija praćena konstantnom vrednosti odbroja u drugom nadgledanom satu. Kao što se moēe potvrditi na Slici 2.20, signal vode i dalje opada, ali mnogo manjom brzinom.



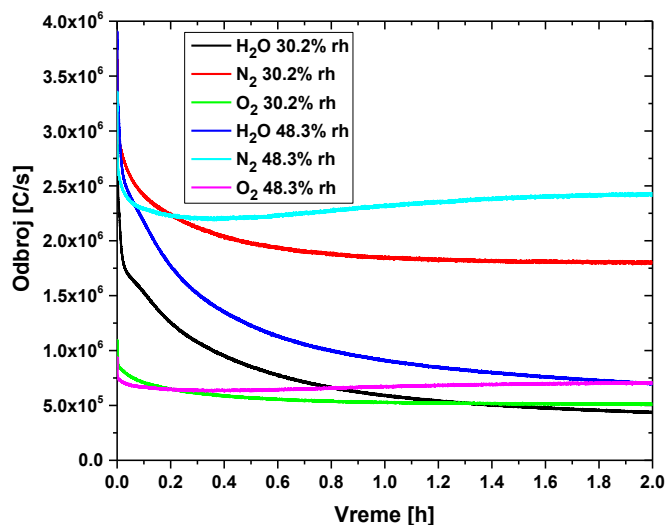
Slika 2.20 Vremenska evolucija molekula vode, azota i kiseonika

Takoēe, da bismo bili sigurni da signal molekula vode potiče uglavnom iz masenog spektrometra i u manjoj meri od vlaēnosti ambijentalnog vazduha, koristili smo ventil *Swagelok* koji nam omogućava da odvojimo eng. *background* tj. pozadinski signal (signal unutar uređaja) od eng. *foreground* tj. ukupnog signala (zbir signala izvan i unutar uređaja) izmerenog pomoću HPR60. Zatvaranje ventila je rućno i u tom periodu dolazi do brzog pada signala. Nakon njegovog zatvaranja (Slika 2.21), moēe se primetiti drastićan pad signala molekula azota i kiseonika, dok se signal molekula vode praktićno uopšte nije promenio. Ovo je dokaz o prisustvu deponovane vode u masenom spektrometru. Dakle, oduzimanjem ukupnog i pozadinskog signala u stanju smo da eliminišemo signal vode koji potiče od vlage deponovane unutar uređaja, što nam daje priliku da predstavimo što preciznije rezultate.



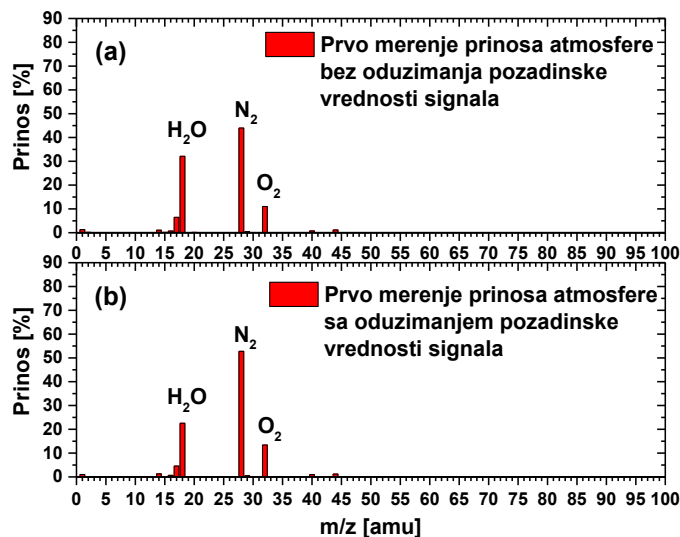
Slika 2.21 Pad signala azota i kiseonika prilikom zatvaranja Swagelok ventila (signal vode ostaje nepromenjen)

Uzimajući u obzir činjenicu da smo otkrili da se značajna količina vlage taloži u masenom spektrometru, vlažnost u prostoriji je merena pomoću higrometra (EKSTECH RHT20 datalogger za vlažnost / temperaturu) kao i unutar protočnog plazmenog mlaza koristeći merač vlažnosti u protoku gasa *eng. Dewpoint meter*. (VAISALA DMT143). Što se tiče merenja atmosfere, pokazalo se da vlažnost sredine u kojoj se instrument nalazi značajno utiče na količinu vlage deponovane u masenom spektrometru. Povećanje vlažnosti okoline direktno je srazmerno povećanju količine vlage u instrumentu što utiče na veći odbroj azota i kiseonika. (Slika 2.22)



Slika 2.22 Poređenje vremenske evolucije molekula vode, azota i kiseonika za različite količine vlažnosti (rh – relativna vlažnost)

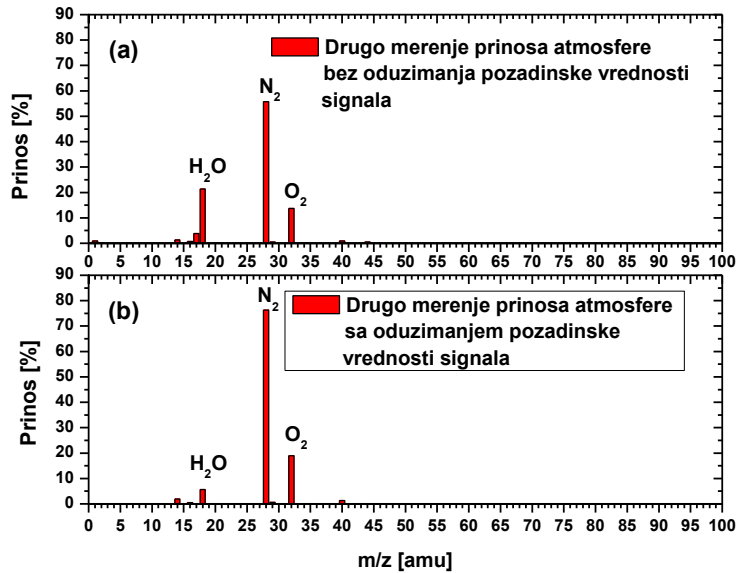
Važnost prireme masenog spektrometra pre početka merenja se može videti i na sledećih nekoliko grafika (Slika 2.23-27) koji predstavljaju uticaj prisustva vode. Odmah nakon što je instrument pripremljen za rad urađen je niz od pet uzastopnih merenja sa međusobnim vremenskim razmakom od 30 minuta.



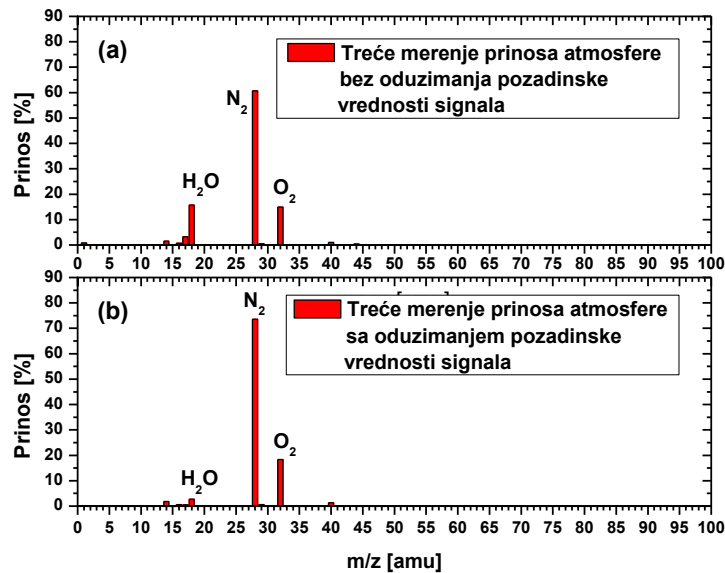
Slika 2.23 Maseni spektar atmosfere prikazan u procentima (prinos) za dva različita slučaja: (a) maseni spektar sa pozadinskom vrednosti signala i (b) maseni spektar bez pozadinske vrednosti signala

Na slici 2.23 prikazan je izmereni maseni spektar atmosfere neposredno nakon dostizanja maksimalne dozvoljene vrednosti pritiska, tj. trenutka kada je instrument spreman za merenje. Ako ne primenimo oduzimanje pozadinskog signala, slika 2.23 (a), možemo primetiti visok procenat odbroja signala H₂O koji remeti odnos masenog sastava atmosfere. Nasuprot ovome, slika 2.23 (b) prikazuje oduzimanje pozadinske vrednosti signala. Zbog promenljivosti signala odbroja vode koji nije konstantan već opada dobijamo i njen visok doprinos u spektru koji daje neprecizno definisan sastav atmosfere.

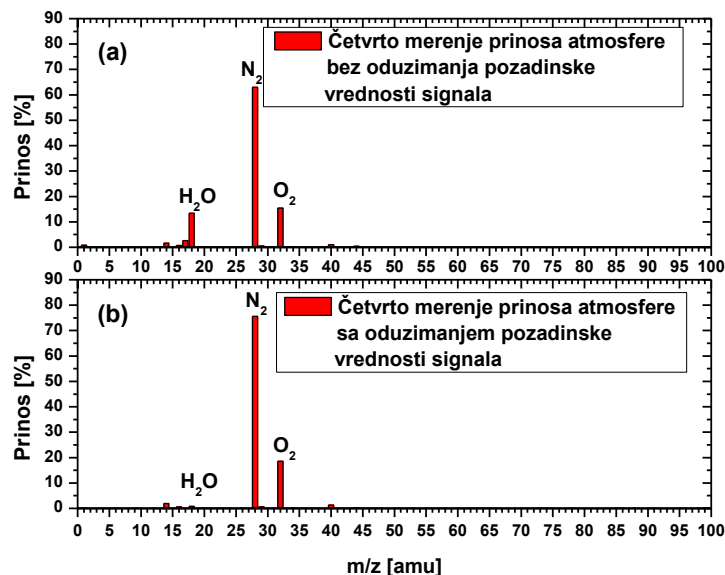
Slike 2.24-27 prikazuju rezultate izmerene atmosfere sa mnogo većom preciznošću zbog sporijeg smanjenja signala vode. Svakim uzastopnim merenjima nakon 30 min, signal vode je manji i zbog sporijeg smanjenja možemo primetiti manji prinos vode u ukupnom masenom spektru, što povećava preciznost samog merenja.



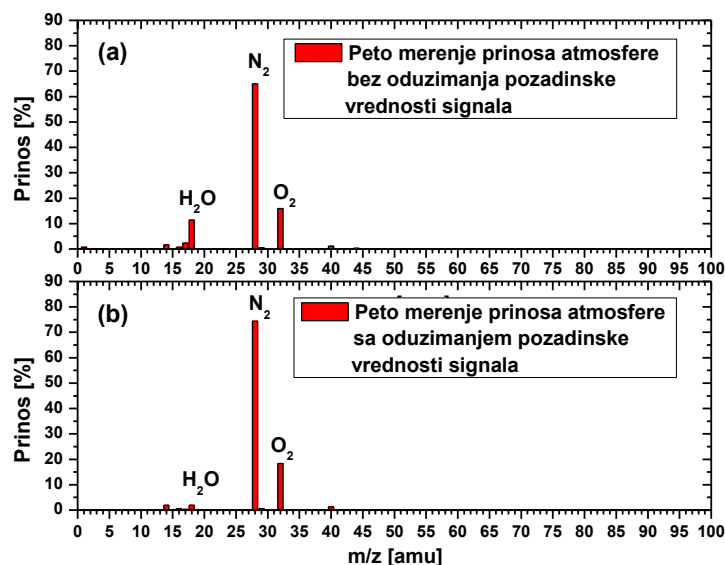
Slika 2.24 Maseni spektar atmosfere snimljen 30 minuta nakon prvog merenja i prikazan u procentima (prinos) za dva različita slučaja: (a) maseni spektar sa pozadinskom vrednosti signala i (b) maseni spektar bez pozadinske vrednosti signala



Slika 2.25 Maseni spektar atmosfere snimljen 60 minuta nakon prvog merenja i prikazan u procentima (prinos) za dva različita slučaja: (a) maseni spektar sa pozadinskom vrednosti signala i (b) maseni spektar bez pozadinske vrednosti signala



Slika 2.26 Maseni spektar atmosfere snimljen 90 minuta nakon prvog merenja i prikazan u procentima (prinos) za dva različita slučaja: (a) maseni spektar sa pozadinskom vrednosti signala i (b) maseni spektar bez pozadinske vrednosti signala



Slika 2.27 Maseni spektar atmosfere snimljen 120 minuta nakon prvog merenja i prikazan u procentima (prinos) za dva različita slučaja: (a) maseni spektar sa pozadinskom vrednosti signala i (b) maseni spektar bez pozadinske vrednosti signala

Prilikom merenja sastava atmosfere pokazano da voda deponovana u masenom spektrometru značajno utiče na od broj ostalih uzorkovanih vrsta. Precizno izmeren sastav atmosfere pomoću masenog spektrometra predstavlja dobar preduslov za analiziranje maseno-energijskih sastava atmosferskih plazmi. Ukoliko se ovaj tip merenja ne izvede ne može se sa sigurnošću tvrditi da su rezultati uzorkovane

atmosferske plazme validni i zato je bitno uvrstiti merenje atmosfere kao pripremno merenje pre svakog eksperimenta.

Kao što se sa prethodnih slika može primetiti za kvantitativni prikaz sastava atmosfere korišćeni su prinosi specifičnih masa (eng. Yield) koje za prezentovanje rezultata iz masene spektrometrije koristi većina istraživača. Prinosi specifičnih masa (relativni doprinos ukupnom signalu) se umesto odbroja u sekundi, koji se dobija direktno od uređaja, koriste da bi se smanjio šum izazvan vremenskim varijacijama plazme. Prinosi su računati kao:

$$Y = \frac{Y_{mass}^i}{\sum_i Y_{mass}^i} \cdot 100 [\%]$$

gde Y_{mass}^i predstavlja odbroj specifične vrste izmerene u spektru (kao što je N_2 , O_2 , H_2O , itd.) koji se kasnije deli sa sumom odbroja svih snimljenih masa (1 amu-100 amu). Ova formula može da se koristi za neutrane i pozitivne i negativne jone. Pošto su u pitanju procenti mora se voditi računa da ne dolazi do brzih promena odbroja određenih vrsta. Brzu promenu odbroja karakteriše neuniformnost količine određenih konstituenata koji su sastavni deo gasa koji se uzorkuje. U našem slučaju do promene dolazi jer deponovana voda u masenom spektrometru prilikom merenja isparava i izlazi iz instrumenta (gde je brz pad signala najizraženiji u prva dva sata rada masenog spektrometra). Smanjenje signala molekula vode u pozadinskom signalu utiče na promenu odbroja konstituenata koji se uzorkuju iz atmosfere. Ukoliko je ta promena brza razlika ukupnog i pozadinskog signala za vodu će biti mala i prilikom računanja procenata masenog sastava dobiće se netačan rezultat, kao što je to bio slučaj kod prvog merenja sastava atmosfere (Slika 2.23). Jako je bitno pre ovog računa ispratiti masene sastave i uvideti bilo kakve drastične promene u odbroju vrsta iz dobijenog spektra. Ukoliko je maseni spektar konzistentan računanje prinosa je moguće izvesti sa velikom preciznošću.

U Tabeli 2.1 prikazane su vrednosti izmerenog sastava atmosfere (Slika 2.27 (b)) i njeno poređenje sa sastavom atmosfere iz literature. S obzirom da je deponovana voda u masenom spektrometru značajno eliminisana oduzimanjem vrednosti signala pozadine, može se reći da postoji saglasnost merenja dobijenih masenim spektrometrom i vrednostima koje u svojoj knjizi prikazuju Valas i Hobs (Wallace i Hobbs, 2006).

Tabela 2.2 Različite procentualne vrednosti konstituenata izmerenih u sastavu atmosfere.

Dominantne vrste u sastavu atmosfere	Merenje HPR-60 masenim spektrometrom	Literatura
(H)	0.22962	-
(H ₂)	0.00193	-
(¹² C)	0.00151	-
(¹⁴ N)	1.87711	-
(¹⁵ N)	0.0069	-
(¹⁶ O)	0.59909	-
(OH)	0.3524	-
(H ₂ O)	1.88283	0.4
(²⁸ N ₂)	74.39597	78.08
(²⁹ N ₂)	0.59707	0.566
(O ₂)	18.35933	20.94
(H ₂ O ₂)	0.07416	
(³⁶ Ar)	0.00392	0.0031
(³⁸ Ar)	0	0.0006
(⁴⁰ Ar)	1.29833	0.93
(⁸⁴ Kr)	0.00172	0.0065
SUMA	99.68189	100.9262

2.4.4 Zaključak

U okviru ove glave opisana je problematika koja je vezana za pripremu masenog spektrometra merenjem masenog sastava atmosfere u laboratoriji. Prikazan je uticaj odabira različitih konfiguracija sistema za uzorkovanje na intenzitet odbroja. Takođe, prikazani su prinosi izmerenih integralnih vrednosti masenih spektara atmosfere u rasponu od 1-100 amu. Ta merenja su izvedena od trenutka u kojem je maseni spektrometar bio tehnički spreman za merenje (vrednosti pritiska u vakuumskom delu spektrometra zadovoljavaju sve tehničke karakteristike za rad uređaja) i vršena su na svakih pola sata. Izvedeni su sledeći zaključci:

- Veći otvori komponenti iz konfiguracije za uzorkovanje daju veći signal pri merenju zbog većeg fluksa uzorkovanih čestica,
- Promene veličina otvora komponenti ne utiču na signal vode koja je deponovana u masenom spektrometru,
- Iako je maseni spektrometar tehnički „spreman“ za uzorkovanje potrebno je dodatno sačekati da signal vode padne na vrednost gde će brzina njenog opadanja biti približno konstantna,
- Jedan od načina na koji može da se proveriti da li je uređaj spreman za merenja je merenje sastava atmosfere gde se koristi račun za prinos i tu se mora voditi računa o konzistentnosti

masenog spektra (ne sme da postoji drastična promena u odbroju konstituenata koji čine maseni spektar – ovo se može utvrditi nakon dva ili više sukcesivnih merenja),

Može se reći da prisustvo vode unutar masenog spektrometra ne može da se izbegne, ali da njen uticaj prilikom merenja može da se svede na minimum.

2.5 Merenje raspodela energija pozitivnih jona kao jedan od načina pripreme masenog spektrometra

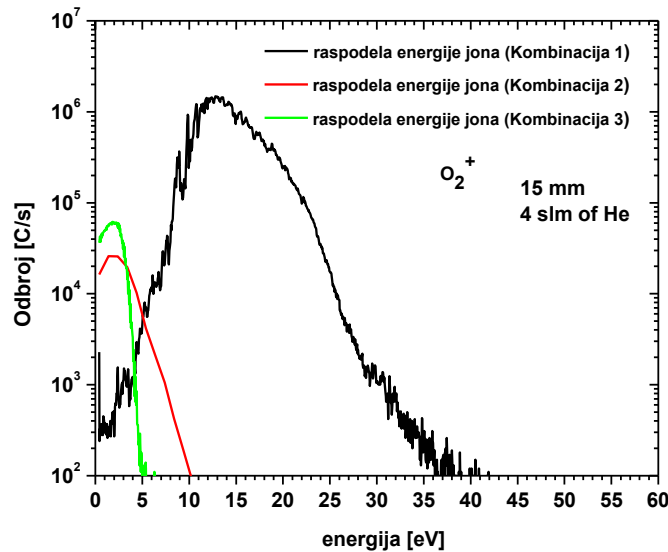
2.5.1 Uvod

Plazme na atmosferskom pritisku karakteriše velika gustina elektrona i mala debljina (<1 mm) granice između plazme i površine (*eng.* Sheath-a). Stoga, ukoliko se odabere veličina otvora koja je dosta veća od veličine sheath-a može doći do prodiranja električnog polja unutar inleta sistema za uzorkovanje a samim tim i do pojave ubrzanja elektrona i pomeranja maksimuma IED ka višim vrednostima, što su pokazali Rees i saradnici pri masenoj analizi RF plazma igle gde su dobili vrednosti maksimuma IED od 50 eV, (Rees *et al.*, 2010). Merenja pri ovim eksperimentalnim uslovima rezultuju dobijanjem nepreciznije/manje-realne slike masenog spektra plazme. Stoga je potrebno pažljivo odabrati kombinaciju inleta i konusa, komponenti za uzorkovanje masenog spektrometra, koje su odgovorne za formiranje molekulskog snopa HIDEN HPR60 masenog spektrometra.

2.5.2 Izbor konfiguracija za uzorkovanje

Na slici 2.28 prikazano je poređenje raspodela energija pozitivnog jona molekula kiseonika O_2^+ za tri različite kombinacije sistema za uzorkovanje. Očigledno je da je najveći odbroj dobijen za Kombinaciju 1. Maksimum IED se nalazi na energiji od 15 eV. Druge dve kombinacije osim manjeg odbroja odlikuju pozicije maksimuma raspodela jona po energijama na 2 eV. Prvi zaključak je da Kombinacija 1 iako daje najveći odbroj nije dobro rešenje za analizu plazmenog mlaza jer dolazi do prodiranja električnog polja unutar inleta (podsetimo se da Kombinacija 1 ima inlet dijametara 0.3 mm). Druge dve kombinacije prikazuju maksimume energija koje odgovaraju očekivanim vrednostima i njih dve su uzete u dalje razmatranje.

Razlika Kombinacija 2 i 3 je u veličini Konusa 2 (0.3 mm naspram 0.4 m). Kombinacija 2 u preliminarnim merenjima dala je dobar signal pozitivnih jona plazmenog mlaza pri rastojanjima manjim od 15 mm posmatrano u odnosu na maseni spektrometar. Međutim, s obzirom na to da je potrebno uskladiti masenu analizu sa ICCD merenjima (praćenje kretanja PAPS-a pri rastojanju većem od 15 mm), odlučeno je da treća kombinacija najbolje odgovara eksperimentu, ali da postoje ograničenja eksperimentalnih uslova izborom drugih parametara, kao što su primenjeni napon i brzina protoka radnog gasa.



Slika 2.28 Poređenje raspodela energija pozitivnog jona molekula O_2^+ tri različite kombinacije sistema za uzorkovanje

2.5.3 Izbor odgovarajućih parametara za potrebe masene analize plazmenog mlaza

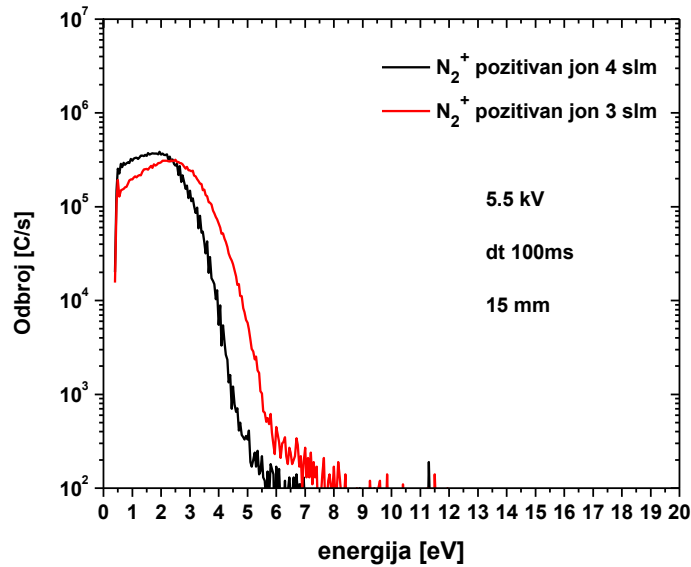
U daljem tekstu biće prikazani podaci merenja koja su urađena korišćenjem Kombinacije 3 otvora za uzorkovanje. Cilj je bio određivanje graničnih vrednosti eksperimentalnih parametara pri kojima ne dolazi do pojave pražnjenja unutar masenog spektrometra:

- koliko blizu izvor plazmenog mlaza može da „priđe“ masenom spektrometru,
- koji maksimalan/minimalan primenjeni napon može da se koristi i,
- koja brzina protoka helijuma može da se koristi.

Za ova merenja odabrana su 3 različita rastojanja plazmenog mlaza u odnosu na maseni spektrometar: 15 mm, 25 mm i 30 mm.

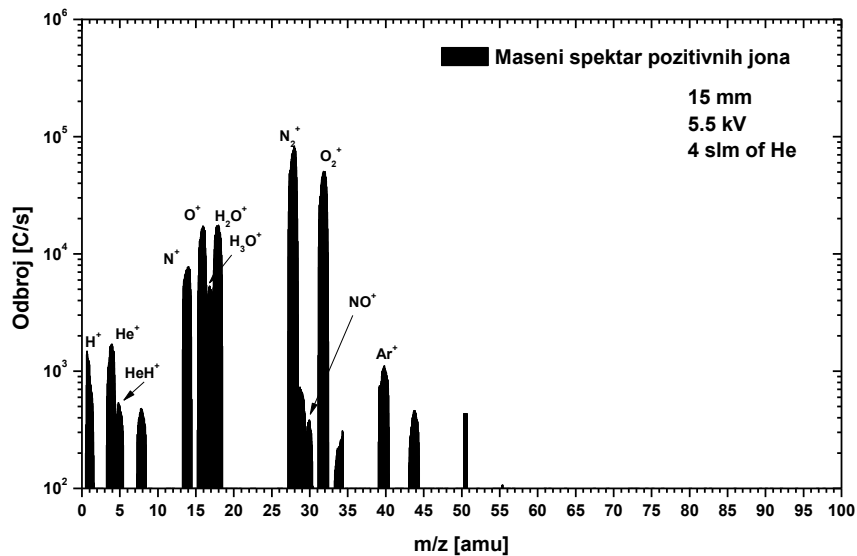
Prvi set prikazuje rezultate za deo eksperimenta gde je ivica cevi plazmenog mlaza bila postavljena 15 mm ispred inleta masenog spektrometra.

Prva vrednost primenjenog napona iznosila je 5.5 kV i kod ovih raspodela energija najzastupljenijih vrsta pozitivnih jona N^+ , N_2^+ , O^+ i O_2^+ , za oba protoka helijuma, nije dolazilo do pojave „repa“, Slika 2.29 (primer za N_2^+). Ako se poredi protoci, za oba se može primetiti da je raspodela energije jednoličnog oblika bez ikakvih deformacija i pojave repa. Jedinu uočljivu razliku predstavlja maksimum raspodele koji je kod 4 slm blago pomeren ka višim energijama (sa 2 eV na 3 eV). Za ovaj set smo izmerili i maseni spektar pozitivnih jona (Slika 2.30).



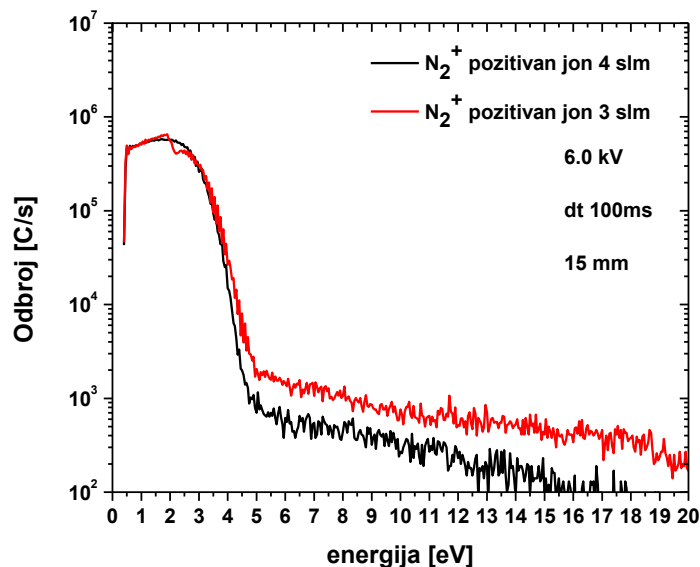
Slika 2.29 Poređenje N_2^+ pozitivnih jona pri primenjenom naponu od 5.5 kV za različite vrednosti protoka radnog gasa

U masenom spektru pozitivnih jona, slika 2.30, najdominantnije vrste koje možemo primetiti N_2^+ , O_2^+ , O^+ , H_2O^+ , N^+ , H_2O^+ i H_3O^+ su praćene nizom manjeg broja atomskih vrsta, kao što su azot i kiseonik. Takođe u spektru se može primetiti za još jedan red veličine manji odbroj signala He^+ , H^+ , NO^+ .

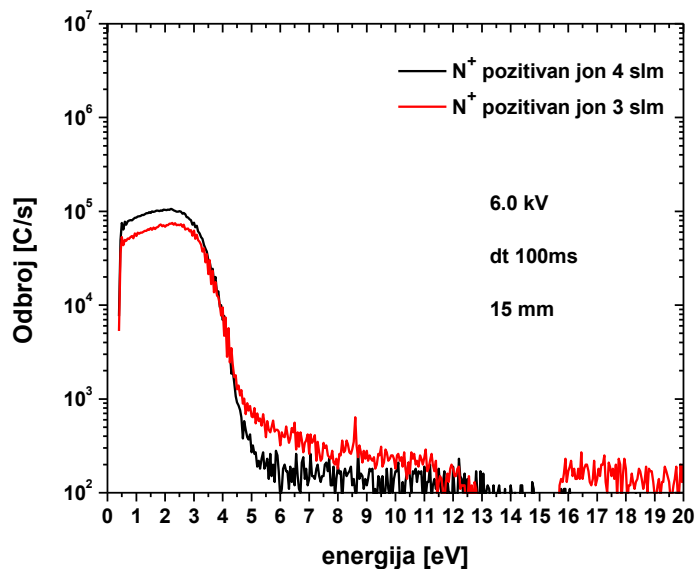


Slika 2.30 Maseni spektar pozitivnih jona za rastojanje od 15 mm, primenjeni napon 5,5 kV, i protok radnog gasa 4 slm

Pri povećanju primenjenog napona na 6.0 kV i za oba korišćena protoka od 3 slm i 4 slm, dolazi do pojave „repa“, Slika 2.31. Rep je izraženiji kod molekulskih vrsta, ali je izražen i kod atomskih vrsta (primer N^+ jona, Slika 2.32). Iz ovih rezultata možemo zaključiti da primena višeg napona dovodi do moguće pojave pražnjenja unutar masenog spektrometra usled pojave repa koji je pomećen ka višim energijama.



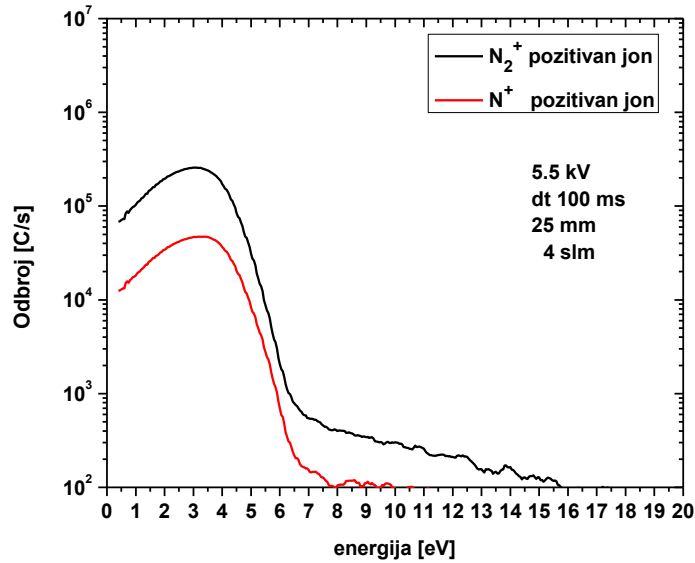
Slika 2.31 Poređenje N_2^+ pozitivnih jona pri primenjenom naponu od 6.0 kV za različite vrednosti protoka radnog gasa



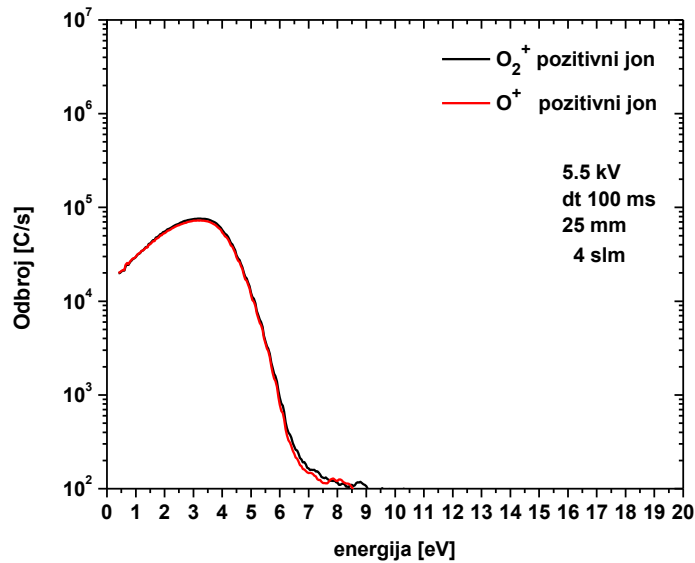
Slika 2.32 N^+ Poređenje N^+ pozitivnih jona pri primenjenom naponu od 6.0 kV za različite vrednosti protoka radnog gasa

Ustanovljeno je da bi jedini odgovarajući primenjeni napon pri rastojanju od 15 mm bio 5.5 kV. Pri nižim vrednostima napona plazma ne pokazuje stabilno ponašanje u odnosu na inlet masenog spektrometra (signal nije stabilan usled „treperenja“ mlaza). Pri višim naponima kao što je pokazano dolazi do pojave „repa“ što sa sobom nosi mogućnost pojave pražnjenja unutar spektrometra. Drugi set prikazuje rezultate za deo eksperimenta gde je ivica cevi plazmenog mlaza bila postavljena 25 mm ispred inleta masenog spektrometra.

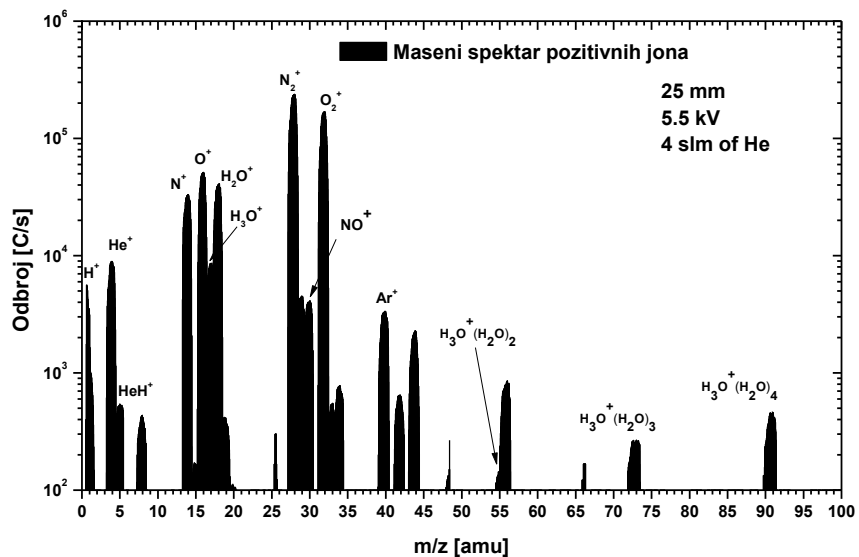
Slike 2.33 i 2.34 prikazuju raspodelu energija pozitivnih jona molekulskih i atomskih vrsta azota i kiseonika za primenjeni napon od 5.5 kV i protoku helijuma od 4 slm. Može se primetiti pojava repa u raspodeli energije molekula azota na Slici 2.33 koja završava na vrednosti od oko 16 eV, dok se za ostale vrste raspodele završavaju na vrednosti od oko 8 eV. Posmatrane distribucije za ovaj set parametara imale su najmanje deformisan oblik, i zato smo te parametre koristili za merenje masenog spektra pozitivnih jona, (Slika 2.35). Možemo primetiti da u spektru najviše dominiraju molekuli N_2^+ , O_2^+ a zatim ih prate njihove atomske vrste čiji je odbroj za red veličine manji, kao i joni molekula vode. Takođe, u spektru se mogu primetiti značajne vrednosti odbroja za He^+ , H^+ , NO^+ , kao i klasteri vode $H_3O^+ \cdot (H_2O)_2$, $H_3O^+ \cdot (H_2O)_3$ i $H_3O^+ \cdot (H_2O)_4$.



Slika 2.33 Raspodela energije N_2^+ i N^+ pozitivnih jona pri naponu 5.5 kV

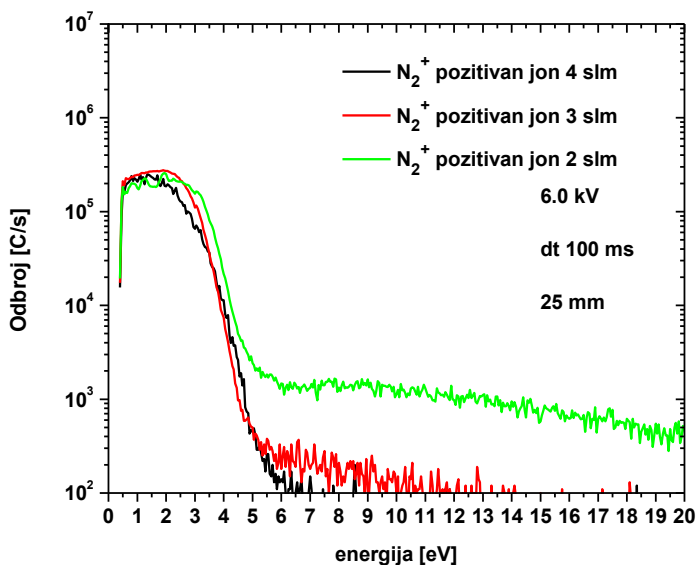


Slika 2.34 Raspodela energije O_2^+ i O^+ pozitivnih jona pri naponu 5.5 kV

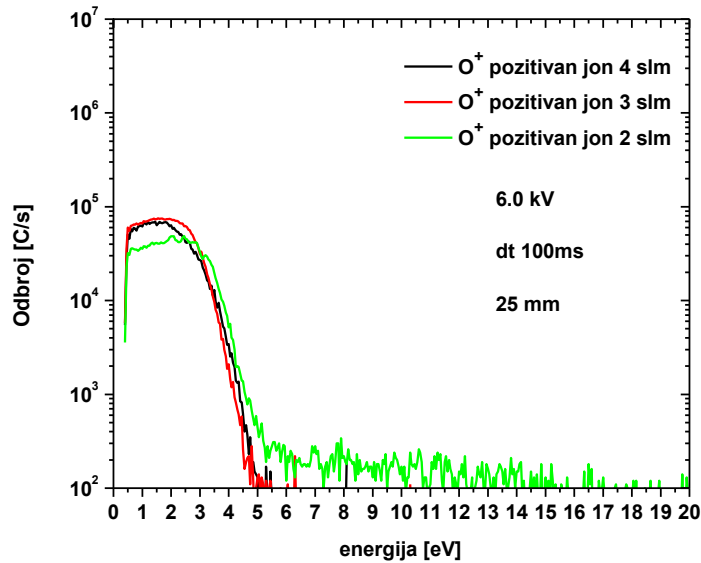


Slika 2.35 Maseni spektar pozitivnih jona za rastojanje od 25 mm, primenjeni napon 5.5 kV, i protok radnog gasa 4 slm

Na Slikama 2.36 i 2.37 može se videti poređenje raspodela energija za tri različita protoka helijuma (2 slm, 3 slm i 4 slm) i primenjenim naponom od 6.0 kV za pozitivne jone molekula azota N_2^+ (Slika 2.36) i atoma kiseonika (2.37). Pojava repa se dešava pri vrednosti energije od 5 eV i dominantna je za najniži protok helijuma (2 slm) što je izraženije kod molekulskih vrsta. Za 3 slm pojava repa je blaga dok se kod 4 slm pojava repa i ne primećuje.



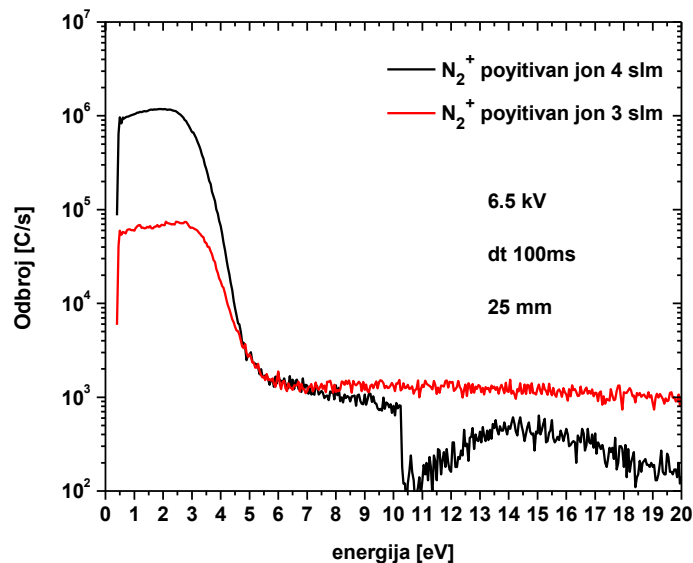
Slika 2.36 Poređenje N_2^+ pozitivnih jona pri primenjenom naponu od 6.0 kV za različite vrednosti protoka radnog gasa



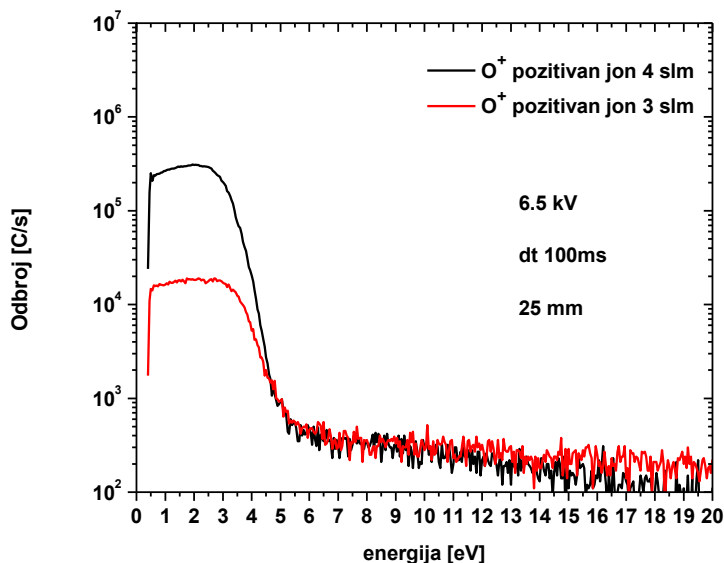
Slika 2.37 Poređenje O^+ pozitivnih jona pri primenjenom naponu od 6.0 kV za različite vrednosti protoka radnog gasa

Za sledeći set merenja eliminisan je protok od 2 slm pod pretpostavkom da će povećanje primenjenog napona pospešiti pojavu pražnjenja unutar masenog spektrometra.

Kako se povećava primenjeni napon, na Slikama 2.38 i 2.39 "rep" raste za izmerene atomske i molekulske vrste (dat je primer za N_2^+ i O^+ jone). Za molekulske vrste odbroj repa je reda veličine 10^3 c/s, dok je kod atomskih vrsta manji ali očigledno izražen.

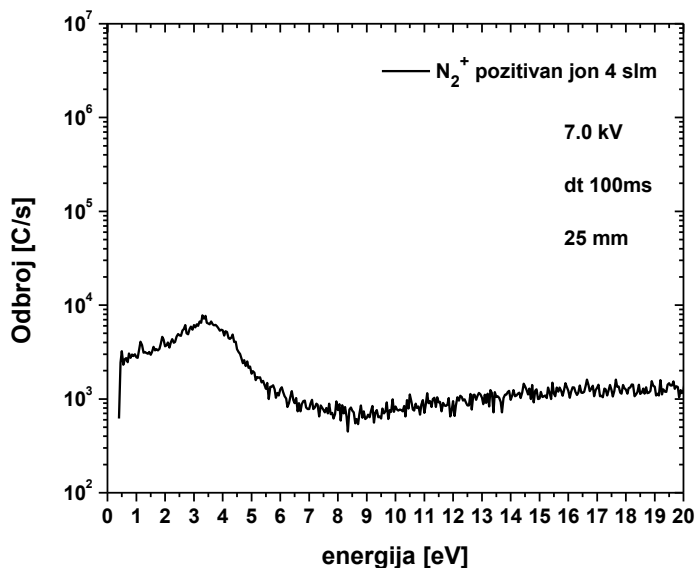


Slika 2.38 Poređenje N_2^+ pozitivnih jona pri primenjenom naponu od 6.5 kV za različite vrednosti protoka radnog gasa



Slika 2.39 Poređenje O^+ pozitivnih jona pri primenjenom naponu od 6.5 kV za različite vrednosti protoka radnog gasa

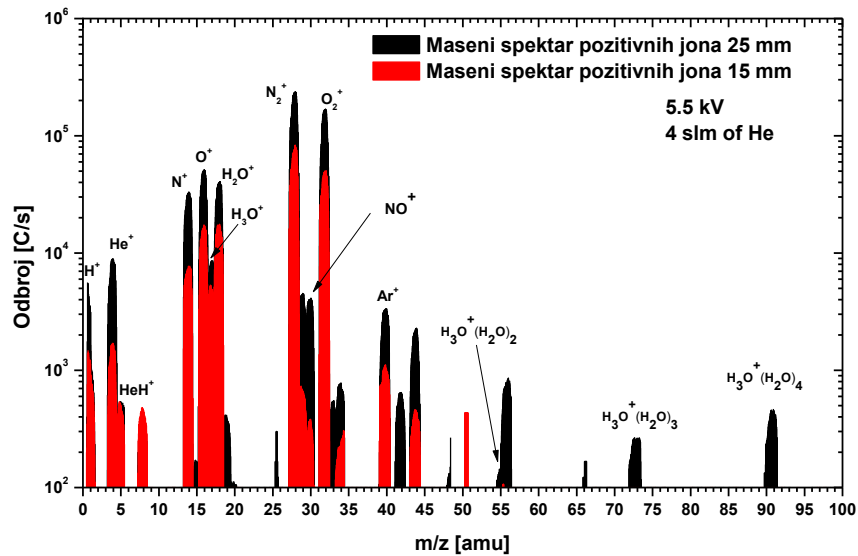
Sa povećanjem vrednosti primenjenog napona na 7 kV, Slika 2.40, maksimalna raspodela energije jona drastično opada, oblik raspodele je deformisan a rep se kreće prema većim energijama sa povećanjem odbroja. To dovodi do zaključka da ove vrednosti energija jona ne bi dale realnu sliku masenih spektra tako da ove parametre eliminišemo u daljim istraživanjima.



Slika 2.40 Poređenje N_2^+ pozitivnih jona pri primenjenom naponu od 7.0kV za različite vrednosti protoka radnog gasa

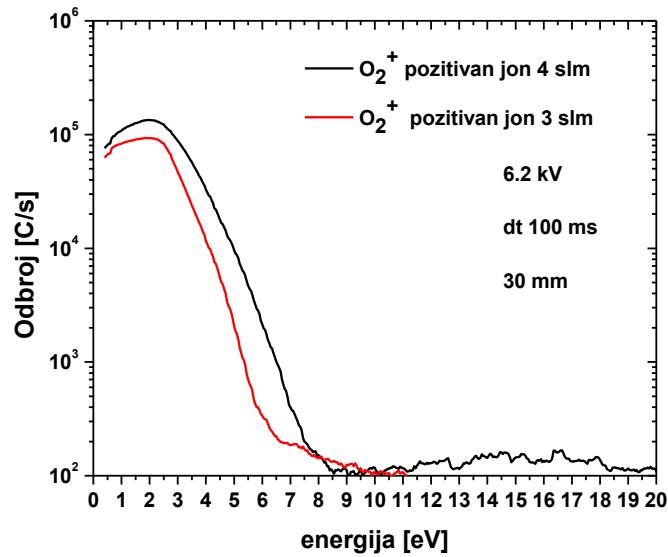
Prema prikazanim rezultatima za rastojanje 25 mm odgovarajući parametri koji bi mogli da se koriste su sledeći: primenjeni naponi 5.5 kV i 6.0 kV, protok radnog gasa od 3 slm i 4 slm. Pri smanjenju protoka na 2 slm pojava repa raste kao i pri povećanju primenjenog napona na vrednostima većim od 6.5 kV.

Ako posmatramo poređenje dva masena spektra za različito rastojanje plazmenog mlaza u odnosu na maseni spektrometar (15 mm i 25 mm), na slici 2.41, može se primetiti nedostatak klastera vode za udaljenost od 15 mm. Prva pretpostavka je da se klasteri vode pojavljuju na vrhu plazma mlaza gde je emisija manjeg intenziteta nego na samom rubu cevi i bili smo u mogućnosti da ih posmatramo na udaljenosti većoj od 20 mm. Druga pretpostavka je da je na kraćem rastojanju od 20 mm pritisak protoka radnog gasa 4 slm dovoljno velik da rasprši klasterne vode i oni jednostavno ne mogu da budu uzorkovani korišćenjem otvora masenog spektrometra od 0.1 mm. Takođe primetna je razlika od jednog reda veličine kod NO^+ .

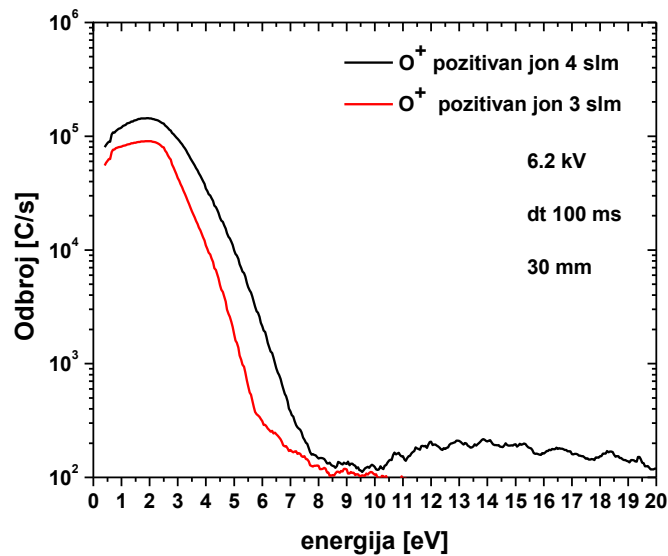


Slika 2.41 Maseni spektar pozitivnih jona – poređenje rastojanja 15 mm i 25 mm, primenjeni napon 5.5 kV, i protok radnog gasa 4 slm

Na Slikama 2.42 i 2.43 prikazani su rezultati za rastojanje plazmenog mlaza 30 mm u odnosu na otvor masenog spektrometra za molekulske i atomske vrste pozitivnog jona kiseonika. Primenjen je napon od 6.2 kV, za dva različita protoka radnog gasa (3 slm i 4 slm). Pokazano je kao i kod prethodnih slučajeva maksimum IED se nalazi na oko 2 eV. Na ovom rastojanju dolazi do izraženije pojave „repa“ pri protoku helijuma od 4 slm (to je izražajnije kod molekulskih vrsta).

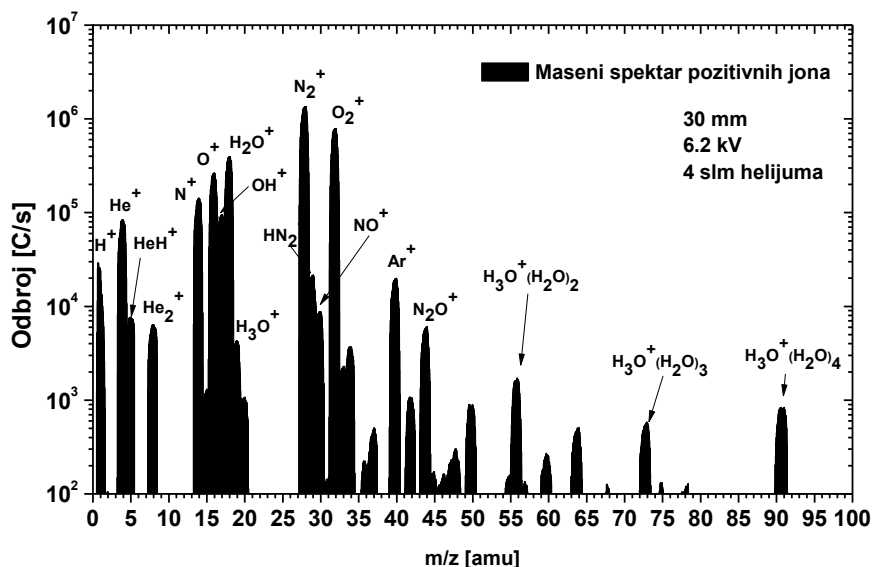


Slika 2.42 Poređenje O_2^+ pozitivnih jona pri primenjenom naponu od 6.2 kV za različite vrednosti protoka radnog gasa na rastojanju 30 mm



Slika 2.43 Poređenje O^+ pozitivnih jona pri primenjenom naponu od 6.2 kV za različite vrednosti protoka radnog gasa na rastojanju 30 mm

Slično kao za rastojanje od 25 mm i kod rastojanja od 30 mm (Slika 2.44) u masenom spektru pozitivnih jona se može uočiti pojava vodenih klastera značajnog signala. S obzirom na činjenicu da su to 2 rastojanja u odnosu na maseni spektrometar kod kojih je moguće posmatranje vremenske evolucije PAPS-a, (pri kraćim rastojanjima PAPS se ne kreće već je pozicioniran na otvor masenog spektrometra; pri tim rastojanjima nije moguće snimanje pozitivnih jona klastera vode) odabrali smo ih za dalju analizu. Postavka sa 15 mm je izostavljena (PAPS se ne kreće već je „zalepljen“ za inlet masenog spektrometra).



Slika 2.44 Maseni spektar pozitivnih jona pri rastojanju od 30 mm, za primenjeni napon 6.2 kV i protok helijuma od 3 slm

2.5.4 Zaključak

U okviru ove glave opisan je odabir odgovarajuće konfiguracije sistema za uzorkovanje masenog spektrometra kao i izbor odgovarajućih parametara koji će se koristiti u masenoj analizi plazmenog mlaza. Ovo je omogućeno snimanjem pozitivnih jona koji sačinjavaju maseni spektar plazmenog mlaza. Snimane su raspodele energija pozitivnih jona koje su nam ukazale kod kojih eksperimentalnih uslova pražnjenje ne prodire u vakuumski deo masenog spektrometra i time ne utiče na preciznost analize masenih spektara. Iz datih merenja mogu se izvući sledeći zaključci:

- Kombinacija 1 sa najvećim otvorom inleta od 0.3 mm ne spada u dobro rešenje jer se dobijaju vrednosti IED čiji je maksimum pomeren ka višim vrednostima energije,
- Kombinacija 2 (otvor inleta 0.1 mm i otvor Konusa1 0.3 mm) može biti dobro rešenje za plazma izvore koji moraju da se uzorkuju iz neposredne blizine što u slučaju plazmenog mlaza gde treba da posmatramo i kretanje PAPS-a nije dobra solucija,
- Kombinacija 3 (otvor inleta 0.1 mm i otvor Konusa2 0.4 mm) se pokazala kao idelna za uzorkovanje plazmenog mlaza do rastojanja od 30 mm pri kojem se kretanje PAPS-a može pratiti,
- Zanimljiv rezultat je da se pri većim rastojanjima plazmenog mlaza u odnosu na maseni spektrometar mogu detektovati klasteri vode (na kraćim rastojanjima npr. 15 mma PAPS je „zalepljen“ za otvor masenog i u tom slučaju nisu detektovani klasteri vode),
- Za dalju analizu masenim spektrometrom i ICCD kamerom odabrani su parametri pri kojim se može posmatrati vremenska evolucija PAPS-a i pri kojim ne dolazi do pomeranja maksimuma energije raspodele jona ka višim energijama (pojava „repa“ kod ovih raspodela još uvek nije dobro objašnjena, ali je pretpostavka da i u tom slučaju može doći do pojave pražnjenja unutar vakuumskeg dela pa su parametri za te slučajeve isključeni iz dalje analize).

3. UTICAJ VODENE PARE UNUTAR PROTOČNOG SISTEMA RADNOG GASA NA FORMIRANJE PAPS-A

3.1 Uvod

Poslednjih godina određeni broj autora je počeo da prati uticaj vlažnosti kao veoma važan parametar za električna pražnjenja na atmosferskom pritisku. Primećene su promene koncentracija reaktivnih vrsta kiseonika (*eng.* reactive oxygen species ROS) i reaktivnih vrsta azota (*eng.* reactive nitrogen species RNS), posebno OH radikala zbog promene vlažnosti, koristeći različite eksperimentalne metode kao što su laserski indukovana fluorescencija LIF, optička emisiona spektroskopija i Rejljevo mikrotalasno rasejanje (Han, 2018; Bruggeman et al., 2010; Lin et al., 2019; Ono i Tokuhiko, 2020).

Ne samo da vlažnost iz ambijentalnog vazduha predstavlja važan parametar, već i vlažnost unutar protočnog sistema radnog gasa koji napaja izvor plazme. Međutim, iako su prethodna istraživanja uticaja vlažnosti dovela do značajnih zaključaka u vezi sa sastavom pražnjenja, njegov uticaj na prostiranje električnog pražnjenja i formiranje PAPS-a još uvek nije publikovan do sada.

U ovoj studiji biće pokazano da nečistoće u obliku vlage deponovane na zidovima cevi unutar protočnog sistema plazmenog mlaza dizajniranog u Laboratoriji za neravnotežne procese i primenu plazme (Maletić *et al.*, 2015) mogu značajno uticati na propagaciju same plazme kao i na njen hemijski sastav. Koristili smo ICCD kameru za snimanje prostorno-vremenskog razvoja pražnjenja u periodu od 12.5 mikrosekundi s obzirom da pobudna frekvencija plazmenog mlaza iznosi 80 kHz. Takođe, da bismo izmerili promene hemijskog sastava masenog spektra, koristili smo maseni spektrometar (HIDEN HPR60).

3.2 Rezultati vremenske evolucije PAPS-a korišćenjem ICCD kamere i masene spektrometrije

Rezultati ove studije biće podeljeni u dva dela prema korišćenim dijagnostičkim metodama:

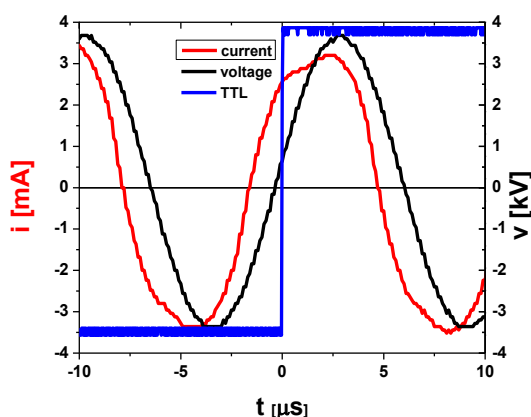
1. ICCD vremenski razložena merenja PAPS-a plazmenog mlaza na atmosferskom pritisku pri različitim koncentracijama molekula vode unutar protočnog sistema radnog gasa (prirodno deponovana vlaga u protočnom sistemu i veštački dodata vodena para u protočni u sistem – korišćenjem ispiralice),
2. Merenja integralnih vrednosti masenih spektara pozitivnih jona plazmenog mlaza pri različitim koncentracijama vlažnosti unutar protočnog sistema radnog gasa (prirodno dodata vlažnost u sistem i veštački dodata vlažnost u sistem – korišćenjem ispiralice).

Glavna ideja bila je istražiti uticaj prisustva vodene pare unutar sistema za dovod gasa na stvaranje i propagaciju PAPS-a. To smo učinili snimanjem brzom ICCD kamerom u dva slučaja. Prvi je kada je u sistemu za protočni sistem radnog gasa (u ovom slučaju 4 slm helijuma) prisutna zaostala vodena para. Ova vodena para rezultat je izlaganja protočnog sistema laboratorijskom vazduhu tokom dužih vremenskih perioda (obično 24 sata). Koncentracija zaostale vodene pare i njeno smanjenje tokom ICCD snimanja i masene spektrometrije izmereni su VAISALA DMT143 meračem vlažnosti u protoku gasa (*eng.* dewpoint transmitter).

Drugi slučaj predstavlja situaciju kada smo pustili da se protočni sistem radnog gasa potpuno osuši (koncentracija vodene pare bila je ispod 20 ppm), a zatim je vodena para veštački dodata i njena koncentracija je merena i kontrolisana tokom ICCD snimanja i masene spektrometrije. Da bismo to uradili dodali smo drugu granu u protočni sistem za dovod radnog gasa. U toj drugoj grani je mali procenat helijuma prolazio kroz vodu unutar ispiralice i unosio veštački kontrolisanu vrednost (uz pomoć protokomera i merača vlažnosti u protoku gasa) vodene pare u glavni protok od 4 slm helijuma.

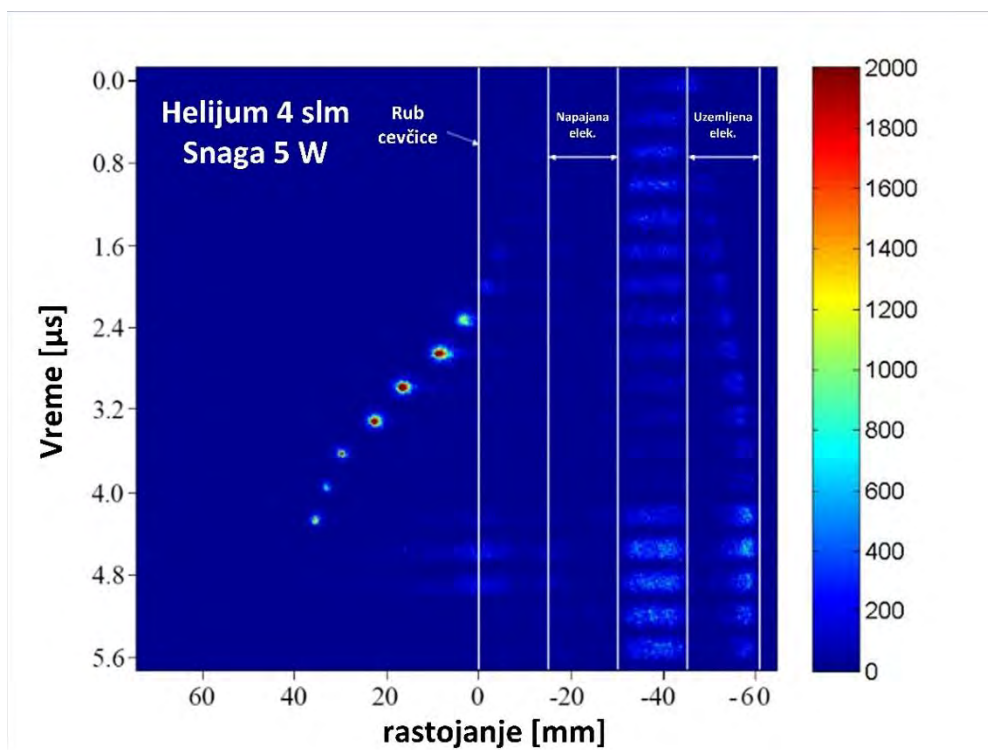
3.3 ICCD – vremenska evolucija PAPS pod uticajem vlažnosti

Prvi deo eksperimenta je urađen na Masarik univerzitetu u Brnu, Republika Češka u okviru COST akcije. Jedina razlika u odnosu na već opisanu eksperimentalnu postavku jeste da je u ovom slučaju korišćena ICCD kamera (PI-MAKS2 1024RB-25-FG43, 16-bitna rezolucija sive skale) sa fotokatodom osetljivom na UV zračenje i UV propusnim objektivom za snimanje intenziteta optičke emisije posmatrane tokom vremenske evolucije PAPS-a. Za sinhronizaciju ICCD kamere i električnih signala koristili smo TTL signal (vidi Sliku 3.1) generisanim iz 2. kanala signal generatora marke RIGOL. Tokom eksperimenta zabeležili smo pražnjenje tokom čitavog perioda od $12.5 \mu\text{s}$ što odgovara frekvenciji od 80 kHz. Međutim, u ovom radu smo predstavili samo pozitivan deo poluperioda ($0.0 \mu\text{s} - 5.6 \mu\text{s}$) intenziteta optičke emisije u kome smo mogli da posmatramo prostiranje PAPS-a. U negativnom delu poluperioda ($5.6 \mu\text{s} - 12.5 \mu\text{s}$) plazma se može posmatrati samo unutar napajane elektrode sa odsustvom PAPS-a i te snimke nismo prikazali.

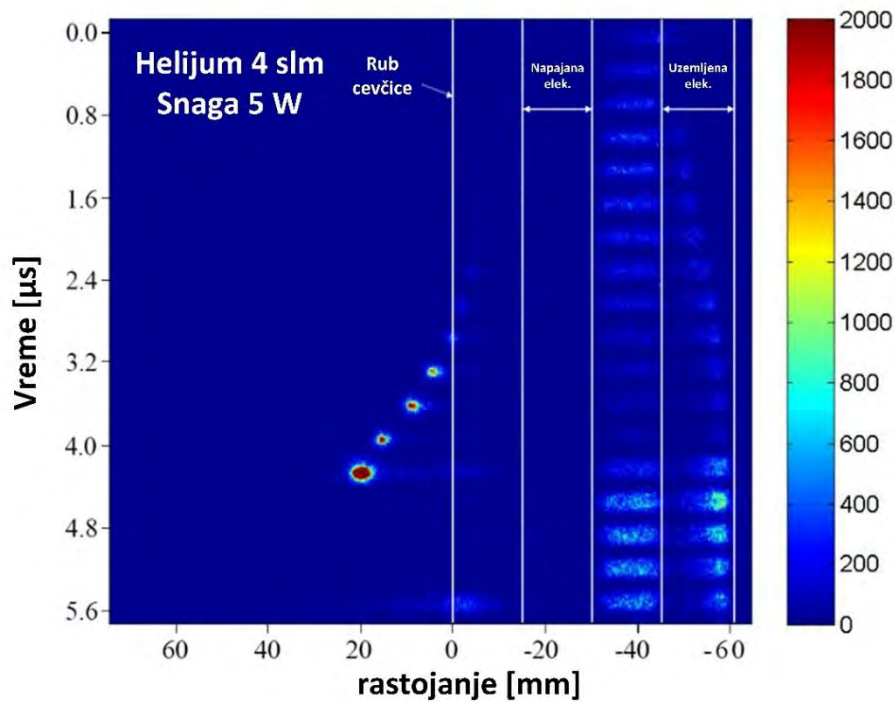


Slika 3.1 Strujno-naponski signali sa TTL signalom za trigerovanje kamere. (Selaković *et al.*, publikacija u pripremi)

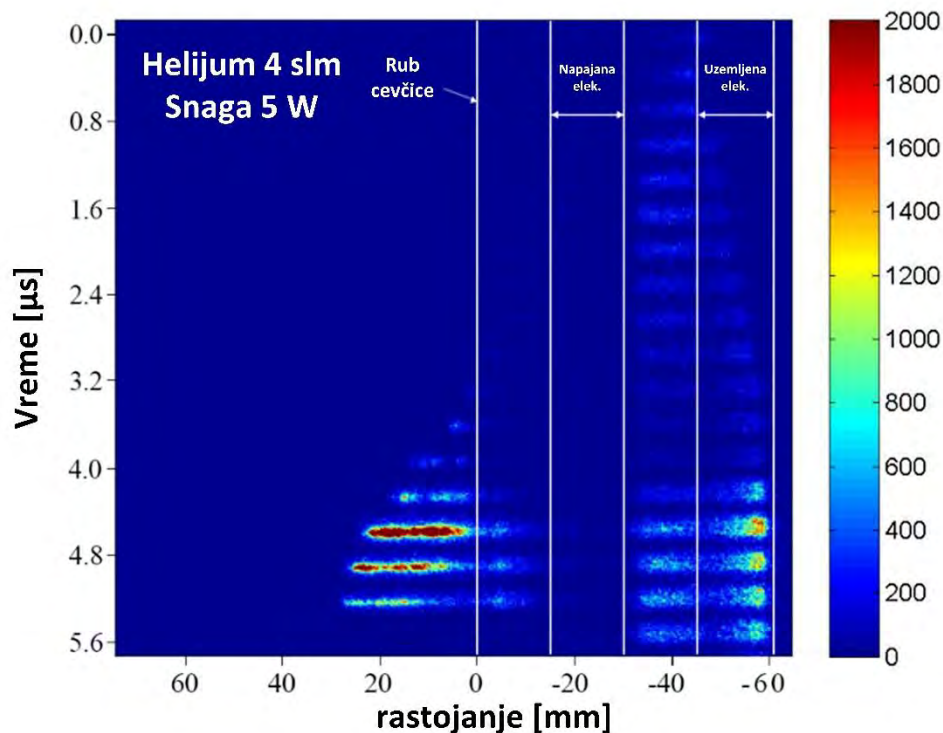
Predstavljeni setovi snimaka vremenske evolucije PAPS-a, dobijeni ICCD kamerom, pokazuju promenu ponašanja električnog pražnjenja. Svi ICCD snimci u eksperimentu su skalirani na isti intenzitet optičke emisije radi lakšeg poređenja. Na Slikama 3.2-4 može se videti deo perioda od $12.5 \mu\text{s}$ ($2.0 \mu\text{s} - 4.4 \mu\text{s}$) unutar kojeg se PAPS pojavljuje, propagira u pravcu protoka radnog gasa izvan ruba cevčice plazmenog mlaza a zatim i nestaje. Tokom pražnjenja primetili smo da merena vrednost vlažnosti unutar protočnog sistema radnog gasa postepeno opada, pa se koncentracija molekula H_2O smanjuje sa 400 ppm–25 ppm (brzina kojom vlažnost opada obrnuto je proporcionalna relativnoj vlažnosti u laboratoriji gde se eksperiment izvodi). Slika 3.2, sa koncentracijom vode od 400 ppm, prikazuje jasno formirane PAPS-ove sa najdužim dometom duž aksijalne ose. Na slici 3.3, gde je koncentracija molekula vode pala na 99 ppm, može se primetiti kraći domet pražnjenja sa primetno većom glavom PAPS-a. Dalje, oko koncentracije od 30 ppm PAPS počinje da gubi svoj prvobitni oblik, postaje sve razmrljaniji pri koncentraciji molekula vode od 23 ppm (Slika 3.4), gde sada imamo izgled prostiranja električnog pražnjenja u vidu razmrljanog traga koji podseća na difuzno kontinualno pražnjenje bez pojave PAPS-a. Ovi setovi ICCD snimaka su napravljeni u periodu od 30 min–45 min nakon formiranja električnog pražnjenja. Izmerena koncentracija vode potiče isključivo od vlažnosti unutar protočnog sistema radnog gasa koja je deponovna na zidovima cevčica. Nakon 45 minuta može se zaključiti da je protočni sistem radnog gasa suv. Suv protočni sistem (ispod 20 ppm molekula vode) onemogućava nam posmatranje PAPS-a. Pretpostavka je da bi veštački dodata vodena para u protočni sistem mogla da bude rešenje formiranja PAPS-a i rezultati tog eksperimenta slede u nastavku.



Slika 3.2 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, protok helijuma 4 slm, koncentracija H_2O unutar protočnog sistema 400 ppm

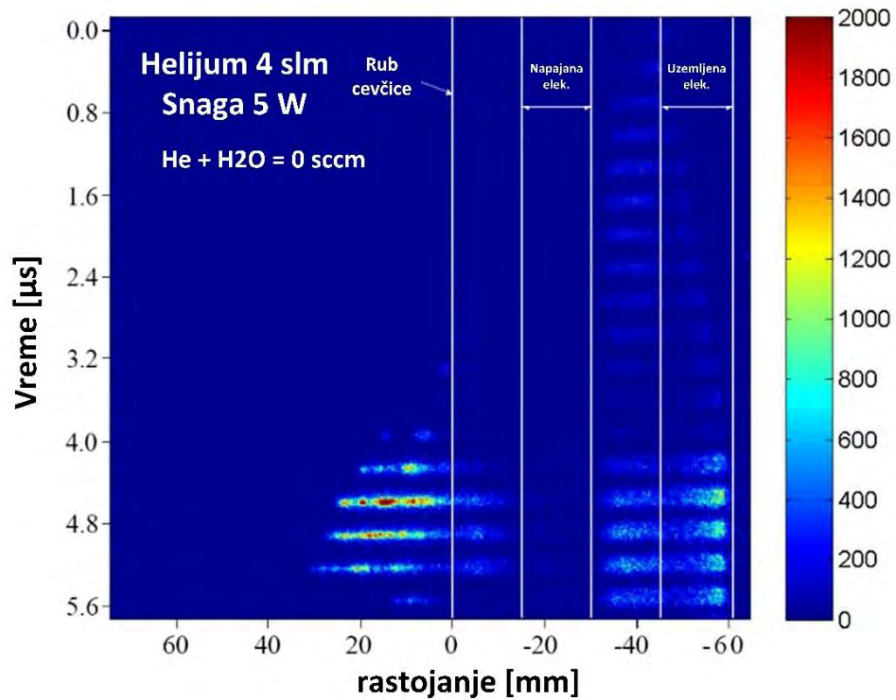


Slika 3.3 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, protok helijuma 4 slm, koncentracija H_2O unutar protočnog sistema 99 ppm.

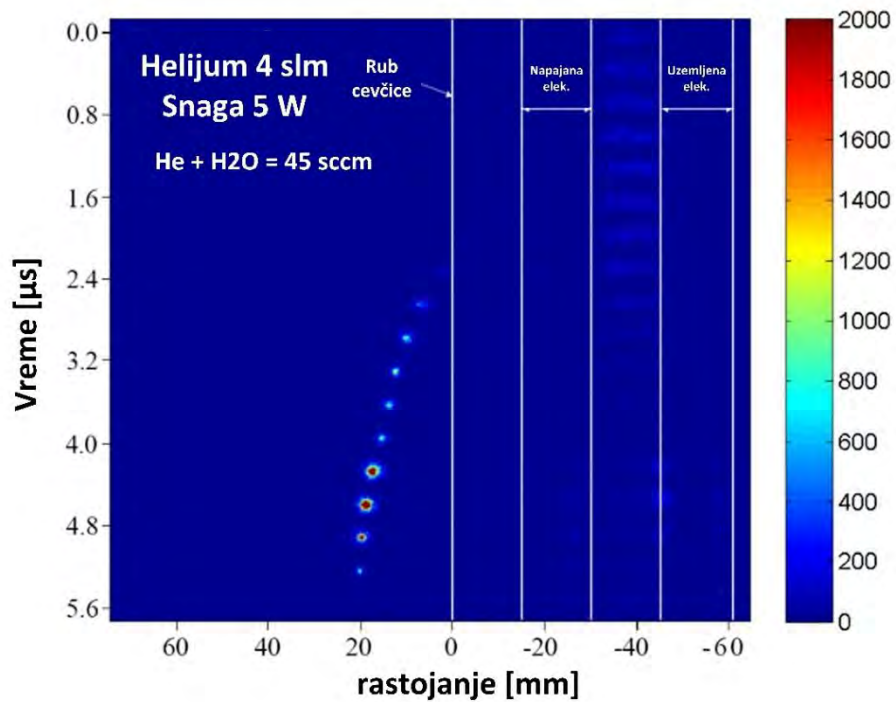


Slika 3.4 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, protok helijuma 4 slm, koncentracija H₂O unutar protočnog sistema 23 ppm. (Selaković *et al.*, publikacija u pripremi)

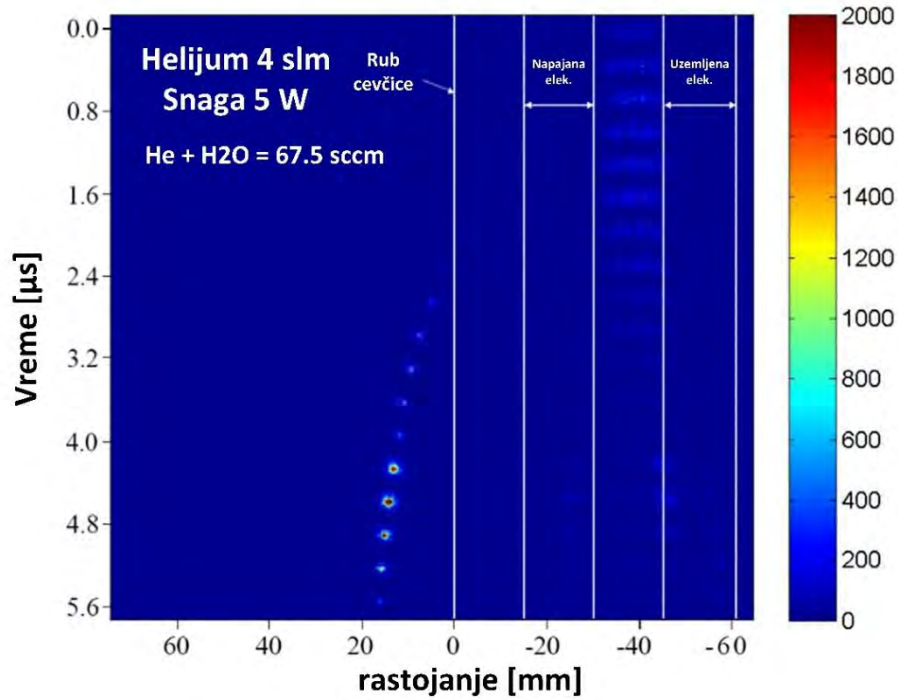
Mali procenat vodene pare dodavan je u protočni sistem (0 sccm–150 sccm) u smešu sa helijumom (4 slm) za kontrolu vlažnosti (koncentracija H₂O) (videti Slike 3.5-8). Ono što prvo može da se primeti (Slika 3.5) u trenutku dodavanja ispiralice sa destilovanom vodom u protočni sistem, gde je protok navlaženog helijuma (cevčica sa helijumom koji prolazi kroz vodu unutar ispiralice) jednak 0 sccm, jeste da je pražnjenje i dalje razmrljano i nema pojave PAPS-a što potvrđuje merenje sa slike 3.4. Sa porastom koncentracije vode dolazi do drastičnih promena u posmatranom električnom pražnjenju gde ono prestaje da bude razmrljano i PAPS se ponovo formira, što je primećeno na ICCD slikama (vidi slike 3.6-7). Na Slici 3.6. mogu se primetiti jasno formirani PAPS koji, za razliku od prvog slučaja, imaju kraći aksijalni domet od skoro 20 mm. Očigledno, pod uticajem povećanja vlažnosti helijuma, aksijalni domet PAPS-a nastavlja da opada (slika 3.7). Ukoliko se povećanje koncentracije H₂O nastavi, sredina postaje zasićenija vodom, tako da PAPS smanjuje svoj domet i postaje manje svetao (videti Sliku 3.8). U nekom trenutku (H₂O>1000 ppm), pod uticajem prezasićenog radnog gasa vodenom parom, električno pražnjenje se gasi, i više ne može biti posmatrano.



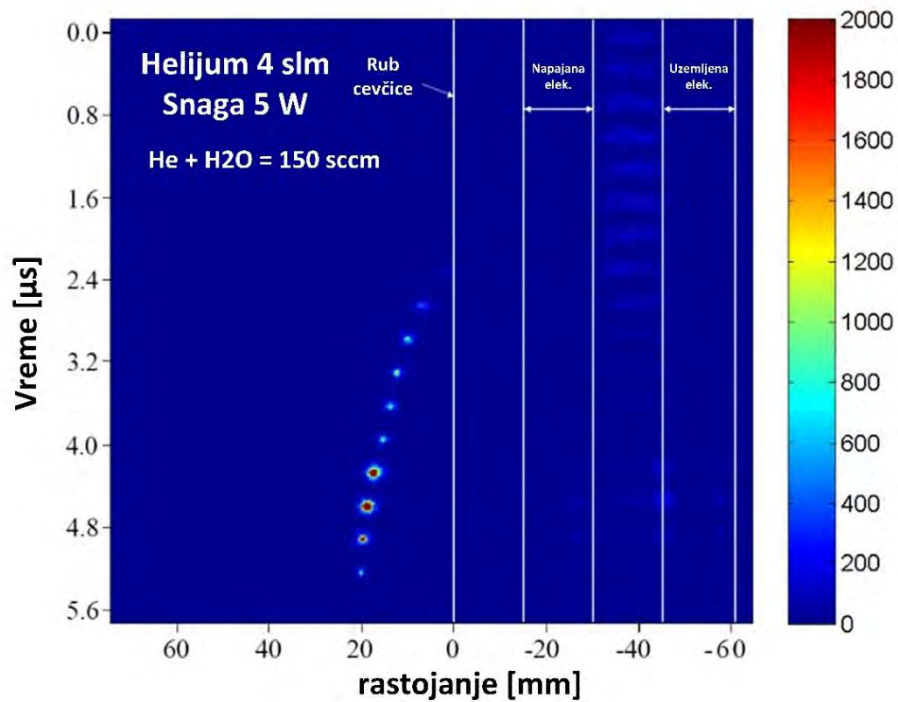
Slika 3.5 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, helijum 4 slm (sa 0 sccm vodene pare), koncentracija H₂O unutar protočnog sistema 22 ppm



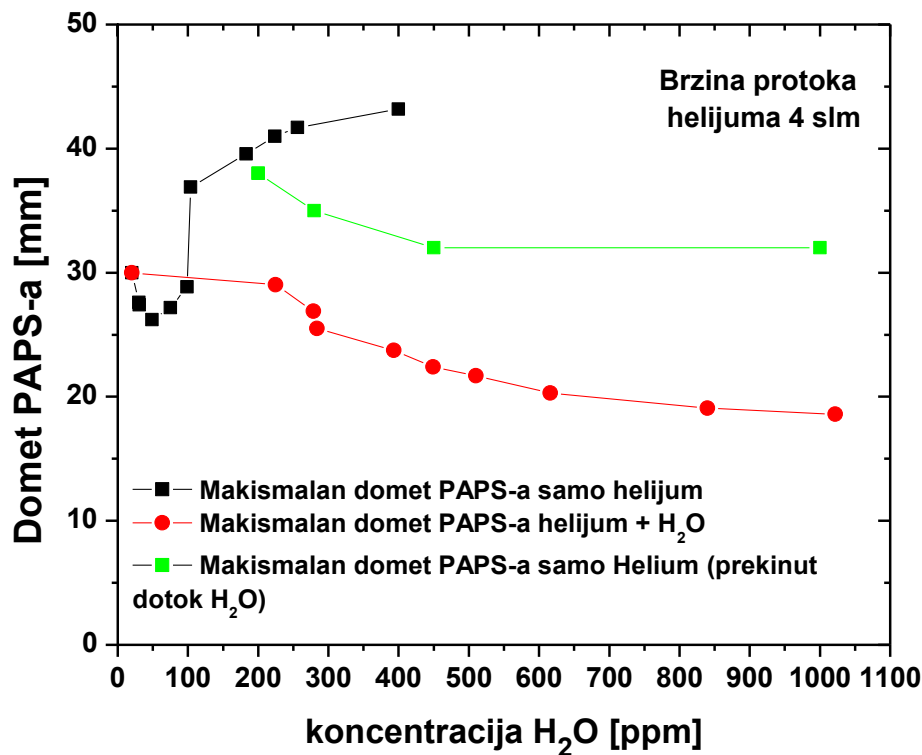
Slika 3.6 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, helijum 4 slm (sa 0 sccm vodene pare), koncentracija H₂O unutar protočnog sistema 45 ppm



Slika 3.7 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, helijum 4 slm (sa 0 sccm vodene pare), koncentracija H₂O unutar protočnog sistema 67.5 ppm



Slika 3.8 Vremenski razloženi ICCD snimci dobijeni pri sledećim parametrima: snaga plazme 5 W, helijum 4 slm (sa 0 sccm vodene pare), koncentracija H₂O unutar protočnog sistema 150 ppm

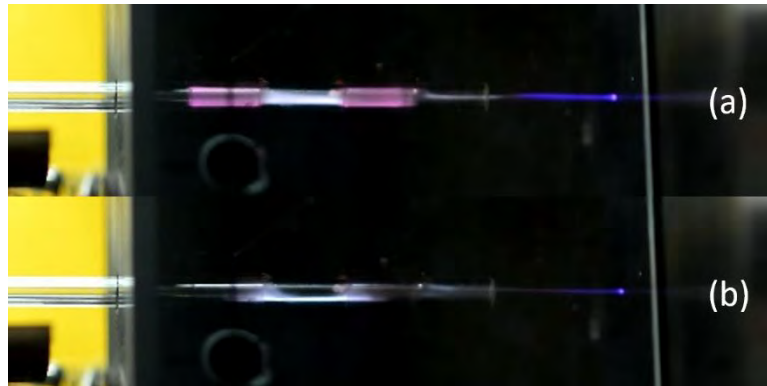


Slika 3.9 Poređenje dometa PAPS-a za dve različite eksperimentalne postavke sa i bez dodavanja vodene pare pomoću ispiralice. (Selaković *et al.*, publikacija u pripremi)

Grafik na Slici 3.9 pokazuje poređenje maksimalnog dometa PAPS-a za različite vrednosti koncentracije molekula H₂O izražene u ppm. Domet PAPS-a meren je od ruba staklene cevčice do vrha pražnjenja plazmenog mlaza koji se prostire u ambijentalni vazduh. Maksimalan domet je primećen za koncentraciju H₂O od 400 ppm (crna kriva). Tokom prosušivanja protočnog sistema radnog gasa pod uticajem proticanja helijuma, domet PAPS se postepeno smanjuje. Domet PAPS-a u okolini i ispod vrednosti od 100 ppm značajno se smanjuje. Minimalni opseg PAPS-a takođe predstavlja najnižu koncentraciju vlažnosti u graničnom području gde PAPS počinje da gubi svoj prvobitni oblik (ispod 30 ppm).

Da bi se proverila konzistentnost dužine električnog pražnjenja koje se prostire izvan staklene cevčice plazmenog mlaza, izvršili smo dodatna merenja. Prvo se protočni sistem radnog gasa osuši, a zatim se doda 1000 ppm molekula vode uz pomoć ispiralice. Nakon toga se demontira grana sa ispiralicom iz protočnog sistema i može se primetiti brzo sušenje protočnog sistema i izduženje električnog pražnjenja koje se prostire u ambijent laboratorije na vrednost sličnoj kao u delu eksperimenta gde nije korišćena ispiralica. Do ove pojave dolazi veoma brzo tj. nije potrebno čekati da se sistem prosuši da bi se plazma „izdužila“. Odgovor je najverovatnije u činjenici da veštački dodati molekuli vode u protoku helijuma ispunjavaju homogeno radni gas duž celog volumena mlaza. Nakon gašenja dotoka navlaženog helijuma momentalno se uspostavlja režim u kojem nema molekula vode već se povećava proizvodnja pobuđenog helijuma koji Penningovom jonizacijom utiče na pražnjenje a samim tim i na „izduženje“ mlaza. Zabeležili smo domet PAPS-a pri koncentraciji vode od 200 ppm za ovaj slučaj i označili ga na grafiku kao zelenu tačku. Preko 1000 ppm molekula vode, okolina je prezasićena, pa se pražnjenje gasi usled gašenja drugih vrsta molekulima vode.

Crvena kriva na grafiku prikazuje maksimalni domet PAPS-a za različite vrednosti H₂O, ali za slučaj kada smo vlažnost dodali veštački korišćenjem ispiralice. U poređenju sa crnom krivom, možemo primetiti da je domet PAPS-a kraći, a takođe je primećena promena boje pražnjenja. U pražnjenju plazmenog mlaza izvan cevčice a posebno unutar providnih elektroda boja pražnjenja imala je roze boju (Slika 3.10 (a)) dok je ta nijansa boje nestala kao i golim okom vidljiv intenzitet u slučaju kada je dodata vodena para (slika 3.10 (b)) (koncentracija molekula vode je u oba slučaja iznosila 400 ppm). Očigledno je da u zoni elektroda i strimera imamo dve različite situacije zbog različitog režima pražnjenja pri identičnoj koncentraciji H₂O. Pored različitih boja koje se mogu primetiti u zoni elektroda evidentna je i razlika u intenzitetu između dva primera.



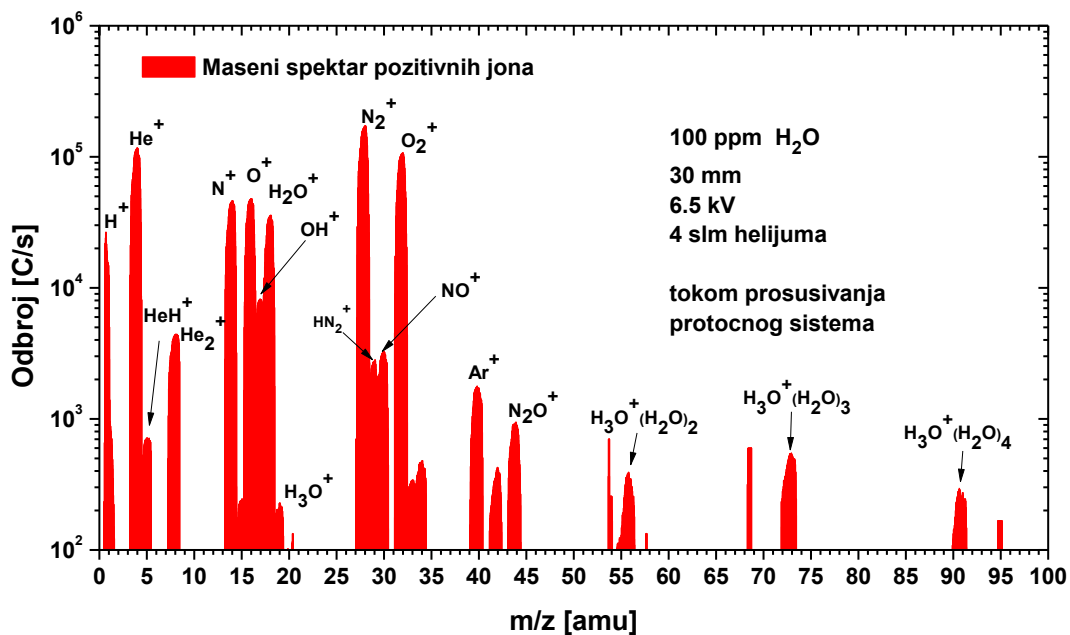
Slika 3.10 Poređenje različite boje i intenziteta električnog pražnjenja plazmenog mlaza u (a) postavci prosušivanja protočnog sistema gde je izmerena koncentracija H₂O bila 200 ppm i (b) sa veštački dodatom vodenom parom korišćenjem ispiralice nakon prosušivanja gde je koncentracija H₂O iznosila 200 ppm. (Selaković *et al.*, publikacija u pripremi)

Može se pretpostaviti da je efekat na različit domet PAPS-a pri istoj koncentraciji vode za prvi i drugi slučaj eksperimenta rezultat različite homogene raspodele molekula vode u zapremini protočnog sistema helijuma (usled sušenja prirodno navlaženog protočnog sistema adsorbovani molekuli vode se skidaju sa zidova pod uticajem helijuma usled opadanja koncentracije da bi popunili i unutrašnji deo protoka radnog gasa kao u slučaju veštačkog navlaživanja). Prethodno su Nikiforov i saradnici pokazali da primese molekularnih gasova utiču na smanjenje temperature elektrona (Nikiforov *et al.*, 2011). Tada se dalje utiče na proizvodnju i formiranje fotona i energijskih vrsta poput metastabilnog helijuma u pražnjenju. Metastabili helijuma igraju ključnu ulogu u procesima pobude, ali zbog gašenja pod uticajem molekula vode koncentracija metastabila pa samim tim i rezultujući intenzitet emisije opadaju. Drugi efekat je vezan za povećane gubitke elektrona usled pojačanog zahvata. Ovo će svakako zavisiti od promenjenog oblika funkcije raspodele po energijama koja mora da zadovolji uslov održavanja plazme a istovremeno je pod udarom snažnog zahvata elektrona na srednjim energijama.

Ako je formiranje metastabila helijuma otežano u električnom pražnjenju plazmenog mlaza u okolnom vazduhu pod uticajem medijuma sa homogenom raspodelom molekula vode koja se veštački dodaje smeši, ukupni intenzitet emisije i procesi pobude takođe će biti smanjeni. Stoga se može zaključiti da se dužina propagacije mlaza izvan cevčice za slučaj eksperimenta sa veštački dodatom vodom skraćuje pod uticajem ovog efekta.

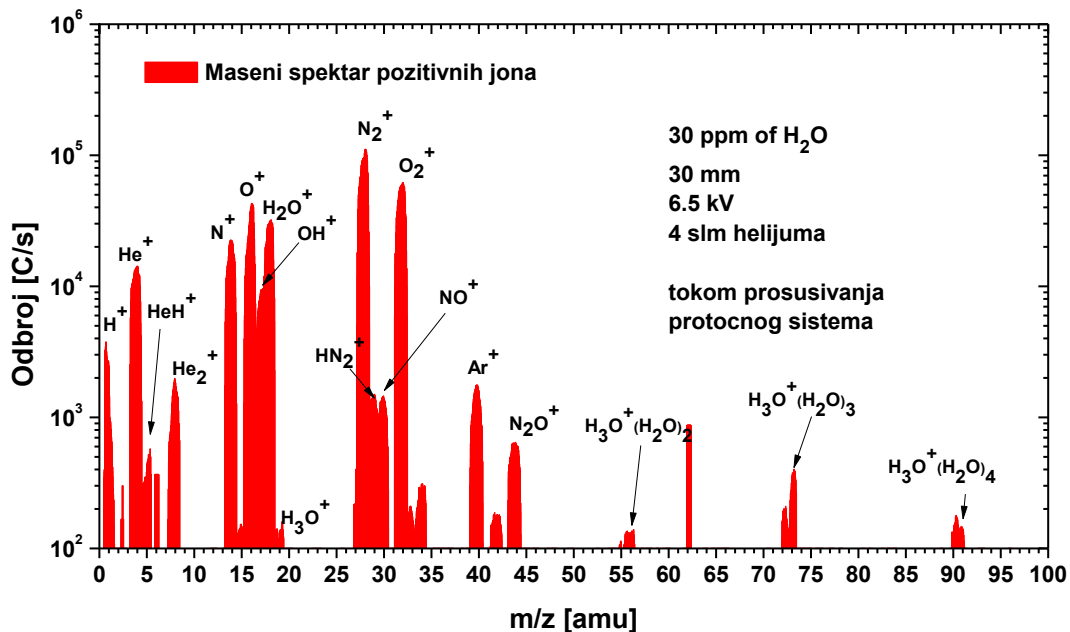
3.4 Masena spektrometrija – uticaj vlažnosti na masene spektre pozitivnih jona

U ovom delu istraživanja izvršeno je merenje masenog sastava pozitivnih jona plazmenog mlaza za iste eksperimentalne uslove koji su korišćeni za merenje intenziteta optičke emisije uz pomoć ICCD kamere. Pozitivni joni koje ovde predstavljamo dobijeni su u mešavini radnog gasa (helijuma), atmosferskog vazduha, kao i vlažnosti i nečistoća adsorbovanih na zidovima sistema protoka dovodnog gasa. Bilo je teško ponoviti uslove vlažnosti od 400 ppm unutar cevčice bez korišćenja ispiralice. Ambijentalni vazduh u laboratoriji u poređenju sa danom kada je izvršeno ICCD snimanje bio je mnogo suvlji. To je dovelo do činjenice da se koncentracija vode u protočnom sistemu plazmenog mlaza dosta brže smanjivala tako da pri vrednosti od 400 ppm nije bilo dovoljno vremena da se izvrše merenja masenim spektrometrom. Svi ostali parametri su uspešno ponovljeni.



Slika 3.11. Maseni spektar pozitivnih jona pri koncentraciji vodene pare od 100 ppm bez korišćenja veštački dodate vodene pare uz pomoć ispiralice. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.5 kV a protok helijuma je iznosio 4 slm. (Selaković et al., publikacija u pripremi)

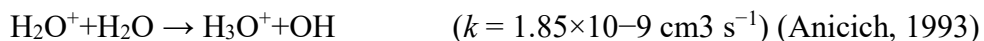
Uzimajući u obzir da helijum ima visok jonizacioni potencijal od 24.6 eV, dominantni pozitivni joni u spektrima potiču od vlage apsorbovane na zidovima protočnog sistema radnog gasa i ambijentalnog vazduha (Bruggeman *et al.*, 2010). Najzastupljeniji pozitivni joni izmereni za uslove koje smo koristili bili su He^+ , N_2^+ , O_2^+ , N^+ , O^+ , OH^+ , HN_2^+ i NO^+ . Upoređujući Slike 3.11 i 3.12, može se primetiti da se zbog isušivanja protočnog sistema pod uticajem helijuma najveća promena u ova dva spektra se primeti kod pada odbroja He^+ za red veličine kao i značajan pad odbroja H_3O^+ i njegovih klastera $H_3O^+(H_2O)_2$ i $H_3O^+(H_2O)_4$.



Slika 3.12. Maseni spektar pozitivnih jona pri koncentraciji vodene pare od 30 ppm bez korišćenja veštački dodate vodene pare uz pomoć ispiralice. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.5 kV a protok helijuma je iznosio 4 slm. (Selaković et al., publikacija u pripremi)

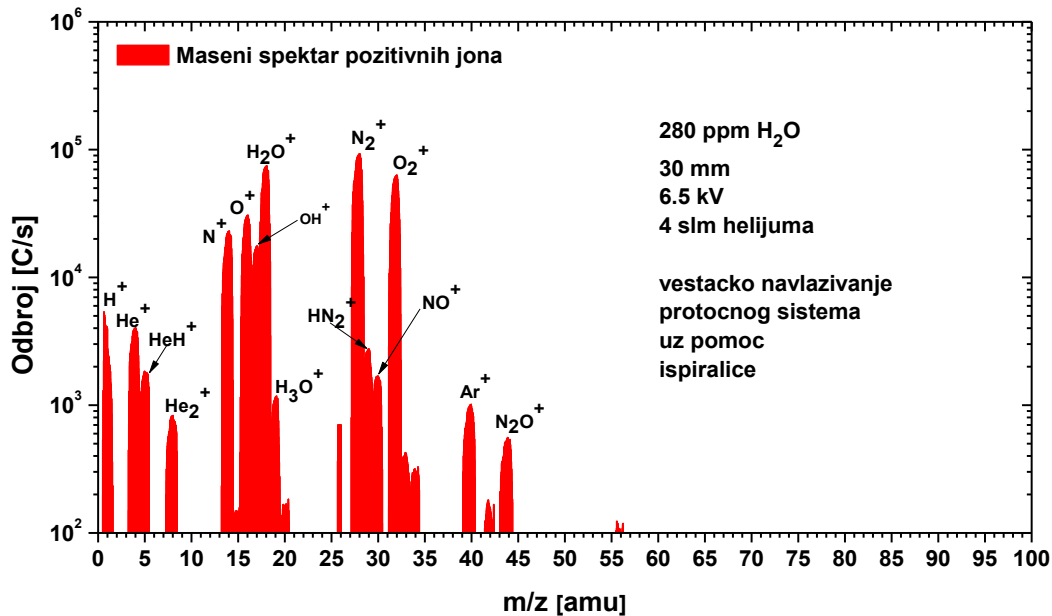
Nakon prosušivanja protočnog sistema u eksperimentalnu postavku je dodata grana u protočni sistem radnog gasa gde se helijum meša sa vodenom parom korišćenjem ispiralice da bi se ponovilo pražnjenje u kojem ponovo formira PAPS. Maseni spektri pri veštački dodatoj vodenoj pari pokazuju značajnu razliku u masenom spektru pozitivnih jona u poređenju sa slučajem gde nije korišćena ispiralica. Prvo što se primeti je porast vrednosti signala H₂O⁺, koji postaje najdominantniji pozitivni jon usled veštački povećane koncentracije vode u He.

Hidratacija jona pod uticajem smeše u kojoj je helijum homogeno zasićen molekulima vode dovodi do rasta određenih vrsta (H₃O⁺, OH⁺, HN₂⁺, HeH⁺) usled dodavanja još veće koncentracije vlažnosti u sistem. Ako se posmatra jon H₃O⁺ za tri različite koncentracije vode (slike 3.13-15), možemo pretpostaviti da on potiče iz sledeće reakcije

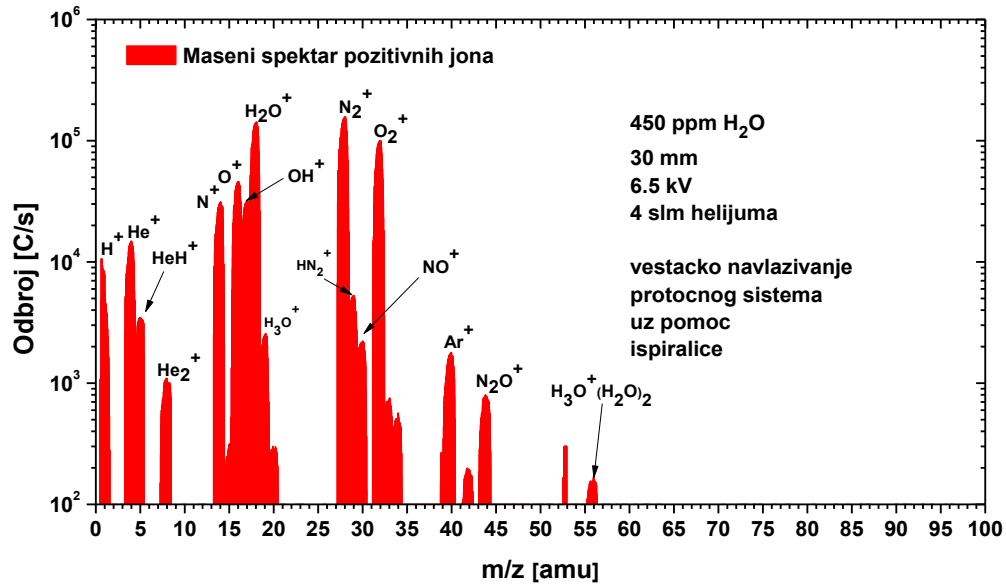


Rast relativne stope broja H₃O⁺ može uticati na smanjenje produkcije vodenih klastera, što su takođe primetili Bruggeman i saradnici. (Bruggeman *et al.*, 2010).

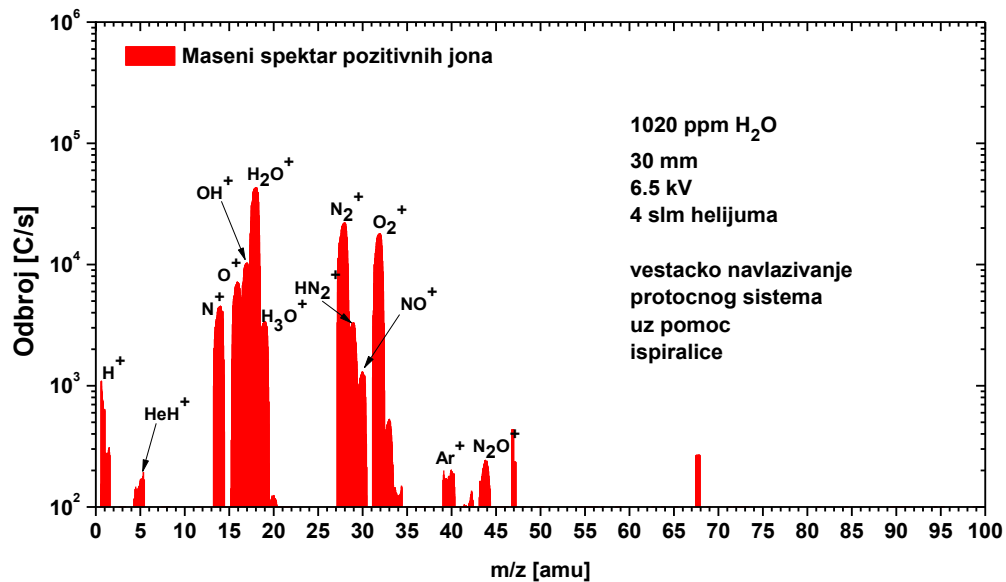
Uloga pozitivnih jona u primeni plazme za tretiranje bioloških uzoraka još uvek nije najbolje objašnjena, kao ni za radikale, ali poznavanje njihovog sastava u masenom spektru kao energijskih čestica može pomoći u fizičko-hemijskim modelima u izračunavanju reakcija za proizvodnju radikala.



Slika 3.13 Maseni spektar pozitivnih jona pri koncentraciji vodene pare od 280 ppm sa korišćenjem veštački dodate vodene pare uz pomoć ispiralice. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.5 kV a protok helijuma je iznosio 4 slm. (Selaković *et al.*, publikacija u pripremi)

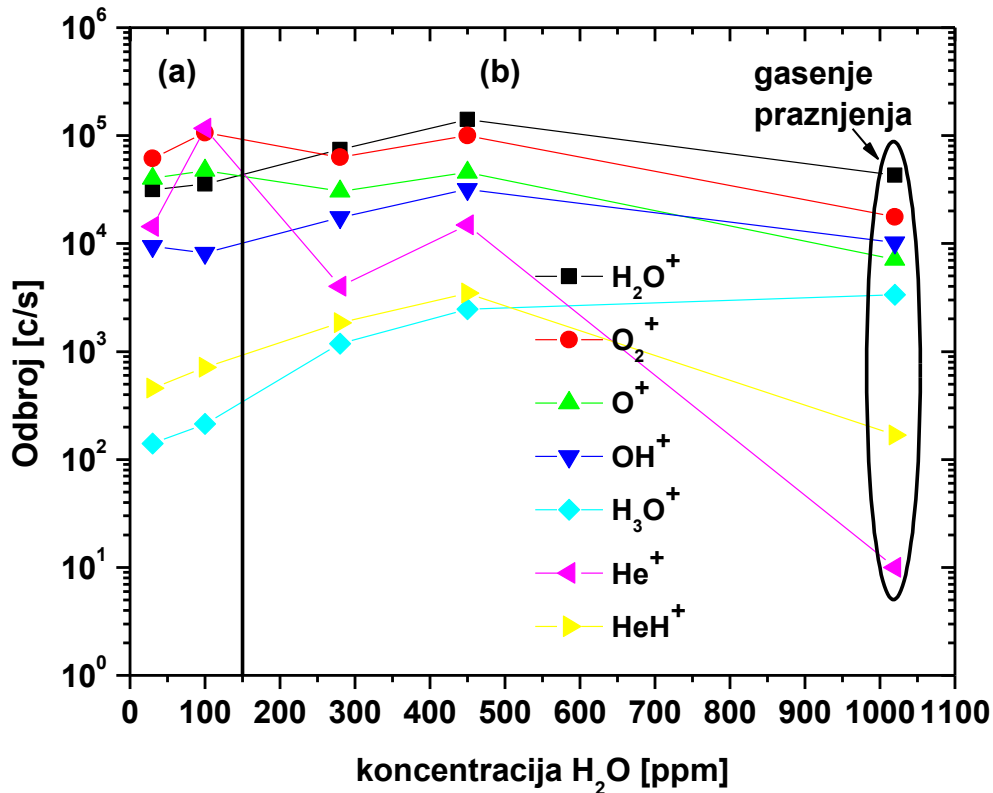


Slika 3.14 Maseni spektar pozitivnih jona pri koncentraciji vodene pare od 450 ppm sa korišćenjem veštački dodate vodene pare uz pomoć ispiralice. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.5 kV a protok helijuma je iznosio 4 slm.



Slika 3.15 Maseni spektar pozitivnih jona pri koncentraciji vodene pare od 1020 ppm sa korišćenjem veštački dodate vodene pare uz pomoć ispiralice. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.5 kV a protok helijuma je iznosio 4 slm.

Povećavanjem koncentracije vode u helijumu smanjuje se proizvodnja He^+ jona što dovodi do povećanja jona H_3O^+ i HeH^+ . To se najbolje može videti na Slici 3.16 gde za najnižu vrednost helijuma (1100 ppm H_2O) H_3O^+ dostiže svoj maksimum. U ovom trenutku vrednosti odbroja svih ostalih vrsta padaju usled gašenja pražnjenja pod uticajem velike koncentracije H_2O .



Slika 3.16. Odbroj pozitivnih jona u funkciji od koncentracije H_2O u He (a) prosušivanje protočnog sistema bez koršćenja ispiralice i (b) veštačko dodavanje vlažnosti u protočni sistem sa korišćenjem ispiralice. (Selaković *et al.*, publikacija u pripremi)

Iz dobijenih spektara za dva različita slučaja može se reći da veštački dodata voda u protočni sistem radnog gasa značajno utiče na promenu u masenom sastavu plazmenog mlaza. Veća prisutnost/homogenost molekula vode u volumenu mlaza utiče na veći broj procesa koji favorizuje proizvodnju pozitivnih jona vode H_2O^+ i njoj srodnih vrsta kao što je H_3O^+ , OH^+ . Pokazano je da povećanje veštački dodate vlažnosti ne povećava već smanjuje proizvodnju klastera. Suprotno ovoj pojavi raste signal njihovog roditeljskog jona H_3O^+ koji je u prvom slučaju prosušivanja prirodno absorbovane vlažnosti imao značajno nižu vrednost.

3.5. Zaključak

U ovom poglavlju opisan je uticaj vlažnosti unutar protočnog sistema radnog gasa (helijuma) plazmenog mlaza na slobodno prostiranje električnog pražnjenja u okolnu sredinu i na formiranje PAPS-a. Videli smo da se koncentracija izmerenog molekula vode u protoku dobija na dva različita načina:

1. sušenjem vode adsorbovane na zidovima polietilenskih cevi i staklenoj cevčici pod uticajem protoka radnog gasa (helijuma),

2. mešanjem helijuma i vodene pare dobijene iz destilovane vode unutar ispiralice.

Iz prikazanih rezultata možemo izvući nekoliko zaključaka:

- apsorbovana vlaga na zidovima protočnog sistema plazmenog mlaza se postepeno suši usled protoka radnog gasa (helijum brzine protoka od 4 slm);
- usled smanjene vlažnosti (samo 20 ppm unutar protočnog sistema radnog gasa) PAPS gubi svoj oblik i plazma se prostire u obliku razmrljanog traga koji podseća na difuzni kontinualni režim;
- veštačko dodavanje vodene pare u protočni sistem radnog gasa vraća formu PAPS-a;
- domet PAPS-a sa veštački dodatom vodenom parom je kraći u poređenju sa prethodnom konfiguracijom gasa;
- boja pražnjenja je različita za dve konfiguracije: 1. je roze sa jačim intenzitetom emisije svetlosti, a 2. je svetlo ljubičasta sa manjim intenzitetom emisije svetlosti.

Veštačkim dodavanjem vodene pare obnovljen je oblik PAPS, ali razlika u posmatranom pražnjenju kao i masenom spektru u oba dela eksperimenta je više nego očigledna. Rezultat pokazuje da otkrivene vrednosti koncentracije molekula adsorbovanih na zidovima protočnog sistema i veštački dodata vodena para u protok helijuma različito utiču na pražnjenje. Pretpostavka je da veštačko dodavanje vlage u protok helijuma dovodi do veće homogenosti vode unutar zapremine protočnog sistema radnog gasa. Shodno tome, javlja se kraći domet PAPS-a jer molekuli vode smanjuju proizvodnju metastabila helijuma i očigledno utiču na različit maseni sastav plazme.

4. SINHRONIZOVANA MERENJA VREMENSKE EVOLUCIJE PAPS-A I POZITIVNIH JONA

4.1 Uvod

Decenija koja je za nama doživela je ekspanziju u istraživanju plazmenih mlazova najrazličitijih konfiguracija sa ciljem najboljeg mogućeg iskorišćenja u različitim vrstama primene. Fenomen „plazma metka“ ili pulsni strimer na atmosferskom pritisku skr. PAPS, koji se javlja kod plazmenih mlazova, je do sada objašnjavan na više načina korišćenjem eksperimenata i modela. Međutim, da bi se stekla šira slika plazmenog mlaza, izvora električnog pražnjenja na atmosferskom sa velikim potencijalom za primene u biomedicini, neophodno je odrediti njegov sastav.

U ovoj disertaciji posmatraćemo evoluciju PAPS-a i pokušati sinhronizacijom ICCD kamere i masenog spektrometra da odredimo sastav „plazma metka“. PAPS se može formirati u maksimumima pozitivnih i negativnih poluperioda naponskog ciklusa kod plazmenih mlazova koji se pobuđuju izvorom naizmenične struje. U slučaju plazmenog mlaza koji istražujemo PAPS se javlja samo tokom pozitivnog poluperioda naponskog ciklusa od 12.5 μ s. Da bi se dobili dobri signali vremenski razloženih snimanja mora se uzeti u obzir više faktora:

- Visok odbroj čestica,
- Stabilan rad izvora plazme,
- Što manja perturbacija plazme pod uticajem masenog spektrometra koja može biti dvojaka: uticaj uzemljenog orifisa kao dodatne elektrode pri previše bliskom rastojanju i uticaj napajanog orifisa kao dodatne elektrode.

U preliminarnim eksperimentima masene spektrometrije plazmenog mlaza ustanovljeno je da je doprinos neutrala stvorenih u plazmenom mlazu nizak a s obzirom da činjenicu da se pri merenjima neutrala mora voditi računa i o prinosu gasa iz ambijenta kao i prinosu unutar masenog spektrometra odlučili smo da je najbolje snimiti vremenski razložene jone kod kojih je ovaj problem ne postoji jer se ne koristi jonizaciona komora pri akviziciji podataka. Što se tiče negativnih jona njihova akvizicija je moguća isključivo korišćenjem napajanja na orifisu i dobar odbroj se dobija pri rastojanjima kraćim od onog za koji smo se odlučili zbog praćenja kretanja PAPS-a. Na kraju su pozitivni joni odabrani kao najbolje moguće rešenje jer se njihova visoka produkcija u odbrojima pokazala kao najbolja.

Vremenski razloženo snimanje jona sinhronizovano TTL signalom može nam dati uvid u rast, maksimum i opadanje odbroja pozitivnih jona. Taj isti TTL signal koristiće se za sinhronizaciju ICCD kamere i strujno-naponskih signala što će omogućiti praćenje najzastupljenijih vrsta iz masenog spektra pozitivnih jona i ICCD snimaka PAPS-a. Eksperimentalna shema je prikazana i opisana u poglavlju Eksperimentalna postavka – Plazmeni mlaz. Pre odabranih rezultata biće prikazan proračun za kašnjenje jona prilikom masene analize.

4.2 Vreme preleta (*eng.* Time of flight - TOF) jona

S obzirom na činjenicu da joni predstavljaju pozitivno naelektrisane čestice atoma i/ili molekula, koji imaju međusobno različitu masu i energiju, različita vremena preleta se moraju uzeti u obzir prilikom snimanja vremenske evolucije masenim spektrometrom. Ukupno vreme preleta jona ima sledeći oblik:

$$t_{ukupno} = t_{atm} + t_{vak} + TOF$$

gde je t_{atm} vreme za koje jon pređe put od izvora plazme do masenog spektrometra, t_{vak} je vreme tokom kog jon provede u preletu unutar masenog spektrometra kroz nizak i visok vakuum (dve međufaze sistema diferencijalnog pumpanja gde postoji gradijent pritiska). TOF predstavlja vreme koje jon preleti

kroz najduži deo masenog spektrometra (maseno-energijski analizator) gde je pritisak najniži (ultra-visok vakuum).

Ovde neće biti prikazane apsolutne vrednosti kašnjenja jona već će biti izračunate korekcije razlika u kašnjenjima između jona različitih masa (lakše čestice su brže u odnosu na teže pa sve čestice ne stižu u isto vreme na detektor). Jiang i saradnici su ustanovili da vremena preleta t_{atm} i t_{vak} prema dobroj aproksimaciji ne zavise od vrsta čestica te se za ovaj tip računa ova vremena mogu zanemariti (Jiang *et al.*, 2019).

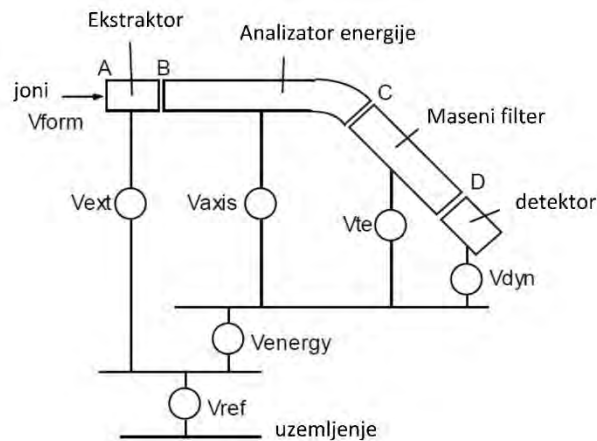
Na kraju t_{ukupno} se računa kao:

$$t_{ukupno} = TOF$$

Vremena preleta različitih jona kroz instrument masenog spektrometra uzeta su u obzir pri određivanju njihove vremenske korelacije u odnosu na pobudni signal. Unete su korekcije prema algoritmu dobijenom od HIDEN Analytical a koji su koristili McKay i saradnici pri korekciji vremenske evolucije jona koju su snimili sa istim tipom masenog spektrometra (McKay *et al.*, 2013) koji se koristi u ovoj disertaciji.

4.3 TOF – vreme preleta u maseno-energijskom analizatoru

U ovoj glavi biće opisan algoritam za računanje vremena preleta jona u maseno energijskom analizatoru. Ovaj proračun dobijen je od HIDEN Analytical kompanije čiji maseni spektrometar je korišćen za masenu analizu prezentovanih rezultata u ovoj disertaciji (Proračun vremena preleta u maseno-energijskom analizatoru - <http://www.hiddenanalytical.com/>). Za određene režime rada važno je znati koliko je vreme preleta potrebno jonima da prođu kroz maseno-energijski analizator (Slika 4.1).



Slika 4.1 Shema maseno-energijskog analizatora EQP. (www.hiddenanalytical.com)

Analizator se može podeliti u četiri oblasti za analizu vremena preleta:

- oblast ekstraktora; dužina s_{ext} , vreme preleta t_{ext}
- oblast energetskog analizatora; dužina s_{en} , vreme preleta t_{en}
- oblast masenog filtera; dužina s_{mass} , vreme preleta t_{mass}
- oblast detektora; dužina s_{det} , vreme preleta t_{det}

Joni prolaze kroz svaku od ovih oblasti sa različitim energijama.

Ukupno vreme preleta kroz analizator je (HIDEN Analytical proračun, 2013):

$$t = t_{ext} + t_{en} + t_{mass} + t_{det} \quad (1)$$

Ovo vreme preleta odnosi se na jone koji imaju jednostruko naelektrisanje. U slučajevima kada postoje joni sa višestrukim naelektrisanjem, efektivna masa jona je stvarna masa podeljena brojem naelektrisanja.

Tokom prolaska jona kroz analizator, on će imati sledeće energije:

U tački I joni nastaju sa energijom eV_{form}

U tački A jon ima energiju $e(V_{form} - V_{ext} - V_{ref})$

U tački B jon ima energiju $e(V_{form} - V_{axis} - V_{energy} - V_{ref})$

U tački C jon ima energiju $e(V_{form} - V_{te} - V_{energy} - V_{ref})$

U tački D jon ima energiju $e(V_{form} - V_{dyn} - V_{energy} - V_{ref})$

Da bi analizator mogao da prenese jon sa ekstraktora na detektor, potrebno je imati:

$$V_{form} = V_{energy} + V_{ref}$$

tako da energije u označenim tačkama postaju:

U tački A $e(V_{energy} - V_{ext})$

U tački B $-eV_{axis}$

U tački C $-eV_{te}$

U tački D $-eV_{dyn}$

Kako su naelektrisanja e i naponi V_{axis} , V_{te} i V_{dyn} uvek suprotnih znakova, energije se mogu opisati kao:

U tački B $|eV_{axis}|$

U tački C $|eV_{te}|$

U tački D $|eV_{dyn}|$

Znaci i opsezi napona zadati su internim parametrima dobijenih podešavanjem (*eng.* tuning) instrumenta koje je neophodno izvršiti za svaki novi tip izvora plazme. (*eng.* Global enviroment).

Oblast ekstraktora

Pretpostavlja se da joni u ovu oblast ulaze sa energijom koja potiče od načina formiranja ili potencijala u kome nastaju. Potencijal na ulazu u ovu oblast je zbir napona na ekstraktoru V_{ext} plus referentni napon V_{ref} , izlazni potencijal je zbir napona ose V_{axis} , napona energije V_{en} i referentnog napona V_{ref} (pogledati dijagram napona). Ova oblast se posmatra kao prostorni pomeraj (*eng.* drift space) (koji će doprineti maloj grešci) kroz koji se joni ubrzavaju, jer u suprotnom analiza postaje veoma komplikovana.

$$V_{in} = V_{ref} + V_{ext}$$

Ukoliko se jon formira sa energijom u potencijalu V_{form} , tada će u ovu oblast ući sa energijom:

$$E_i = e(V_{form} - V_{in})$$

Tako da je

$$\frac{mV^2}{2} = e(V_{form} - V_{in})$$

Dajući ulaznu brzinu

$$u = \sqrt{\frac{2e(V_{form} - V_{in})}{m}}$$

Da bi jon prolazio kroz energijski filter u sledećoj oblasti, potrebno je da ima $|eV_{axis}|$ kinetičku energiju. Izlazna brzina iz ove oblasti (B) je

$$v = \sqrt{\frac{|2eV_{axis}|}{m}}$$

Ukoliko je dužina oblasti s_{ext} , može se reći

$$s_{ext} = \frac{u+v}{2} t_{ext} \quad \text{gde je } t_{ext} \text{ vreme preleta}$$

Dakle

$$t_{ext} = \frac{2s_{ext}}{\sqrt{\frac{2e(V_{form} - V_{in})}{m}} + \sqrt{\frac{|2eV_{axis}|}{m}}}$$

$$t_{ext} = \frac{s_{ext}\sqrt{2m}}{\sqrt{2e(V_{form} - V_{in})} + \sqrt{|2eV_{axis}|}}$$

Zamenom za V_{in} i V_{form}

$$t_{ext} = \frac{s_{ext}\sqrt{2m}}{\sqrt{e(V_{energy} - V_{ext})} + \sqrt{|eV_{axis}|}} \quad (2)$$

• Oblast energijskog analizatora

Joni preleću ovu oblast konstantnom brzinom.

Prolazeći kroz energijski analizator, joni moraju proći ovu oblast sa kinetičkom energijom od $|eV_{axis}|$, što daje brzinu

$$v_{en} = \frac{s_{en}}{t_{en}} = \sqrt{\frac{|2eV_{axis}|}{m}}$$

Vreme preleta

$$t_{en} = s_{en} \sqrt{\frac{m}{|2eV_{axis}|}} \quad (3)$$

- **Oblast masenog filtera**

Joni prolaze kroz ovu oblast sa energijom definisanom naponom energije preleta V_{te} koji se može podešavati. Njihovo vreme preleta je

$$t_{mass} = s_{mass} \sqrt{\frac{m}{|2eV_{te}|}} \quad (4)$$

- **Oblast detektora**

Joni ulaze u ovu oblast sa energijom od 3 eV i ubrzavaju se do potencijala detektora pre nego što udare u konvertujuću dinodu (1. dinoda). Za ovu analizu se uzima nulta ulazna energija, što ne izaziva značajnu grešku.

Pošto se joni pretvaraju u elektrone na prvoj dinodi, ubrzavaće ih samo V_{dyn} i sila koja deluje na jone je

$$\frac{|eV_{dyn}|}{s_{det}}$$

Što daje ubrzanje

$$f = \frac{|eV_{dyn}|}{ms_{det}}$$

koristeći

$$s = ut + \frac{ft^2}{2}$$

gde je s udaljenost a u početna brzina.

Pod pretpostavkom da je početna brzina jednaka nuli, zamenom se dobija

$$s_{det} = \frac{|eV_{dyn}|t_{det}^2}{2ms_{det}}$$

$$t_{det} = s_{det} \sqrt{\frac{2m}{|eV_{dyn}|}} \quad (5)$$

- **Vreme preleta**

Zamenom (2), (3), (4) i (5) u (1) dobija se:

$$t = s_{ext} \frac{\sqrt{2m}}{\sqrt{e(V_{energy} - V_{ext}) + \sqrt{|eV_{axis}|}}} + s_{en} \sqrt{\frac{m}{|2eV_{axis}|}} + s_{mass} \sqrt{\frac{m}{|2eV_{te}|}} + s_{det} \sqrt{\frac{2m}{|eV_{dyn}|}}$$

Tabela 4.1 Rastojanja s_n za prelet jona u oblastima sonde maseno-energijskog analizatora EQP.

Rastojanje	Standardna sonda	Produžena (321) sonda	Produžena (400) sonda	Produžena (600) sonda
s_{ext}	0.040 m	0.040 m	0.040 m	0.040 m
s_{en}	0.274 m	0.381 m	0.460 m	0.660 m
s_{mass}	0.200 m	0.200 m	0.200 m	0.200 m
s_{det}	0.016 m	0.016 m	0.016 m	0.016 m

Da bi se koristio ovaj izraz treba postaviti V_{energy} na ekvivalentni potencijal za energiju jona (npr. za 10 eV jon treba postaviti V_{energy} na 10 V). Uobičajena vrednost za V_{axis} je -40 V, za V_{te} je 3.0 V a za V_{dyn} je -1200 V. *Hiden* preporučuje da se napon ekstraktora podesi na nisku vrednost, pa bi za potrebe procene nula bila prihvatljiva. Masa je vrednost atomske jedinice mase (*eng.* amu) jona pomnožena sa masom protona ($1.6 \cdot 10^{-27}$). Kada se na podatke primenjuju dati izrazi dobijeni za prelet jona u EQP, potrebno je učitati vrednosti internih parametara instrumenta.

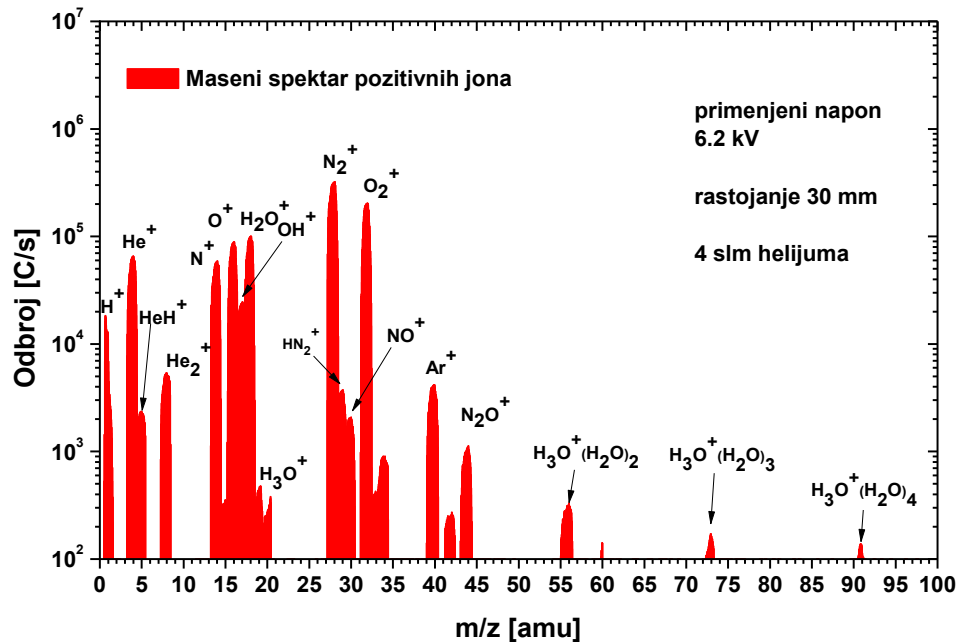
4.4 Rezultati vremenski evolucije PAPS-a

Prikažaćemo rezultate vremenske evolucije jona za najduže rastojanje pri kojem je plazmeni mlaz mogao da bude uzorkovan (30 mm). Pri tom rastojanju je uticaj masenog spektrometra bio sveden na minimum a kretanje PAPS-a je bilo moguće bez većih perturbacija (primećena je veća površina kao i manja brzina PAPS-a u odnosu na slučaj kada se plazmeni mlaz prostire slobodno u ambijent bez mete).

Eksperimentalni uslovi su bili sledeći:

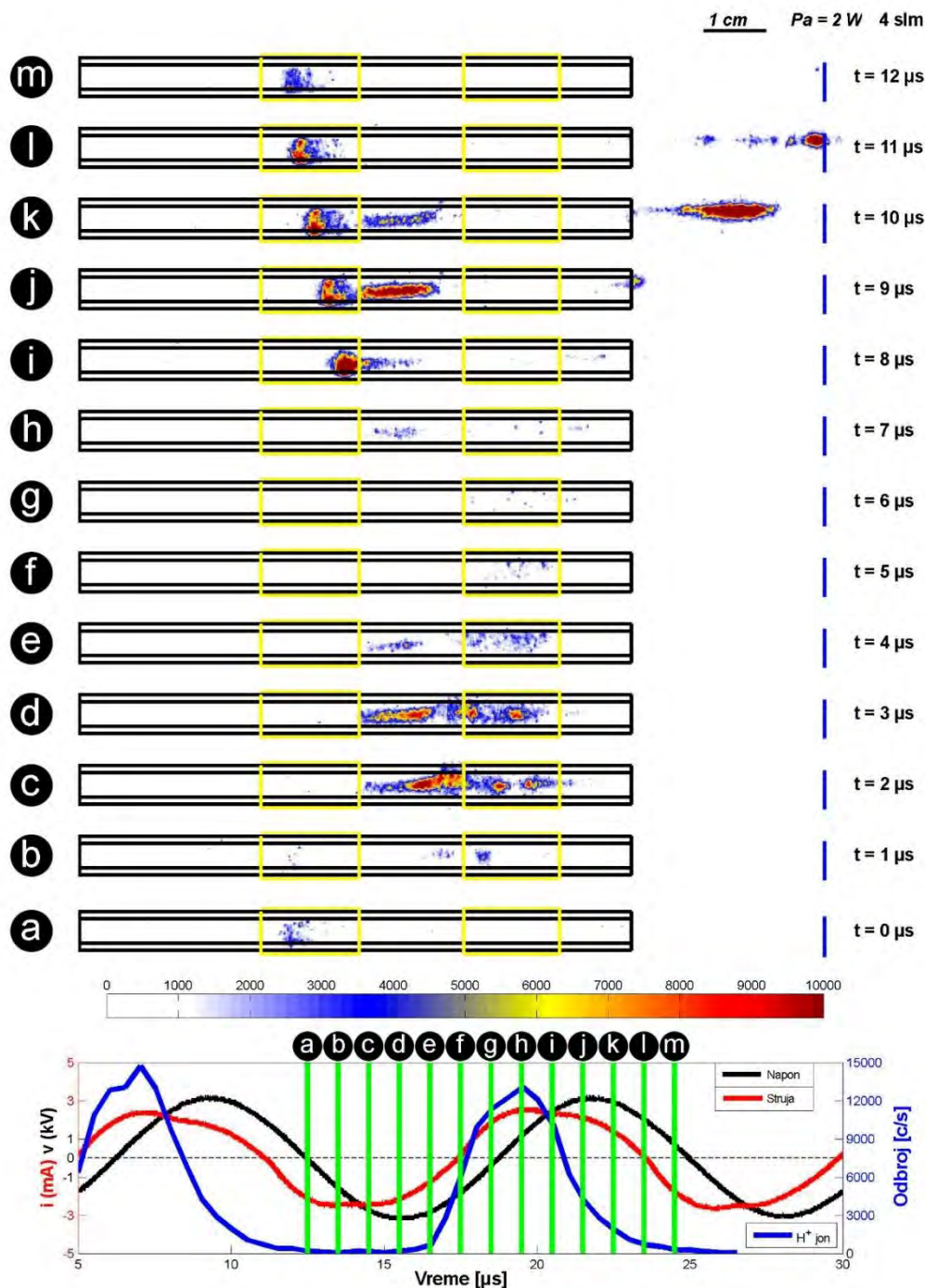
- varirane su 2 brzine protoka radnog gasa (helijuma): 3 slm i 4 slm,
- fiksiran je primenjeni napon na 6.2 kV jer pri tom naponu plazmeni mlaz nije bio perturbovan od strane masenog spektrometra.

Pre početka merenja sinhronizovanih snimaka vremenske evolucije jona i PAPS-a potrebno je uraditi identifikaciju masenog spektra pozitivnih jona. Na Slici 4.2 prikazan je tipičan primer masenog spektra pozitivnih jona dobijenih iz plazmenog mlaza pri sledećim eksperimentalnim uslovima: primenjeni napon na napajanoj elektrodi iznosio je 6.2 kV, rastojanje od masenog spektrometra iznosilo je 30 mm i brzina protoka helijuma je bila 4 slm. Najzastupljenije vrste jona su atomske i molekulske vrste azota (N_2^+ , N^+) i kiseonika (O_2^+ , O^+) praćene vrstama NO^+ , OH^+ , H^+ , H_3O^+ , N_2O^+ koje bi mogle da imaju potencijalnu ulogu u interakciji plazme sa ćelijom.



Slika 4.2 Maseni spektar pozitivnih jona. Rastojanje ruba cevčice plazmenog mlaza od inleta masenog spektrometra iznosio je 30 mm, primenjeni napon na napajanoj elektrodi iznosio je 6.2 kV a protok helijuma je iznosio 4 slm. (Selaković *et al.*, publikacija u pripremi)

ICCD kamera je korišćena za dobijanje vremenski razloženih snimaka prostiranja plazme unutar staklene cevčice, providnih elektroda i PAPS-a ispred inleta masenog spektrometra. Slike plazme dobijene tokom jednog perioda od 12.5 μ s prikazane su na Slici 4.3 Može se videti da se za rastojanje od 30 mm plazma ne prostire kontinualno, već je sačinjena iz niza paketa plazme tzv. PAPS-ova koji se formiraju u pozitivnom delu poluperioda signala napona i struje. Rezultati masene spektrometrije predstavljeni su za rastojanje od 30 mm. Fotografije su snimane pri koraku od 0.5 μ s, međutim radi bolje preglednosti predstavljen je manji broj fotografija sa korakom od 1 μ s. Da bi se dobila svaka pojedinačna fotografija korišćena je ekspozicija kamere od 2 μ s gde je širina gejta iznosila 25 ns. Korišćena je i integracija na čipu pomoću koje je 160 fotografija integrisano u jednu da bi se dobio prikazani intenzitet. Vrednost pojačanja na čipu iznosila je 250. Kao i kod ICCD snimaka i vremenski razloženi snimci pozitivnih jona su snimani sa korakom od 0.5 μ s. Širina gejta masenog spektrometra podešena je na 0.1 μ s, a toliko je iznosilo i kašnjenje gejta trigeru. Ekspozicija masenog spektrometra, tj. vreme za koje maseni spektrometar prikuplja podatke u jednoj tački, „eng. dwell time“ iznosila je 100 ms.

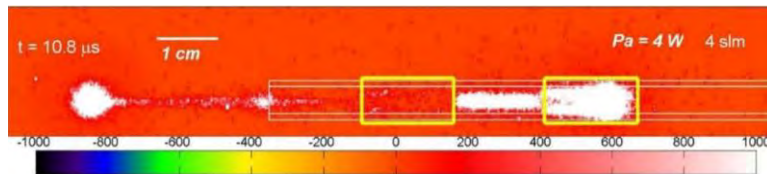


Slika 4.3 ICCD fotografije prostiranja PAPS-a sinhronizovane sa vremenskom evolucijom H^+ jona. Mala latinična slova na zelenim linijama označavaju poziciju trigovanja. Eksperimentalni parametri su bili: 30 mm rastojanje od plazmenog mlaza do masenog spektrometra, primenjeni napon je bio 6.2 kV, protok helijuma je iznosio 4 slm

Zelene linije na strujno-naponskim krivama predstavljaju pozicije trigera (označene malim latiničnim slovima (a-m) koja odgovaraju prikazanim vremenima ($0 \mu\text{s}$ - $12 \mu\text{s}$)) TTL signala kojim su ICCD kamera, HPR60 maseni spektrometar i osciloskop sinhronizovani. Plava kriva postavljena je uporedno sa strujno-naponskim krivama i predstavlja vremensku evoluciju H^+ jona.

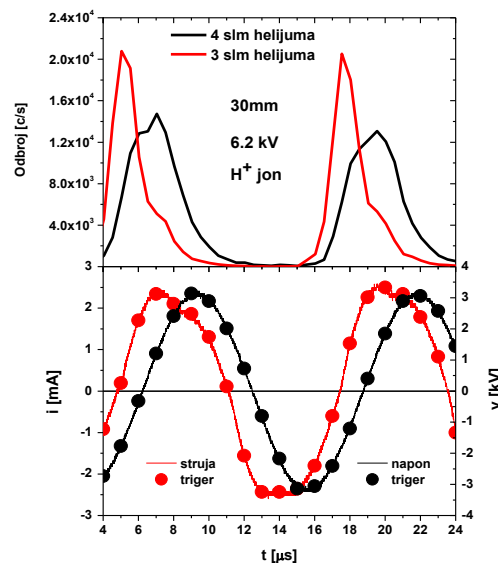
PAPS se pojavljuje, propagira i nestaje za pozitivan deo poluperioda u tačkama triger a, b, c, d, e, f i g kao i pri uzlaznim tačkama pozitivnog poluperioda h i i nije zapaženo formiranje PAPS-a. Pozitivan jon vodonika se u tačkama j, k, l i m smanjuje prema minimumu, ali u određenom procentu ulazi u sastav PAPS-a (tačka l predstavlja momenat kad je PAPS na otvoru masenog spektrometra i tu se vidi da H^+ nije u minimumu odbroja).

Maksimalna vrednost jona vodonika je u tački h (odbroj od oko 12000 c/s) i u toj tački ne postoji formacija PAPS-a. Ovo dovodi do toga da je veća zastupljenost vodonika u kanalu slabijeg intenziteta plazmenog mlaza. Ovaj kanal spaja glavu PAPS-a sa električnim pražnjenjem u međuelektrodnoj oblasti. Pojavu kanala slabijeg intenziteta u svojoj studiji pokazali Gherardi i saradnici Slika 4.4 (Gherardi et al., 2015.) (ovaj kanal se na Slici 4.3 ne primeti zbog skaliranja koje je izvršeno radi lakšeg praćenja kretanja PAPS-a)..



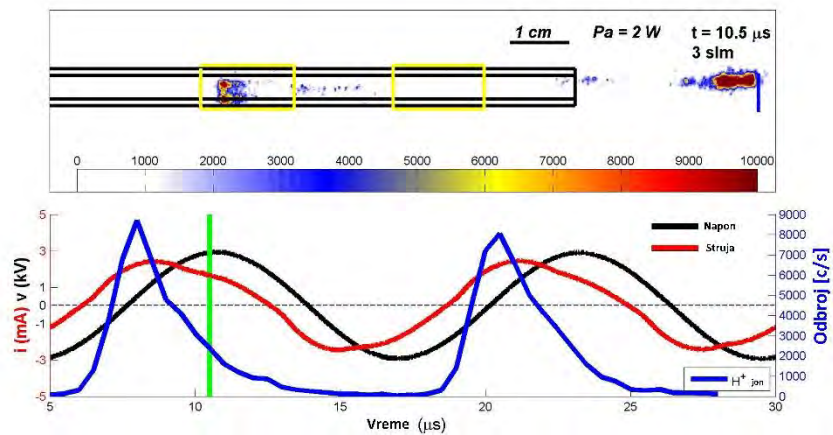
Slika 4.4 ICCD snimak PAPS-a spojenog kanalom sa električnim pražnjenjem između međuelektrodne oblasti. (Gherardi et al., 2015.)

Na slici 4.5 prikazano je poređenje vremenski razloženih jona vodonika pri istim eksperimentalnim uslovima gde je variran samo protok helijuma (3 slm i 4 slm). Osim što pri manjoj brzini protoka radnog gasa pozitivni jon vodonika dostiže veći maksimum može se primetiti i razlika u fazi gde jon pri nižem protoku kasni za 1.5 μs . Možemo pretpostaviti da je za razliku kašnjenje zadužen radni gas koji pri većem intenzitetu protoka „gura“ pozitivne jone vodonika ispred u odnosu na niži protok.



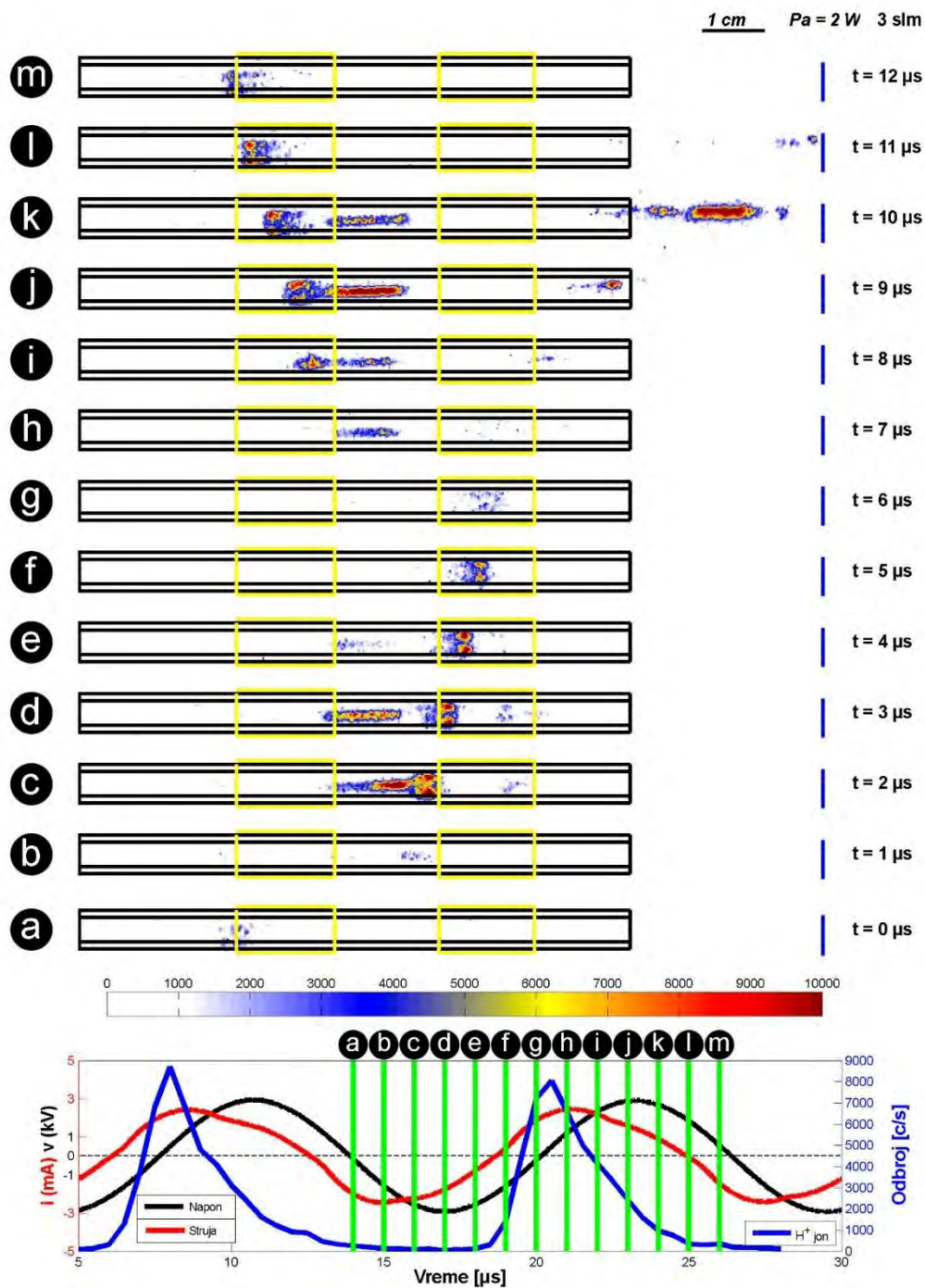
Slika 4.5 Poređenje vremenske evolucije H^+ jona (gore) sinhronizovanih sa strujno-naponskim signalima (dole) za dva različita protoka radnog gasa 3 slm i 4 slm; ostali eksperimentalni parametri su bili konstantni: primenjeni napon od 6.2 kV, 30 mm rastojanje između plazmenog mlaza i masenog spektrometra.

Na slici 4.6 (3 slm) prikazan je trenutak vremenske evolucije pražnjenja u kojem PAPS dodiruje otvor masenog spektrometra i ovaj snimak je sinhronizovan sa vremenski razloženim H^+ jonom. Može se primetiti da u trenutku kada je triger na maksimumu pozitivnog dela naponskog signala odbroj jona je oko 2000 c/s i opada ka svom minimumu.

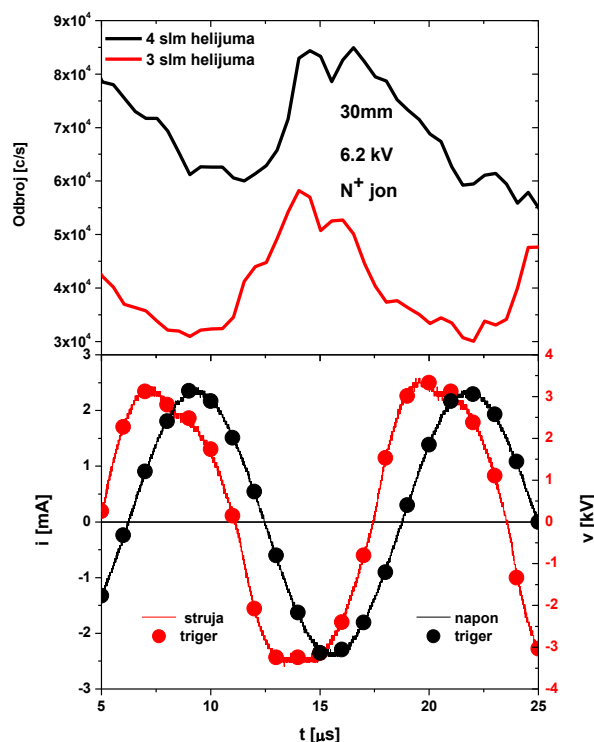


Slika 4.6 Sinhronizovan snimak vremenske evolucije H^+ pozitivnog jona i PAPS-a za vreme trigera 10.5 μs (zelena linija) (Selaković *et al.*, publikacija u pripremi)

Sinhronizovani ICCD snimci sa jonom vodonika i strujno-naponskim signalima za protok helijuma od 3 slm prikazani su na Slici 4.7. Zbog preglednosti i u ovom slučaju su prikazane vrednosti sa korakom od 1 μs . U poziciji I (11 μs) može se primetiti PAPS koji nestaje (zbog toga smo prikazali međukorak na prethodnoj slici) i osim toga može se konstatovati da su razlike vremenske evolucije pražnjenja prikazanog za dva različita protoka minimalne. U tački k se u oba slučaja pojavljuje PAPS „izduženog“ oblika.



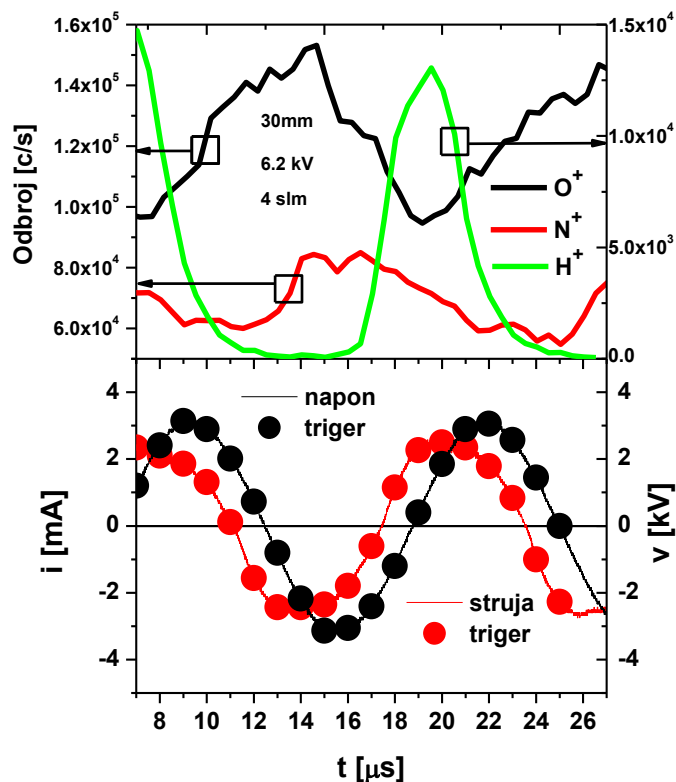
Slika 4.7 ICCD fotografije prostiranja PAPS-a sinhronizovane sa vremenskom evolucijom H^+ jona. Mala latinična slova na zelenim linijama označavaju poziciju trigerovanja. Eksperimentalni parametri su bili: 30 mm rastojanje od plazmenog mlaza do masenog spektrometra, primenjeni napon je bio 6.2 kV, protok helijuma je iznosio 3 slm (Selaković *et al.*, publikacija u pripremi)



Slika 4.8 Poređenje vremenski evolucije N^+ jona (gore) sinhronizovanih sa strujno-naponskim signalima (dole) za dva različita protoka radnog gasa 3 slm i 4 slm; ostali eksperimentalni parametri su bili konstantni: primenjeni napon od 6.2 kV, 30 mm rastojanje između plazmenog mlaza i masenog spektrometra. (Selaković *et al.*, publikacija u pripremi)

Za razliku od „lakog“ hemijskog elementa, pozitivnog jona vodonika, kod atomskog azota (Slika 4.8) je uticaj vrednosti protoka na kašnjenje između vremenski razloženih jona manjeg intenziteta. Takođe, u slučaju atoma azota, odbroji pri većoj brzini protoka (4 slm) radnog gasa su za 3×10^4 veći od odbroja pri manjoj brzini (3 slm). Što se tiče same sinhronizacije sa strujno-naponskim signalima može se primetiti da intenzitet odbroja N^+ jona ima svoj maksimum u minimumu vrednosti strujno-naponskih signala. Iz ovoga sledi da je u sastavu PAPS-a, tačka I, prisutna značajna koncentracija atoma ali je maksimalna izmerena vrednost u sastavu „repa“ tj. kanala nižeg intenziteta emisije (minimalna vrednost ne pada na nulu kao kod vodonika, već je reda veličine 10^4 c/s).

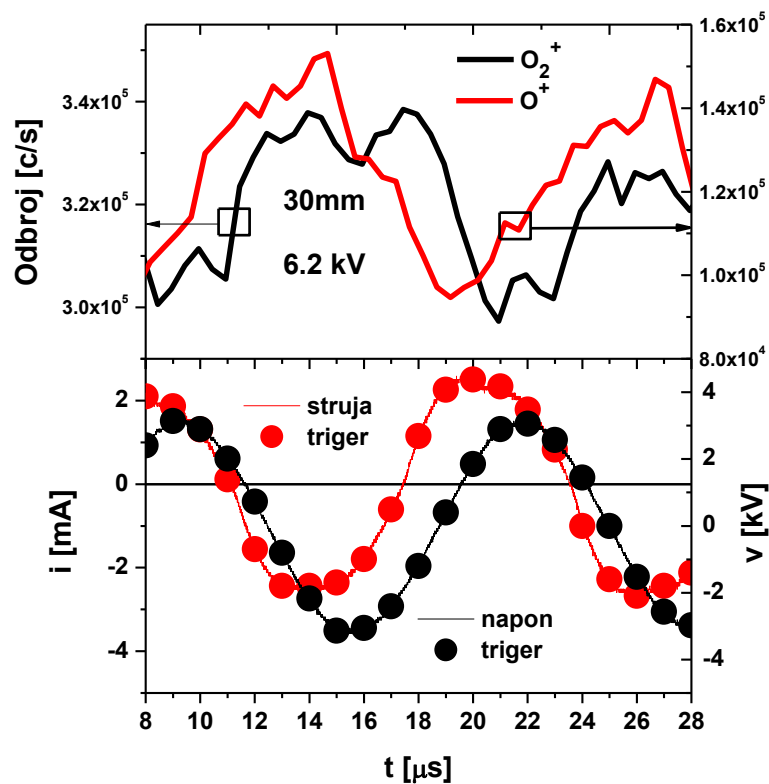
Na Slici 4.9 prikazani su uporedni snimci vremenske evolucije atomskih vrsta pozitivnih jona H^+ , N^+ i O^+ sinhronizovanih sa strujno-naponskim signalima. Iz dobijenih merenja se vidi da jon atomske vrste kiseonika kasni $2.7 \mu s$ za jonom atomske vrste azota i u kontra-fazi je sa jonom atomske vrste vodonika. U trenutku kada PAPS dodiruje otvor masenog spektrometra, tačka I (crvena tačka triger) koja je u blizini nule struje na slici 4.7), jon je u uzlaznoj putanji odbroja i njegova koncentracija u sastavu „plazmenog metka“ je do sada najdominantnija ($\sim 1.3 \times 10^5$ c/s). u odnosu na druge atomske vrste. Kao i kod jona atomske vrste azota i kod kiseonika minimum ne pada na nulu. Pretpostavka je da kod težih čestica ne postoji dovoljno vremena da koncentracija padne na nizak nivo do sledećeg ciklusa, kao što je to slučaj sa vodonikom zbog razlike u masi.



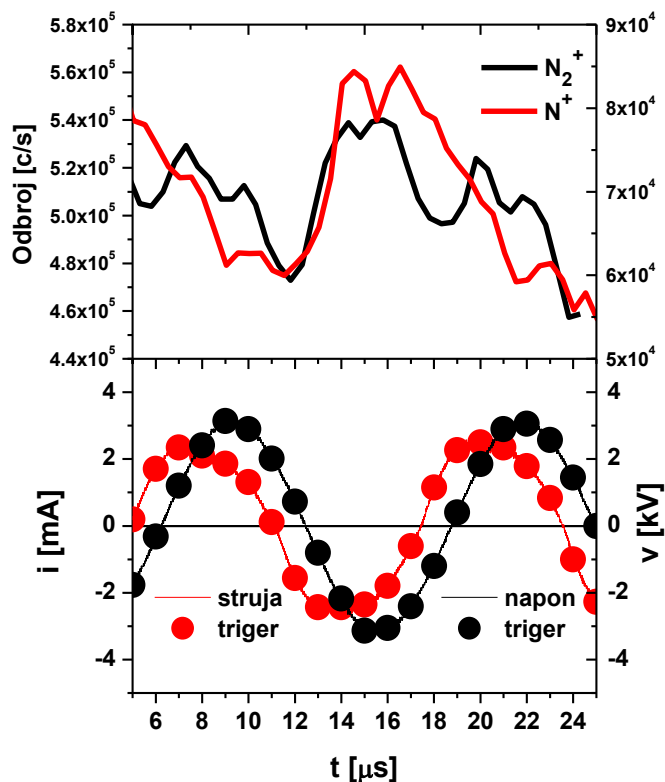
Slika 4.9 Poređenje vremenske evolucije pozitivnih jona atomskih vrsta H^+ , N^+ i O^+ (gore) sinhronizovanih sa strujno-naponskim signalima (dole) za protoka radnog gasa od 4 slm, primenjeni napon od 6.2 kV, 30 mm rastojanje između plazmenog mlaza i masenog spektrometra. (Selaković *et al.*, publikacija u pripremi)

Na slici 4.10 prikazani su signali pozitivnih jona O_2^+ i O^+ (Slika 4.10 (gore)) i talasni oblici primenjenog napona i struje (Slika 4.10 (dole)). Vidimo da signali za jone nisu u fazi i da O^+ jon kasni skoro $2 \mu\text{s}$. Oba ova jona su istovremeno van faze sa trenutnim signalima struje i napona, a O^+ je sa njim skoro u kontra fazi. Odbroj molekulske vrste jona kiseonika je oko 3 puta veći u odnosu na atomsku vrstu. Ono što još može da se primeti jeste da molekularni jon kiseonika ima dva izražena maksimuma gde je prvi maksimum u fazi sa maksimumom atomskog jona kiseonika.

Molekulski i atomski jon azota, N_2^+ i N^+ (Slika 4.11), prikazuje signale koji su u fazi s tom razlikom što molekularni jon ima dva izražena maksimuma nepravilnog oblika i signal ima širinu skoro celog perioda od $12.5 \mu\text{s}$.



Slika 4.10 Poređenje vremenske evolucije pozitivnih jona atoma i molekula O^+ i O_2^+ (gore) sinhronizovanih sa strujno-naponskim signalima (dole) za protoka radnog gasa od 4 slm, primenjeni napon od 6.2 kV, 30 mm rastojanje između plazmenog mlaza i masenog spektrometra. (Selaković *et al.*, publikacija u pripremi)



Slika 4.11 Poređenje vremenske evolucije pozitivnih jona atoma i molekula N^+ i N_2^+ (gore) sinhronizovanih sa strujno-naponskim signalima (dole) za protoka radnog gasa od 4 slm, primenjeni napon od 6.2 kV, 30 mm rastojanje između plazmenog mlaza i masenog spektrometra. (Selaković *et al.*, publikacija u pripremi)

4.5 Zaključak

U ovoj glavi opisana su merenja vremenske evolucije PAPS-a i najzastupljenijih jona od kojih je sačinjen korišćenjem sinhronizacije ICCD kamere i masenog spektrometra pomoću TTL signala i uporedo su praćeni i električni signali. Urađeno je poređenje za dva različita protoka radnog gasa na fiksnom rastojanju plazmenog mlaza u odnosu na maseni spektrometar i pri fiksnom primenjenom naponu. Dobijeni su sledeći zaključci:

- pored najzastupljenijih azotnih i kiseoničnih vrsta spektar plazmenog mlaza sačinjavaju i: He^+ , HeH^+ , NO^+ , OH^+ , H^+ , H_3O^+ , N_2O^+ kao i manje koncentracije vodenih klastera.
- Ustanovili smo da promena protoka ne utiče u značajnoj meri na prelet merenih jona osim u slučaju vodonika, koji kao lak hemijski element pod uticajem promene protoka biva fazno pomeren tj. primetno je kašnjenje,
- PAPS je u većoj ili manjoj meri sačinjen od svih prikazanih vrsta. Kod posmatranih čestica nije primećen maksimalan odbroj u trenutku kada PAPS dolazi na otvor masenog spektrometra.

5 MNOGOSTRUKI PLAZMENI MLAZ

5.1 Uvod

Ovo poglavlje ćemo posvetiti masenoj spektrometriji mnogostrukog plazmenog mlaza (MPM), izvora plazme na atmosferskom pritisku, koji je dizajniran i konstruisan u Istraživačkoj grupi za industrijske primene plazmi Univerziteta u Bolonji, Italija. U okviru zajedničkog istraživanja MPM je dijagnostikovao pomoću maseno-energijskog spektrometra a uporedo je urađena električna karakterizacija i vremenska evolucija prostiranja pražnjenja korišćenjem ICCD kamere. (Stancampiano *et al.*, 2018).

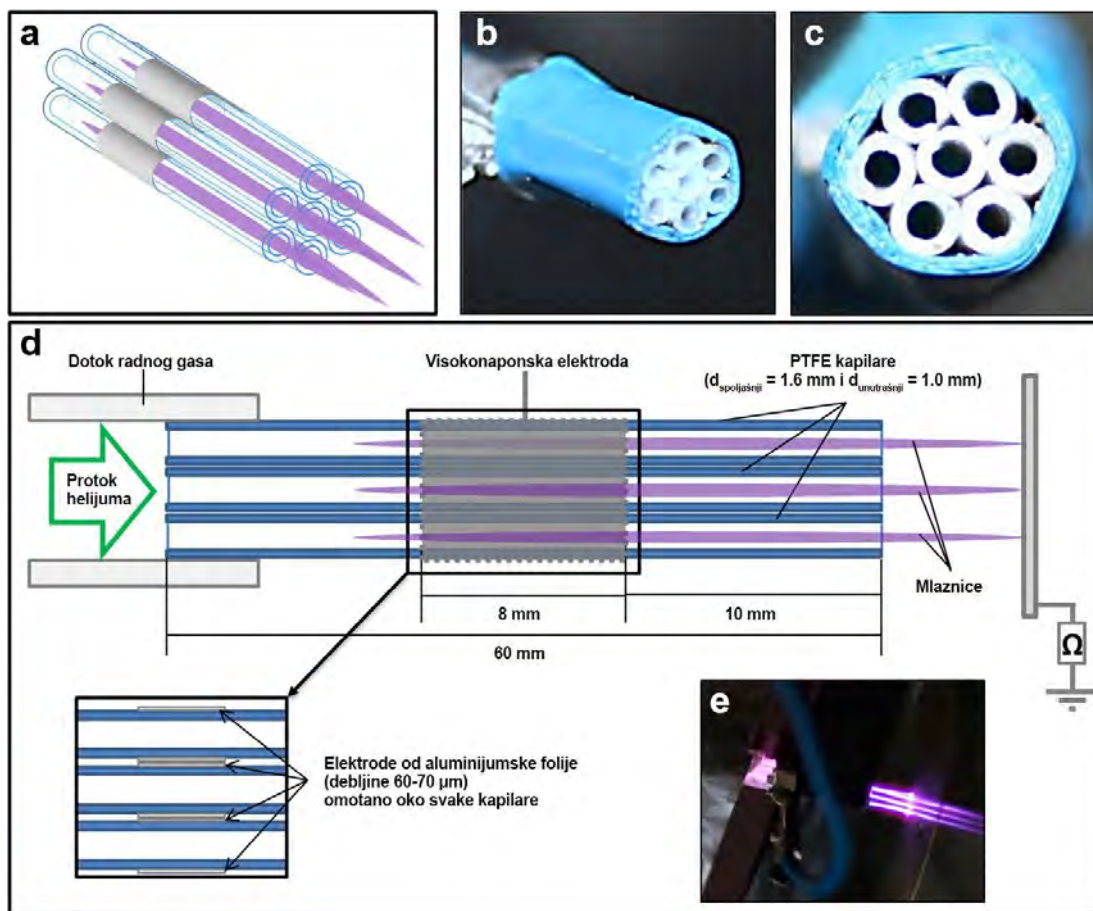
Kao jedna od većih mana atmosferskih plazmenih mlazova jeste nemogućnost primene na većim površinama tretiranog uzorka s obzirom na to da je prečnik samog mlaza reda veličine nekoliko milimetara. Jedno od rešenja ovog problema je dizajniranje izvora plazme koji poseduje više plazma mlaznica – mnogostruki plazmeni mlaz koji bi bio pogodan za tretman velikih ravnih površina (Zhang *et al.*, 2014; Kim *et al.*, 2015; Eden i Park, 2006) i uzoraka sa komplikovanom geometrijom (Fan *et al.*, 2012). Uz postizanje željenog cilja, gde su MPM-i pokrivali veću površinu od konvencionalog tj. jednostrukog plazmenog mlaza, dolazi do pojave novog fenomena a to je međusobna interakcija mnogostrukih mlazova. Ova interakcija se ogleda u međusobnom spajanju pojedinačnih mlaznica u jedan zajednički mlaz ukoliko su oni u neposrednoj blizini jedni drugih. Ovaj fenomen (*eng. jet-to-jet coupling*) dovodi do pojave pražnjenja sa jedinstvenim karakteristikama, različitim od slučaja gde postoji samo jedan mlaz, (Kim *et al.*, 2011; Furmanski *et al.*, 2011; Kim *et al.*, 2012; Stancampiano *et al.*, 2018) koji otvara vrata za nove vrste primena.

Kada se radi o primenama, da bismo mogli da izvučemo maksimum iz MPM-a, potrebno je da se u potpunosti objasni pojava i sam fenomen interakcije plazma mlaznica. Dosadašnja istraživanja vezana za ovaj fenomen su uglavnom dobijena iz podataka prikupljenih snimanjem brzim ICCD kamerama, Šlirenovom fotografijom i optičkom emisionom spektroskopijom (Yambe *et al.*, 2003; Nie *et al.*, 2009; Eden *et al.*, 2006; Cao *et al.*, 2009; Furmanaki *et al.*, 2011; Kim *et al.*, 2012; Fan *et al.*, 2012; Kim *et al.*, 2015; Qian *et al.*, 2014; Babaeva *et al.*, 2014; Bianconi *et al.*, 2014; Chang *et al.*, 2014; Zhou *et al.*, 2013; Ghasemi *et al.*, 2013; Zhang *et al.*, 2014). Manji broj studija sadrže informacije za ovaj tip električnih pražnjenja iz kojih su dobijene informacije o temperaturi podloge, analize tretiranog supstrata, potencijal inaktivacije bakterija i merenje dvosmernih električnih polja tokom njihovih primena (Kim *et al.*, 2012; Bianconi *et al.*, 2014; Robert *et al.*, 2015). Do sada je publikovan značajan broj istraživanja vezanih za masenu spektrometriju atmosferskih pražnjenja (Bruggeman *et al.*, 2010; Malović *et al.*, 2010), ali do ove studije (Stancampiano *et al.*, 2018) nije urađena masena analiza nijednog MPM-a. Kompjuterskim modelovanjem (Babaeva i Kushner, 2014) pokazano je da mlaznice mnogostrukih mlazova međusobno deluju putem elektrostatičkih, hidrodinamičkih i fotolitičkih sila i pokazalo se da njihove interakcije zavise prvenstveno od toga koliko su kapilare gusto postavljene kao i na sam broj koji sačinjava izvor. Plazma jonizacioni talasi npr. dva mlaza koja interaguju, iako se elektrostatički odbijaju, bivaju konfinirani usled ujedinjenog protoka helijuma koji protiče kroz svaku mlaznicu posebno a gde je E/N iznad vrednosti samoodržanja. Kod trostrukog plazmenog mlaza spoljašnja dva mlaza imaju tendenciju konvergiranja ka centralnom mlaznom kanalu gde pridruženi jonizacioni kanal postaje jači.

Ovom studijom želimo da pružimo neke nove uvide u fenomen interakcije mlaznica mnogostrukog plazmenog mlaza sa pozicije masene spektrometrije. Biće prikazana integrisana merenja spektara pozitivnih i negativnih jona za različite modove rada mnogostrukog plazmenog mlaza. Takođe prikazaćemo razloženu vremensku evoluciju prostiranja mlaza unutar perioda pobudne frekvencije od 80 kHz kao i električne karakteristike prilikom formiranja kombinovanog mlaza od strane sedam mlaznica.

5.2 Eksperimentalna postavka

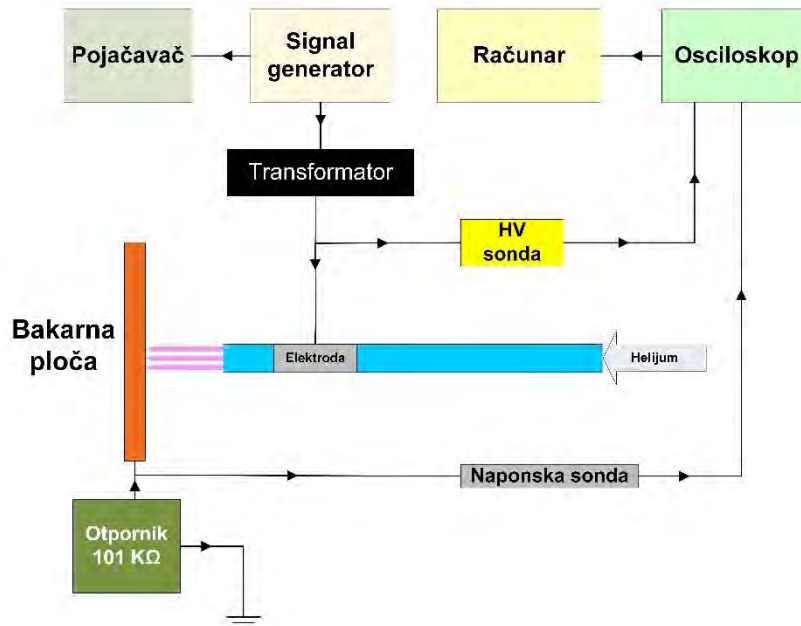
Mnogostruki plazmeni mlaz (MPM) koji smo koristili u eksperimentu predstavlja niz od sedam mlaznica koje propagiraju slobodno u ambijentalnu sredinu i imaju mogućnost da zajedno formiraju kombinovani mlaz visokog intenziteta. Na Slici 5.1 prikazana je eksperimentalna postavka kao i fotografije samog uređaja. Jezgro izvora sačinjavaju sedam identičnih sintetičkih fluoropolimernih PTFE (PoliTetraFluorEtilen) kapilara spoljašnjeg prečnika 1.6 mm i unutrašnjeg prečnika 1.0 mm. Svaka kapilara je obmotana posebno aluminijumskom folijom širine 8 mm (Slika 5.1 (a)) tako da možemo da kažemo da se MPM sastoji od sedam cilindričnih elektroda. Elektrode su međusobno električno povezane i napajaju se istovremeno pomoću visokonaponske elektrode omotane oko njih. Elektroda je napravljena od aluminijumske folije širine 8 mm i debljine 70 μm . Kapilare su dodatno fiksirane pomoću nekoliko slojeva izolir trake koje formiraju plavi omotač MPM-a (Slika 5.1 (b i c)).



Slika 5.1 Shema eksperimentalne postavke i fotografije MPM-a: (a) shema MPM-a sa sedam mlaznica; (b) fotografija izvora; (c) frontalni prikaz izlaznog dela izvora; (d) shema aksijalnog poprečnog preseka izvora; (e) fotografija električnog pražnjenja izvora tokom eksperimenta; (Stancampiano *et al.*, 2018.)

U ovoj studiji MPM je napajan visokonaponskim sinusoidnim signalom frekvencije 80 kHz dok je tokom eksperimenta primenjeni napon na visokonaponskoj elektrodi variran u opsegu vrednosti od 5 kV do 6.4 kV. Električno kolo za napajanje izvora plazme smo već detaljno opisali u poglavlju „**2.1.1 Električno kolo izvora i protok gasa**“. Glavna razlika u odnosu na već opisano električno kolo u ovoj eksperimentalnoj postavci jeste drugačiji tip uzemljene elektrode. Kao što se može videti na

Slici 5.2 bakarna ploča, koja osim što predstavlja metu MPM-a, ima ulogu uzemljene elektrode. Na ploču je povezan i otpornik od 101 kΩ pa smo u toj tački kola mogli da merimo pad napona koristeći naponsku sondu i da sračunamo snagu predatu plazmi. Ova postavka je korišćena za određivanje električne karakterizacije kao i za iCCD snimanja MPM-a. Pri masenoj spektrometriji MPM-a meta izvora je bio sam spektrometar (sačinjen od nerđajućeg čelika) tako da nismo bili u mogućnosti da merimo snagu i u tom slučaju glavni parametar koji smo varirali bio je primenjeni napon na elektrodi.



Slika 5.2 Električno kolo MPM-a

Helijum je korišćen kao radni gas i ravnomeran protok se dovodio u svih sedam mlaznih kapilara. Za obezbeđivanje ravnomernog protoka radnog gasa tokom eksperimenta unutar MPM-a je neposredno ispred kapilara umetnut mali komad sušera. Ukupan protok (zbir protoka kroz sve kapilare) je variran između 2 slm i 7 slm prema odabranom režimu rada. Pre merenja masenim spektrometrom da bismo dobili najstabilnije parametre pri kojima bismo imali prostiranje svih sedam mlaznica ravnomerno koristili smo uzemljenu elektrodu od nerđajućeg čelika - čelična ploča (Slika 5.1 (d)), postavljenu na udaljenosti od 9 mm od izlaznog otvora MPM-a. Pored stabilnijeg formiranja plazme dobijena je i uporediva geometrijska i električna konfiguracija sa onom koja je ostvarena tokom analize masenim spektrometrom.

Tokom masene analize koristili smo režim sekundarne jonske masene spektrometrije (SIMS +/-) sa ciljem ispitivanja masenih spektra pozitivnih i negativnih jona. SIMS +/- režim funkcioniše bez puštanja u rad internog izvora jonizacije masenog spektrometra, tako da dobijeni pozitivni i negativni maseni spektri potiču isključivo iz električnog pražnjenja MPM-a. Pre početka merenja maseni spektrometar je posebno prilagođen (*eng. tuned*) da bismo dobili dobar intenzitet signala. Takođe, za obezbeđivanje stabilnog signala bilo je neophodno da se izvrši poravnavanje/centriranje izvora plazme u odnosu na otvor za uzorkovanje pre svakog seta merenja. MPM je postavljen ispred otvora za uzorkovanje i usmerili

smo ga normalno u odnosu na prednju ploču MBMS-a na sledećim rastojanjima: 5 mm (verzija sa jednim mlazom), 7 mm (verzija sa 3 mlaznice) ili 9 mm (verzija sa 7 mlaznica). Koristili smo sledeću konfiguraciju otvora i konusa za uzorkovanje: prednji otvor=0.3 mm; konus1=0.3 mm; konus2=1.0 mm. Ovaj set je izabran da bi se postigao odgovarajući nivo signala čak i za konfiguraciju jednog jeta, pa su poređenja mogla biti napravljena između svih verzija MPM-a. Pre započinjanja eksperimenata bilo je potrebno proveriti interakciju plazme sa MBMS sistemom, tj. da li je dolazilo do pojave pražnjenja unutar masenog spektrometra na pritisku nižem od atmosferskog. To je učinjeno praćenjem raspodele energija jona kiseonika i azota. Pokazalo se da je maksimum raspodele energije jona reda veličine od svega par elektron-volti što je bio dovoljan dokaz da su uzorkovane vrste jona dobijene iz plazme na atmosferi i da nisu formirane usled pražnjenja unutar masenog spektrometra. Merenja su počinjala za sledeće vrednosti pritiska u tri faze: $P_1=6.5 \times 10^0$ Torr, $P_2=2.4 \times 10^{-5}$ Torr i $P_3=4.1 \times 10^{-7}$ Torr. Prikazani maseni spektri jona predstavljaju prosečne vrednosti tri različita ciklusa skeniranja pri istim radnim uslovima.

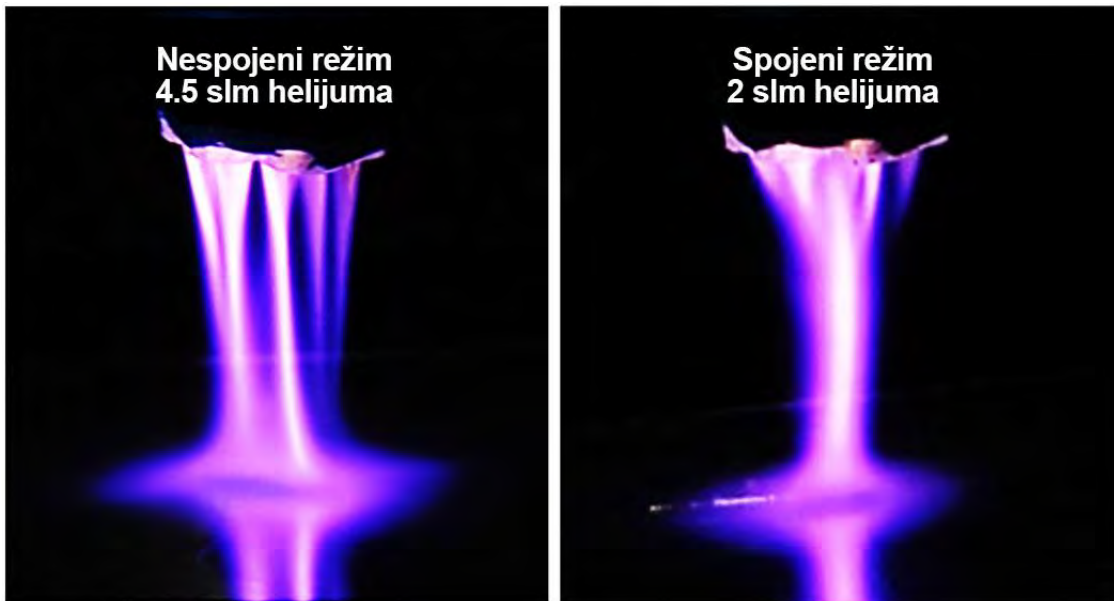
ICCD merenja vršena su u eksperimentalnoj postavci gde je MPM bio usmeren normalno na uzemljenu bakarnu ploču pri rastojanju od 9 mm. Uloga bakarne ploče je bila ta da nam obezbedi slične uslove kao što smo imali pri merenjima sa masenim spektrometrom. (Opis akvizicije ICCD fotografija objašnjen je u poglavlju 2.3 ICCD kamera.

Za potrebe snimanja režima spojenih mlazova gde su radni uslovi imali širi spektar (mogli smo da variramo vrednost napona dosta više u odnosu na režim nespojenih mlazova a da se režim ne promeni) urađena su snimanja ICCD kamerom za duža vremena ekspozicije od jednog period gde smo posmatrali promenu u emisioniom intenzitetu mlazova za različiti set parametara.

Dva različita operativna režima (Slika 5.3) su moguća kod mnogostrukog plazmenog mlaza koji smo koristili u eksperimentu:

- **Nespojeni režim** (sedam dobro definisanih mlaznica plazme koje su odvojene jedna od druge);
- **Spojeni režim** (svih sedam mlaznica se spajaju u jedinstveni tzv. kombinovani intenzivni mlaz).

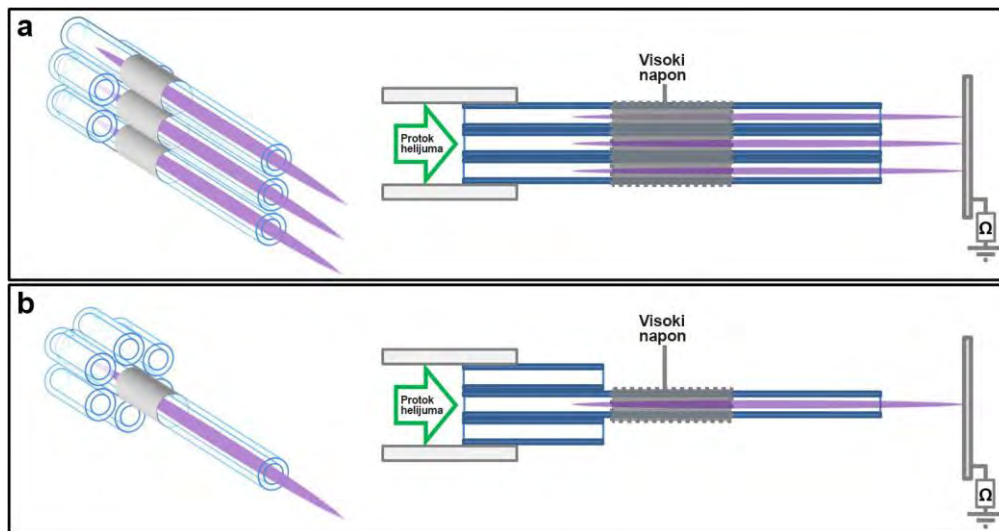
Prelazak iz nespojenog u spojeni režim i obrnuto moguće je uraditi variranjem napona i variranjem protoka. Ove prelaske je mnogo lakše izvesti kada MPM nema metu (maseni spektrometar, bakarnu ili čeličnu ploču) tj. kada se mlaz/mlaznice slobodno prostiru u okolnu sredinu. U takvoj situaciji nismo mogli da variramo parametre previše: svih sedam mlaznica se pojavljivalo samo za određeni napon, pri smanjenju napona neke od mlaznica su se gasile dok su se pri povećanju napona mlaznice spajale u spojeni režim. Zbog nastalih ograničenja rešili smo da napon fiksiramo na vrednost pri kojoj će promena protoka uticati na promenu režima MPM-a. U ovoj studiji, prelazak sa jednog operativnog režima na drugi postizali smo variranjem protoka radnog gasa (npr. pri rastojanju 9 mm od uzemljene mete: protok od 4.5 slm je korišćen za dobijanje nespojenog režima; protok od 2 slm je korišćen za dobijanje spojenog režima).



Slika 5.3 Fotografija MPM-a tokom pražnjenja u (a) nespojenom režimu i (b) spojenom režimu; (Bianconi *et al.*, 2014.)

Da bismo bolje razumeli efekte fenomena spajanja mlaznica, dve modifikovane verzije izvora plazme su takođe korišćene u ovom radu (Slika 5.3). Modifikovane verzije se zasnivaju na istoj kapilarnoj konfiguraciji, koja kao i prvobitna verzija ima oblik pčelinjeg saća.

Razlika kod prve modifikovane verzije bila je u tome što su se kod MPM-a napajale samo tri kapilare (Slika 5.4 (a), dok smo kroz nenapajane kapilare samo propuštali protok helijuma) i on je prilagođen tako da bismo istražili efekat broja spojenih mlaznica. Razmak između izvora plazme i uzemljene ploče smanjen je na 7 mm dok smo ukupni protok gasa povećali (4 slm za spojen režim i 7 slm za nespojen režim) da bi se postigao stabilniji rad (bez oscilacija plazme i padova ponovljive električne karakteristike izvora plazme).



Slika 5.4 Shematski prikaz modifikovanih verzija MPM-a: (a) verzija trostrukog mlaza sa tri napajane elektrode; (b) verzija jednostrukog mlaza sa jednom napajanom elektrodom; (Stancampiano *et al.*, 2018.)

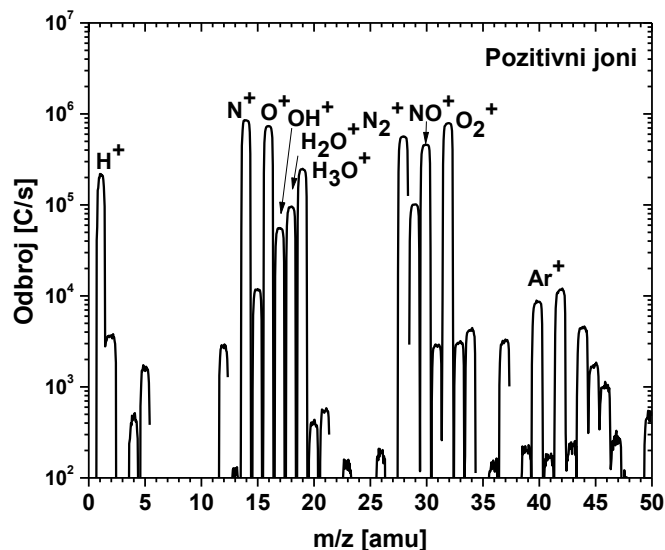
Takođe, istražili smo i drugi modifikovani izvor plazme, jednostruki plazmeni mlaz (slika 5.4 (b)), sa samo jednom elektrodom na centralnoj kapilari (kroz ostale kapilare smo kao u prethodnoj modifikaciji propuštali protok helijuma), kako bi se uporedio MPM sa verzijom izvora sa jednom napajanom kapilaram. U ovom slučaju je razmak između izvora plazme i uzemljene ploče smanjen na 5 mm i ukupni protok gasa je fiksiran na 4.5 slm da bi se postiglo stabilnije pražnjenje.

5.3 Pregled rezultata masene spektrometrije za različite konfiguracije MPM-a

Svi maseni spektri pozitivnih i negativnih jona koje ćemo prikazati u ovom poglavlju predstavljaju skup produkata plazme dobijene usled mešanja radnog gasa, u ovom slučaju helijuma, i konstituenata ambijentalnog vazduha sa određenom relativnom vlažnošću (u najvećoj meri su to atomske i molekulske vrste azota i kiseonika). Procesi koji su zasluženi za proizvodnju pozitivnih i negativnih jona su disocijativna jonizacija i disocijativno vezivanje elektrona (disocijativni attachment). U narednim poglavljima biće prikazani i analizirani maseni spektri za svaku konfiguraciju izvora posebno.

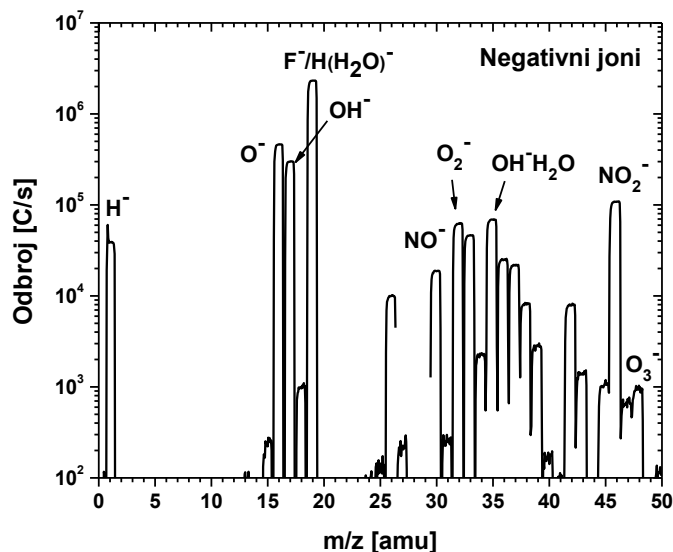
5.4 MPM sa sedam mlaznica

Na Slici 5.5 prikazan je maseni spektar pozitivnih jona za spojeni režim MPM-a. U bogatom hemijskom sastavu preovlađuju joni azotnih vrsta N , N_2^+ , NO^+ kao i joni kiseoničnih i vodoničnih vrsta H^+ , O^+ , O_2^+ , OH^+ , H_2O^+ , H_3O^+ . Ovaj sastav pozitivnih jona je u priličnom slaganju sa masenim spektrom koji su snimili McKay i saradnici. (McKay *et al.*, 2013) u njihovom plazmenom mlazu. Što se tiče klastera vode u masenom spektru snimljeni su samo $H(H_2O)^+$ i $H(H_2O)_2^+$. U električnom pražnjenju sa niskim stepenom vlažnosti snimljen je prilično visok signal H_3O^+ za čiju proizvodnju je zaslužena sledeća reakcija $H_2O^+ + H_2O \rightarrow H_3O^+ + OH$ ($k = 1.85 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$) (Bruggeman *et al.*, 2010; Anicich, 1993). Joni vodenih klastera sa većom molekulskom masom stvaraju se pretežno putem sudara manjih jonizovanih klastera vode sa molekulom vode ($H^+(H_2O)_n + H_2O \rightarrow H^+(H_2O)_{n+1}$) i raspadanjem većih klastera ($H^+(H_2O)_n \rightarrow H^+(H_2O)_{n-1} + H_2O$) (Bruggeman *et al.*, 2010; Anicich, 1993). Dovođenje vlažnosti u protok radnog gasa pogoduje pojavi reakcija koje stvaraju klustere višeg reda što može dovesti do njihovog većeg intenziteta u masenim spektrima nekih konfiguracija pražnjenja na atmosferskom pritisku (Bruggeman *et al.*, 2010). Ovo važi za formiranje klastera pozitivnih i negativnih jona. U našem slučaju tokom merenja vlažnost u vazduhu laboratorije je bila između 30% i 50%, ali procenat vode u protoku helijuma je vrlo nizak (reda nekoliko ppm). Ovo bi mogao biti razlog zbog kojeg nismo mogli da dobijemo klustere višeg reda u našem eksperimentu.



Slika 5.5 Maseni spektar pozitivnih jona spojenog režima MPM-a sa sedam mlaznica (primenjeni napon 5 kV, inicijalni napon 4.14 Vpp, razmak 5 mm i protok helijuma 4.5 slm)

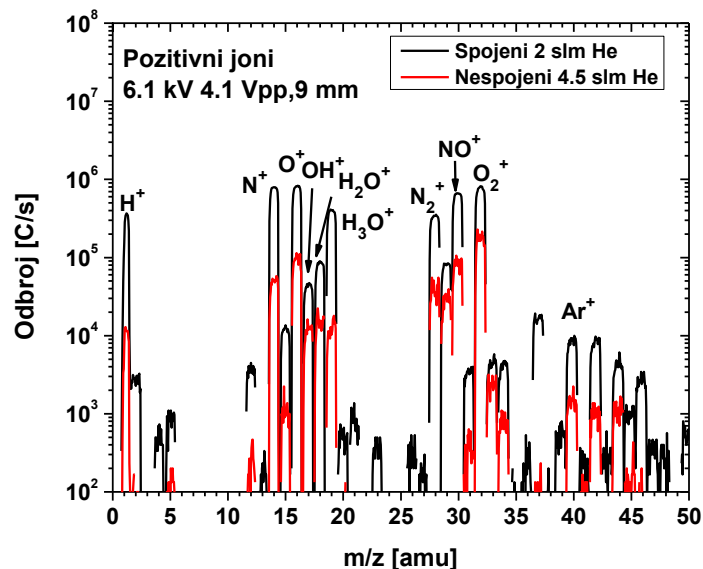
Najzastupljenije vrste negativnih jona (Slika 5.6) O^- , O_2^- , O_3^- , OH^- , H^- , NO^- i NO_2^- snimljene su za identičan set parametara pri istom režimu MPM-a kao i za pozitivan spektar jona, i mogu biti od velikog značaja za biomedicinske primene (Graves, 2012; Bauer i Graves, 2016). Ono što je zanimljivo, nijedna od ovih vrsta nije najdominantnija u spektru. Najveći odbroj kod negativnih jona pripada masi 19 amu. Ova masa može se pripisati $H(H_2O)^-$ klasteru ali takođe može da bude i F^- . Fluor je poznat po svojoj elektronegativnosti a kod ispitivanog izvora plazme su kapilare sačinjene od PTFE-a i velika je verovatnoća da je usled električnog pražnjenja koje je bilo u direktnom kontaktu sa njima došlo do termalne dekompozicije materijala pa je maseni spektrometar detektovao visok signal F^- . Što se tiče $H(H_2O)_n^-$ kao i $OH(H_2O)_n^-$ klastera oni su formirani disocijativnim elektronskim atačmentom praćenim hidratacijom. Za reakciju pri kojoj dobijamo H^- jon potreban je rejt koeficijent koji je veći od OH^- jona ($k_H^- = 7.8 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1}$ naspram $k_{OH}^- = 2.6 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$). Takođe, za reakciju $H^- + H_2O \rightarrow OH^- + H_2$ je potreban dva reda veličine viši rejt koeficijent od k_H^- što dovodi do zaključka da OH^- joni i njegovi klasteri imaju veću zastupljenost u masenom spektru.



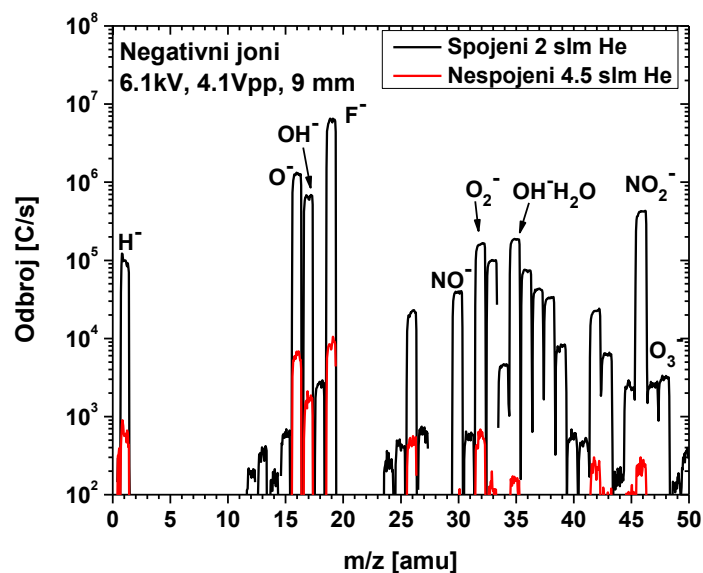
Slika 5.6 Maseni spektrar negativnih jona spojenog režima MPM-a sa sedam mlaznica (primenjeni napon 5 kV, inicijalni napon 4.14 Vpp, razmak 5 mm i protok helijuma 4.5 slm)

Slike 5.7. i 5.8. prikazuju poređenje dva različita režima masenih spektara pozitivnih i negativnih jona za isti set parametara. Kod pozitivnih jona možemo primetiti prilično sličan hemijski sastav kod oba režima s tom razlikom da je odbroj kod nespojenog režima generalno manji za red veličine u odnosu na spojeni režim. Kod negativnih jona se može reći da je sastav drugačiji i da je razlika u odbroju između dva režima još značajnija (dva reda veličine) dok neke vrste kao što su ozon i NO_2^- nisu uopšte detektovane ili je njihov odbroj bio značajno manji u nespojenom režimu. Što se tiče signala F^- on je u oba režima bio najdominantniji negativni jon. S obzirom da promena brzine helijuma predstavlja glavni parametar u promeni dva režima iz jednog u drugi može se zaključiti da sam protok radnog gasa kao i čestice iz okolnog ambijenta koje difunduju u mlaz sa strane imaju veliki uticaj na formiranje sastava ovih spektara. Već je pokazano (Babaeva i Kushner, 2014) da se pri većim protocima helijuma smanjuje uticaj čestica koje difunduju sa strane u pražnjenje što kao rezultat daje mlaznice formirane uglavnom od helijuma pa je samim tim i odbroj helijuma veći u datom spektru.

U slučaju gde smo imali manju brzinu radnog gasa od 6.1 ms^{-1} za spojeni režim u odnosu na 13.6 ms^{-1} u nespojenom režimu dolazilo je do spajanja helijumskih mlaznica a samim tim i do interakcije i spajanja jonizacionih frontova. Pri ovim uslovima može se očekivati veća difuzija konstituenata vazduha u helijumske kanale. Prema Darni i saradnicima, (Darny *et al.*, 2017), negativni joni verovatno nastaju na samom obodu cilindričnih helijumskih kanala, gde je mešanje sa vazduhom najveće. Relativno visoka frekvencija (80 kHz) kola za napajanje koje pokreće MPM i relativno dugoživuće vrste negativnih jona koje su snimljene, kao što su NO_2^- (~1 s) i OH^- (~1 ms) (Sekimoto i Takayama, 2012), može dovesti do nakupljanja negativnih jona u prostoru između MPM-a i mete.



Slika 5.7 Poređenje masenih spektara pozitivnih jona spojenog i nespojenog režima MPM-a sa sedam mlaznica (primenjeni napon 6.1 kV, inicijalni napon 4.1 Vpp, razmak 9 mm i protok helijuma 2 slm i 4.5 slm respektivno)

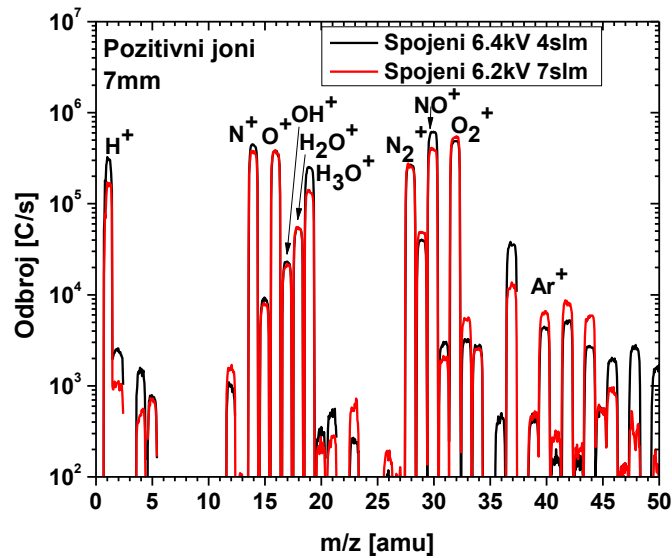


Slika 5.8 Poređenje masenih spektara negativnih jona spojenog i nespojenog režima MPM-a sa sedam mlaznica (primenjeni napon 6.1 kV, inicijalni napon 4.1 Vpp, razmak 9 mm i protok helijuma 2 slm i 4.5 slm respektivno)

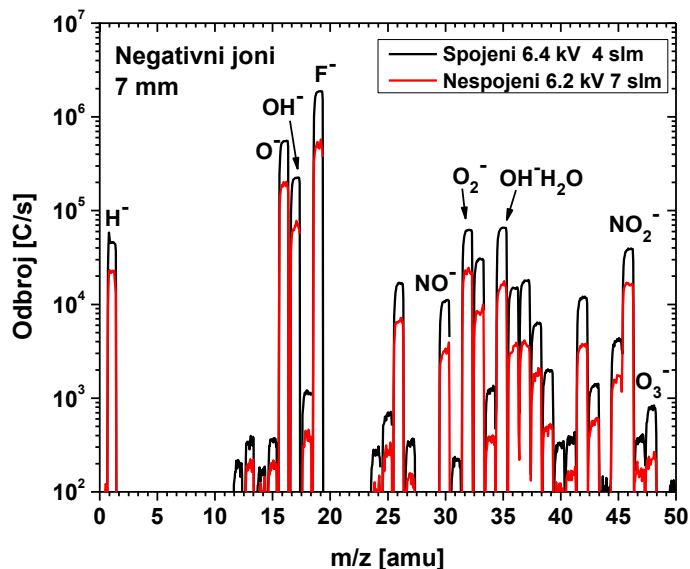
5.5 MPM sa tri mlaznice

Modifikovana verzija MPM-a kod koje su bile aktivne tri napajane mlaznice je korišćena sa ciljem da se naglasi složenost interakcije izvora sa metom. Ovako modifikovan izvor predstavlja sličnu verziju izvora koju su koristili Babaeva i Kushner. (Babaeva i Kushner, 2014). Kao i kod MPM-a sa sedam mlaznica i kod ove verzije izvora smo režim menjali promenom protoka helijuma (spojeni režim 4 slm, nespojeni režim 7 slm) pri fiksiranom primenjenom naponu od 6.1 kV. Da bismo obezbedili sličan nivo signala

kao kod verzije sa 7 mlaznica približili smo izvor na 7 mm od otvora za uzorkovanje masenog spektrometra. Poređenje pozitivnog spektra (Slika 5.9) dva različita režima za ovu verziju MPM-a prikazuje vrlo malu razliku u spektrima, a za neke jone je ona prilično zanemarljiva (N_2^+ , O_2^+ , H_2O^+ , O^+). Kod negativnih jona (Slika 5.10) razlika u spektrima je nešto veća (2.5 puta je manji signal kod nespojenog režima u odnosu na spojeni režim) ali u poređenju sa nespojenim režimom kod 7 mlaznica gde je razlika između režima par redova veličina može se reći da je produkcija negativnih jona kod ove modifikacije značajnija. Ovi rezultati nam pokazuju da se i sa manjim brojem mlaznica može postići spojeni režim koji proizvodi snažniji i pojačani jonizacioni talas koji stremi ka visokoj produkciji koncentracije jona (naročito negativnih), što je u skladu sa rezultatima koje pruža model (Babaeva i Kushner, 2014).



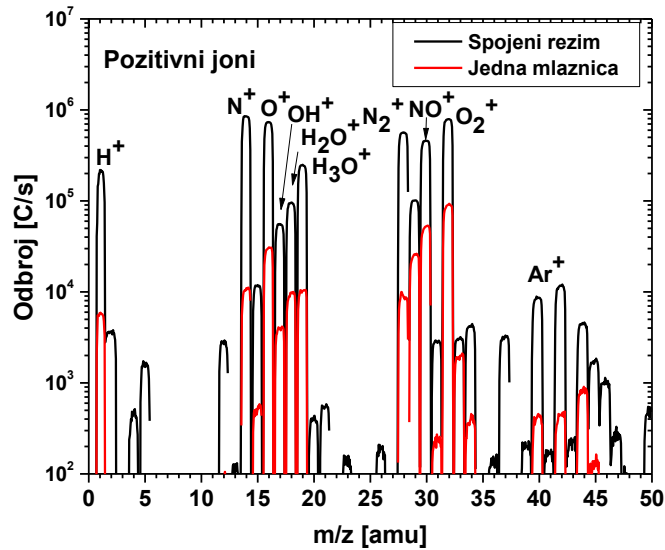
Slika 5.9 Poređenje masenih spektara pozitivnih jona spojenog i nespojenog režima MPM-a sa tri mlaznice (primenjeni naponi 6.4 kV i 6.2 kV, napon dat signal generatorom 4.1 Vpp, razmak 7 mm i protok helijuma 4 slm i 7 slm respektivno)



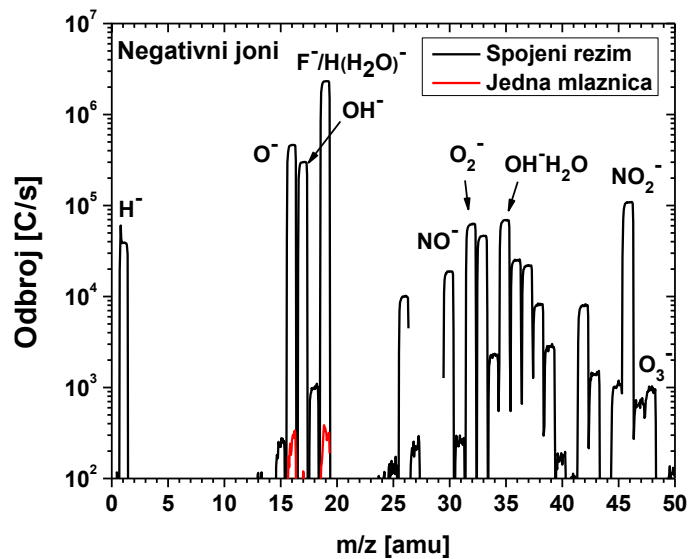
Slika 5.10 Poređenje masenih spektara negativnih jona spojenog i nespojenog režima MPM-a sa tri mlaznice (primenjeni naponi 6.4 kV i 6.2 kV, napon dat signal generatorom 4.1 Vpp, razmak 7 mm i protok helijuma 4 slm i 7 slm respektivno)

5.6 MPM sa jednom mlaznicom

Uloga ove modifikacije bila je pružanje uvida u vrednost nivoa ukupnog signala masenog spektra tj da li postoji razlika u odnosu na spojeni režim kod izvora sa sedam mlaznica. Rastojanje MPM-a u odnosu na maseni spektrometar je smanjeno na 5 mm radi dobijanja dobrog nivoa signala sa jednom mlaznicom pa je pri istim uslovima snimljen maseni spektar pozitivnih i negativnih jona za oba navedena slučaja. Na ovom rastojanju bilo je nemoguće dobiti nespojeni režim sa sedam mlaznica pa taj slučaj nije istražen. Slike poređenja dve modifikacije za spektre pozitivnih i negativnih jona (Slike 5.11. i 5.12.) na prvi pogled pružaju uvid u to da je ukupni signal spojenog režima veći u redovima veličina. U slučaju pozitivnih jona, u jednom mlazu postoji značajan broj jona, međutim, spektar se značajno razlikuje (što se može uočiti u smanjenoj produkciji azotnih vrsta u odnosu na kiseonične vrste) od onog u spojenom režimu. Čak i ako bismo pokušali da u idealnim uslovima pomnožimo jednu mlaznicu sedam puta ne bismo dobili vrednost signala koja je dobijena u spojenom režimu. Produkcije negativnih jona, osim niskih vrednosti signala O^- i F^- , praktično i nema kod jedne mlaznice.



Slika 5.11. Poređenje masenih spektara pozitivnih jona spojenog režima sa sedam mlaznica i jedne mlaznice MPM-a (primenjeni napon 5 kV, inicijalni napon 4.14 Vpp, razmak 5 mm i protok helijuma 4.5 slm)



Slika 5.12. Poređenje masenih spektara negativnih jona spojenog režima sa sedam mlaznica i jedne mlaznice MPM-a (primenjeni napon 5 kV, inicijalni napon 4.14 Vpp, razmak 5 mm i protok helijuma 4.5 slm)

5.7 ICCD i električna karakterizacija

ICCD kameru smo koristili za dobijanje vremenske evolucije pražnjenja spojenog i nespojenog režima MPM-a. Vremenski razložene fotografije za oba režima prikazane su na Slici 5.13. Da bismo izbegli saturisanje emisionih intenziteta za spojeni režim je podešeno skaliranje na 10000 a za nespojeni režim skaliranje na 5000. Ukoliko bismo još smanjili vrednost skale, moglo bi se tokom celog perioda primetiti postojanje slabog plazma kanala koji povezuje MPM i metu (bakarna ploča). Za oba režima imamo

predstavljenih devet iCCD fotografija usklađenih na istoj vremenskoj poziciji tj. vremenu u kojem je triggerovan signal. ($t=0 \mu\text{s}$ je pozicionirano na početku pozitivnog polu-perioda sinusoidnog naponskog signala).

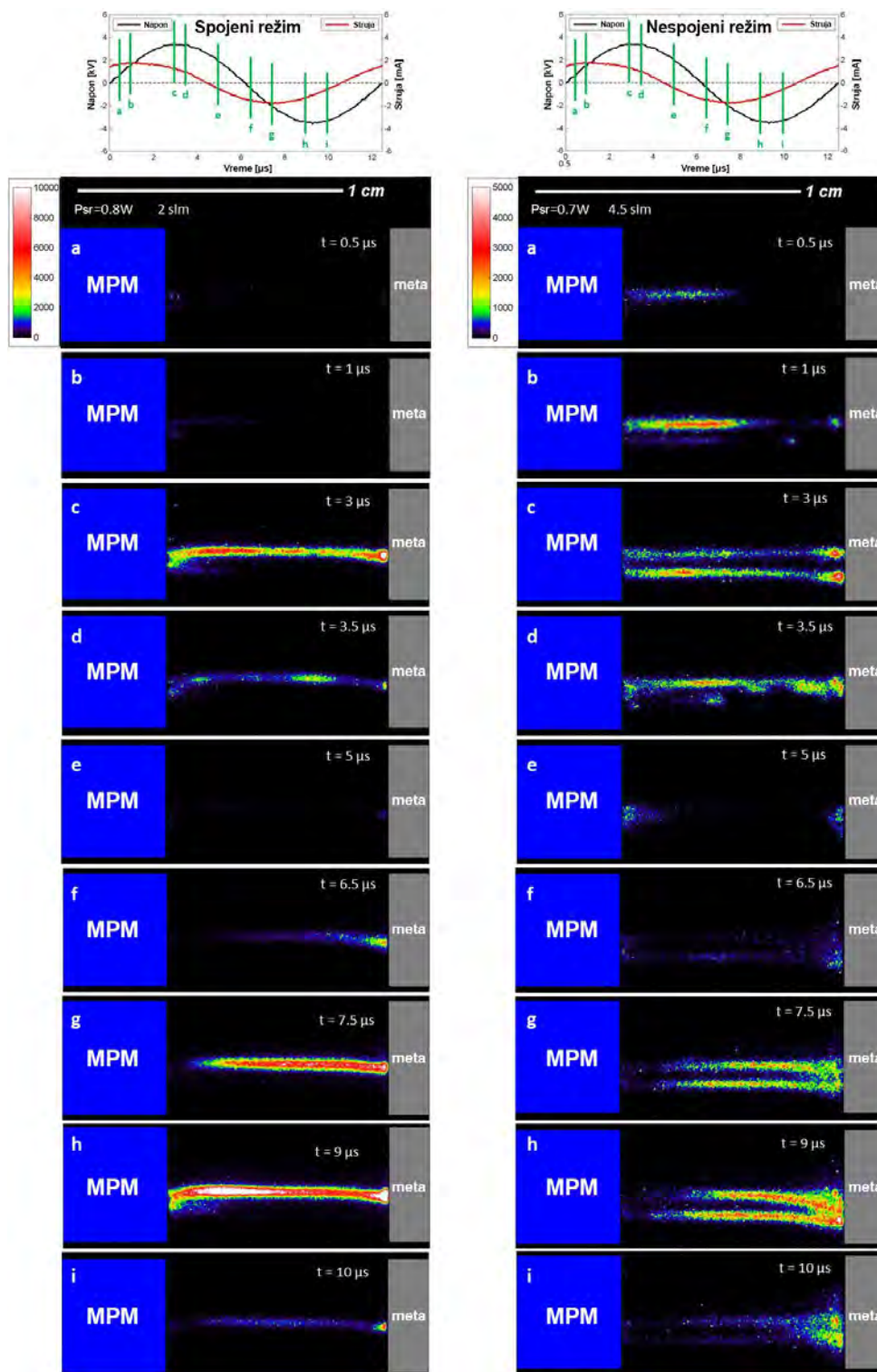
Na početku Slike 5.13., postavljeni su strujno-naponski signali na kojima su zelenim linijama prikazane pozicije trigera koje odgovaraju iCCD fotografijama koje su na slici i prikazane (označene malim latiničnim slovima od a do i). Prikazani set fotografija predstavlja dinamičko ponašanje atmosferskog pražnjenja koje udara u vidljivu površinu mete. U prve tri slike oba režima (Slika 5.13. (a)–(c) koja odgovara delu pozitivnog poluperioda od 0–0.3 μs) može se primetiti prostiranje plazme od izlaza iz MPM-a do uzemljene mete, koja se širi unutar protoka helijuma visoke koncentracije. Sama raspodela koncentracije helijuma se razlikuje kod dva snimljena režima zbog različite količine protoka i kao što je i očekivano, dovodi do pojave višestrukih jonizacionih frontova kod nespojenog režima (iako nije moguće uočiti ih sve zbog preklapanja usled ugla snimanja iCCD kamerom). Kod spojenog režima samo jedan jonizacioni front je uočen. Struja je merena preko uzemljene mete. Jedini signal koji je snimljen bio je signal struje koji je predstavljao struju koja prolazi kroz centralni kanal MPM-a. Sistem nije pokazao pojavu dodatnih strimera koji se mogu pojaviti kao dodatni pikovi na sinusoidnom talasu strujnog signala. Pojava većeg intenziteta emisije na vremenskoj skali polovine perioda $T=6.25 \mu\text{s}$ takođe nije bila dovoljno brza da bi se snimila pojava oštrog pika kod strujnog signala. Slično ponašanje se može videti i kod naponskog signala u slučaju pozitivnog i negativnog polu-perioda.

Ako obratimo pažnju na razlike u fotografijama dva režima, možemo videti da kod spojenog režima propagacija plazme počinje kasnije nego kod nespojenog režima, i da pražnjenje do mete stiže za oko 2 μs kasnije. Posledica ove razlike je verovatno zbog različitog procenta molekula vazduha koji je veći u spojenom režimu i bilo bi potrebno veće električno polje da bi se obezbedilo brže prostiranje. Zbog toga se kod nespojenog režima uslov prostiranja pražnjenja postiže ranije a kod spojenog režima pri višim vrednostima naponskog signala. U spojenom režimu jonizacioni front brže prelazi rastojanje unutar vremenskog intervala između dve akvizicije (2.5 μs i 3 μs) u odnosu na nespojeni režim (0 μs –1.5 μs , akvizicija nije prikazana). Ovu pojavu mogli bismo da opišemo kombinovanjem dva faktora: u nespojenom režimu jonizacioni front se prostire kasnije, kada je vrednost napona bliža pozitivnom piku, a električno polje u datom rastojanju je većeg intenziteta; viši stepen nečistoća vazduha u spojenom režimu može dovesti do povećanja brzine širenja jonizacionog fronta kao što je u literaturi prikazano za male primese azota ili veće frakcije vazduh /helijum (Douat *et al.*, 2012; Bourdon *et al.*, 2016). Brzina protoka gasa u velikoj meri može uticati na širenje vrsta koje nastaju u plazmi (Pei *et al.*, 2013). Konkretno u našem eksperimentu, brzina prostiranja pražnjenja je mnogo veća od brzine protoka gasa (ms^{-1} naspram kms^{-1}) što dovodi do zaključka da možemo da odbacimo direktnu povezanost (koja nije posredovana varijacijama režima protoka i nečistoćama vazduha) između različitih protoka i brzine prostiranja jonizacionih frontova snimljenih za dva režima. Nakon što su strimeri prešli rastojanje između MPM-a i mete, dolazi do formiranja snažnih svetlosnih provodnih kanala između mete i visoko-naponske electrode. Oni dostižu maksimum intenziteta u maksimumu pika pozitivnog dela polu-perioda naponskog signala (3.1 μs) za oba režima, ali rezultuje značajno višim intenzitetom kod spojenog režima (podsetimo se drugačijeg skaliranja za dva režima na Slici 5.13.).

U drugoj fazi (3.5 μs –6.5 μs , slike 5.13 (d)–(f), kod oba režima dolazi do kvazi-gašenja emisije plazme iz jonizovanih kanala. Treba naglasiti da, u tački vremena 6.25 μs (početak negativnog dela polu-perioda ciklusa), čak i ako se detektuje samo mala emisija svetlosti, ostaci provodljivih jonizovanih kanala su još uvek prisutni u svim regionima u kojima je strimer prošao. Prema tome, kada napon koji napaja visokonaponsku elektrodu obrne polaritet, potencijal preko preostalih kanala trenutno se obrće i elektroni i joni se sada ubrzavaju u suprotnom smeru. Kao posledica toga, u trećoj fazi (6,5 μs –9 μs , slike 5.13 (f)–(h), prostiranje sekundarnog strimera u suprotnom smeru od površine mete ka visokonaponskoj elektrodi kroz preostale provodljive jonizacione kanale koji su ostali od primarnog strimera može biti uočen.

Prostiranje katodno usmerenog strimera imamo za oba polu-perioda strujno-naponskih signala. Ono što je u literaturi pokazano (Ries *et al.*, 2014; Kovačević *et al.*, 2018; Darny *et al.*, 2017; Yuanfu *et al.*, 2018) pri merenju interakcije plazmenih mlazova sa provodnim i polu-provodnim metama se u velikoj meri slaže sa zapažanjem iz naše studije. Ono što je u našem slučaju zanimljivo jeste da se može primetiti pojava prostiranja mnogostrukih strimera u nespojenom režimu zbog još uvek prisutnog razdvajanja između preostalih provodnih kanala. Ries i saradnici (Ries *et al.*, 2014) su pokazali da pozicija gde prostiranje u suprotnom pravcu prestaje može zavisiti od različitih parametara među kojima je brzina protoka gasa (što je veća brzina protoka gasa to je prostiranje u suprotnom pravcu kraće). Za slučaj spojenog režima, Slika 5.13, možemo primetiti da, pri sporijem protoku helijuma, sekundarni strimer koji se prostire u suprotnom pravcu jasno prelazi rastojanje, dok kod nespojenog režima glava strimera prilazi neposrednoj blizini izlaza MPM-a ali daje emisiju slabijeg intenziteta u ovom regionu. Strimeri u oba slučaja daju maksimum intenziteta u $9 \mu\text{s}$ u skladu sa negativnim pikom napona. Ako poredimo slaganje sa masenom spektrometrijom, intenzitet emisije spojenog režima je uvek viši u odnosu na nespojeni režim zbog većeg stepena jonizacije.

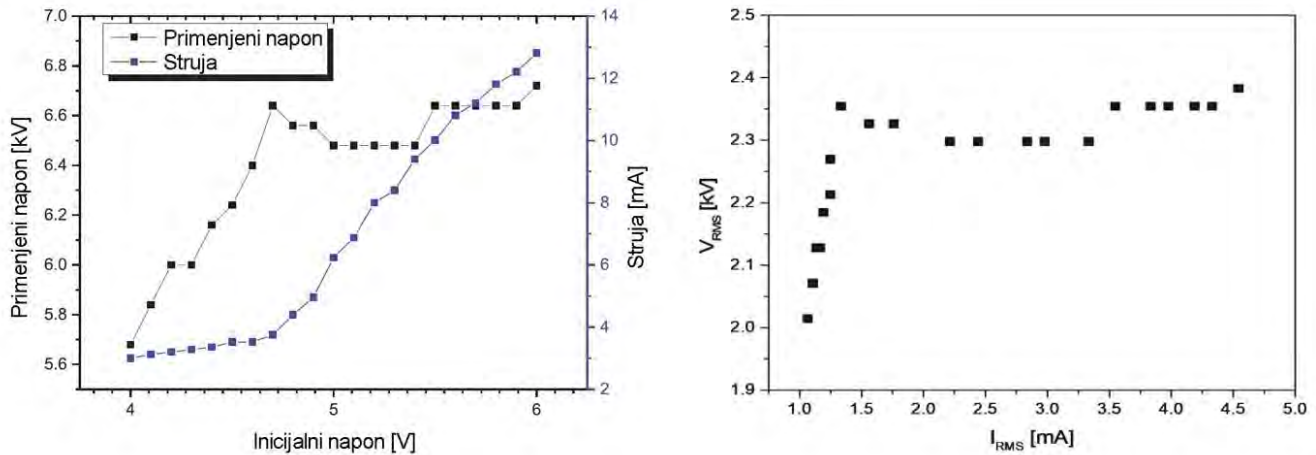
U četvrtoj fazi ($9 \mu\text{s} - 12.5 \mu\text{s}$, slike 5.13 (h) i (i)) dolazi do sporog nestanka plazma kanala koji proizvodi sekundarni strimer. Moramo napomenuti da u takođe u ovom režimu preostali provodni jonizacioni kanali su i dalje prisutni nakon završetka negativnog dela polu-periode. Ovo će voditi nove jonizacione frontove istim putem kuda su se prethodni prostirali. Kako su naveli Darny i saradnici. (Darny *et al.*, 2017), kritična uloga u indukovanju snažne modifikacije širenja protoka gasa može biti pripisana velikim i dugživućim negativnim jonima (npr. O_2^- , O_3^- , NO_2^-) tokom ponavljajućih operacija u kHz režimu kao u našem slučaju. Pošto znamo iz analize masenom spektrometrijom, da su negativni joni prisutni u relativno velikim količinama, posebno u spojenom režimu, skloni smo da poverujemo da ovi joni igraju glavnu ulogu u indukciji fenomena spajanja mlaza i jednog mlaza koji je ovde ispitivan.



Slika 5.13 Spojeni režim (leva kolona, He 2 slm) i nespojeni režim (desna kolona, He 4.5 slm) naponski i strujni talasni oblici (gore) sa iCCD akvizicijama (dole) u različitim vremenskim intervalima (rastojanje MPM u odnosu na metu 9 mm). Vreme prikupljanja iCCD akvizicija je označeno zelenim linijama i slovima (a) - (i) na talasnim oblicima strujno-naponskih signala

Na slici 5.14. su prikazane vrednosti struje i napona, dok smo odabrane ICCD fotografije prikazali na slici 5.15. Na slici 5.14. (levo) može se videti zavisnost napona (V_{p-to-p}) i struje (I_{p-to-p}) (eng. *peak to peak*

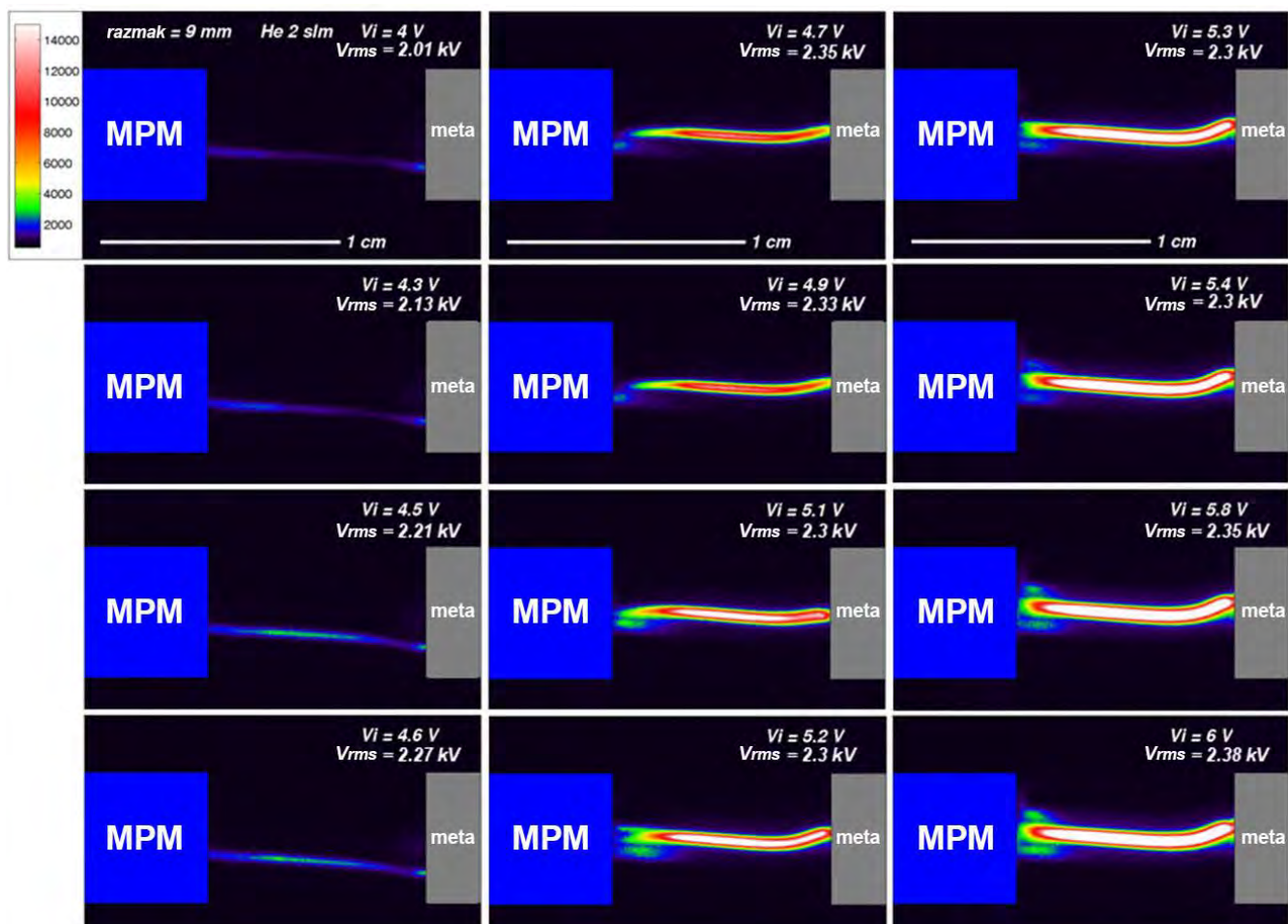
vrednosti) u funkciji napona signal generatora. Slika 5.14 (desno) prikazuje strujno-naponsku karakteristiku MPM-a dobijenu tokom rada spojenog režima. Ova merenja su nam bila dobar pokazatelj nelinearnosti strujnog kola prema kojoj ne postoji direktna korelacija između inicijalnog napona signal generatora i snimljenog napona na visokonaponskoj sondi. Zapravo, nakon određene vrednosti, snimljen napon prestaje da raste uprkos povećavanju inicijalnog napona, te nakon vrednosti od 4.6 V inicijalnog napona on dostiže plato.



Slika 5.14 Snimljene vrednosti napona i struje sa inicijalnim naponom podešenim na signal generatoru (levo) i snimljeni rms napon nad strujom (desno), tokom rada u spojenom režimu (MPM sa sedam mlaznica, He 2 slm, razmak 9 mm).

Sa druge strane, struja ima dva različita trenda i njen nagib se menja u tački 4.6 V. Nakon ove vrednosti, struja počinje da raste brže sa povećavanjem napona datim signal generatorom. Slično ovome, iCCD fotografije prikazuju pražnjenje koje postepeno raste sa intenzitetom emisije i širinom mlaza u opsegu od 4 V do 4.6 V primenjenog napona. U interval od 4.6 do 4.7 V dolazi do značajnije varijacije pražnjenja, koje postaje vidno sjajnije i šire. Dalje povećavanje primenjenog napona ne indukuje porast snimljenog napona ali vodi do širenja kanala MPM-a kao i do porasta emisije svetlosti koju možemo povezati sa snimljenim porastom signala struje.

S obzirom da se provodljivost jonizovanog kanala po svojoj prilici povećava sa naponom, lako je pretpostaviti da se ovaj prelazak na tip „poput difuznog“ pražnjenje kao u našem slučaju događa između 4.6 i 4.7 V napona zadatog signal generatorom. Ovo ponašanje se može objasniti ulogom provodnog kanala plazme koji povezuje izvor plazme i uzemljenu metu; jonizovani kanal se u stvari može posmatrati kao veza male otpornosti između izvora i mete.



Slika 5.15. ICCD fotografije ($12.5 \mu\text{s}$ otvor gejta) -a u nespojenom režimu (MPM sa sedam mlaznica, He 2 slm, razmak 9 mm) za različite inicijalne napone.

S druge strane, pad napona pod uticajem impedanse kanala i protoka struje ograničava vrednost koji se snima napajanoj elektrodi. Činjenica da ovaj napon dostiže plato dok se struja povećava govori o tome da se ukupna impedansa plazma kanala smanjuje sa povećanim primenjenim naponom. Ova hipoteza je u saglasnosti sa snimcima iCCD-a koji prikazuju plazma kanal sa povećanim intenzitetom emisije, verovatno zbog povezanosti sa višim stepenom jonizacije i samim tim sa većom provodljivošću.

5.9 Zaključak

U ovom poglavlju opisan je mnogostruki plazmeni mlaz - MPM, izvor plazme koji radi na atmosferskom pritisku. Jedan od glavnih fenomena koji smo želeli da ispitamo bio je uvid u kompleksnu interakciju mlaznica mnogostrukog mlaza. Korišćenjem masenog spektrometra, iCCD kamere i električnih merenja urađena je analiza kojom je pokazano da je varijacijom brzine protoka radnog gasa moguće kompletno promeniti prirodu pražnjenja ovog izvora. Izvučeni su sledeći zaključci:

- Od niza nezavisnih mlaznica moguće je uspostaviti dva različita režima – spojeni i nespojeni, koji sa sobom nose značajne razlike u broju jonizacionih frontova, vremenske evolucije i masenog sastava jona,
- Bogata hemija dugoživećih negativnih jona predstavlja rezultat koji nam sugeriše na različite varijacije usled uticaja mešanja helijuma sa okolnim vazduhom (u spojenom režimu odbroj je za dva reda bio veći u odnosu na nespojeni režim),

- Posmatrana je obrnuta propagaciju sekundarnih strimera od provodne mete ka plazma izvoru i povezana je sa prisustvom zaostalih provodnih jonizovanih kanala koji prate prvi strimer.
- Pojava višestrukih sekundarnih strimera koji su posmatrani u slučaju nespojenog režima što sugerise na ponavljajuću jonizaciju ovih kanala, relativno stabilnih u prostoru, tokom rada MPM-a,
- Kod spojenog režima, nelinearno ponašanje pražnjenja je uočeno pri povećavanju inicijalnog napona,
- Nakon određene vrednosti inicijalnog napona dolazi do pojave tinjavog pražnjenja karakterisanog strujom visokog intenziteta i stabilnim naponom na napajanoj elektrodi. Ovaj rezultat implicira da formiranje plazma kanala dovoljno visoke provodnosti koji se ponaša kao prolazna konekcija između uzemljene mete i izvora plazme.

6 DIFUZNO PRAŽNENJE KOPLANARNE POVRŠINSKE BARIJERE – DCSBD

6.1 Uvod

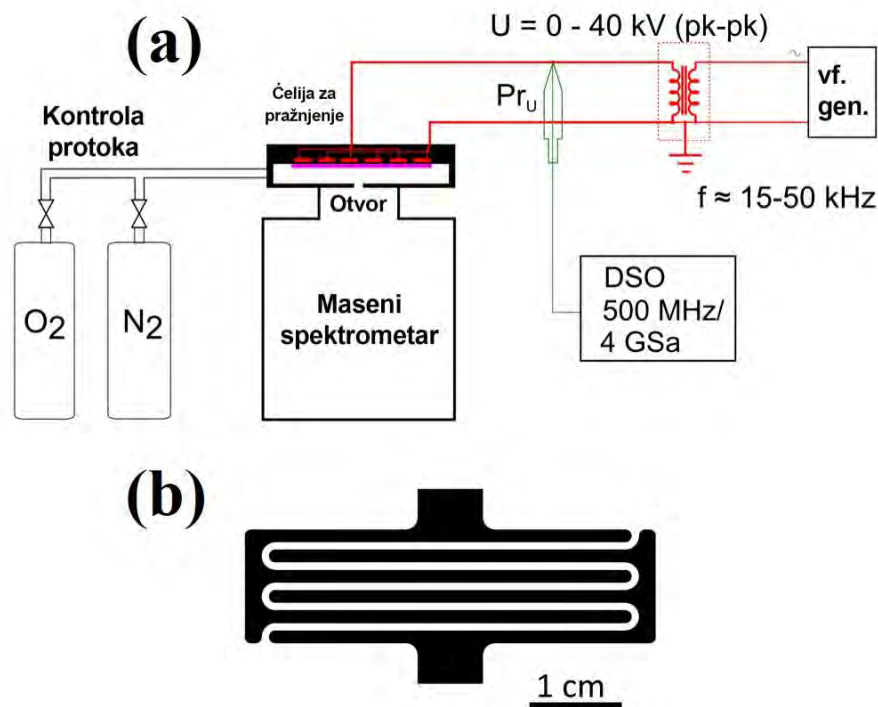
Za razliku od prethodna dva poglavlja gde je obrađena masena spektrometrija dva različita tipa plazmenih mlazova na atmosferskom pritisku u ovom poglavlju ćemo uraditi masenu analizu izvora plazme, tzv. difuzno pražnjenje koplanarne površinske barijere (*eng. diffuse coplanar surface barrier discharge* - DCSBD). DCSBD, izvor niskotemperaturne plazme koji funkcioniše na atmosferskom pritisku, je dizajniran i konstruisan u odseku za Plazma nanotehnologije i bioaplikacije na Masarik Univerzitetu u Brnu, Republika Češka. Tokom zajedničke kolaboracije sa kolegama sa Masarik Univerziteta urađena je masena spektrometrija DCSBD-a u Laboratoriji za gasnu elektroniku, Instituta za fiziku u Beogradu (Čeh et al., 2017).

Dielektrična barijerna pražnjenja (*eng. Dielectric barrier discharges* - DBDs) spadaju u grupu izvora atmosferskih plazmi koje već dugo imaju širok spektar primena u industriji (Kogelschatz et al., 2003). Veliki broj istraživanja urađen je da bi se ovi tipovi plazme što bolje razumeli i lakše kontrolisali variranjem parametara koji utiču na njihov rad (Bruggeman et al., 2013). DCSBD koji ćemo koristiti u eksperimentu predstavlja posebnu vrstu DBD izvora kod kojeg su elektrode u potpunosti ugrađene unutar dielektrika u plan-paralelnoj geometriji (Šimor et al., 2002). Pražnjenje kod ovog izvora se pojavljuje u formi mnoštva individualnih mikropražnjenja iznad dielektrične ploče (Čeh et al., 2015). Ova mikropražnjenja dovode do pojave submilimetarski tankog mikroskopskog difuznog sloja guste plazme velike snage koja nije u kontaktu sa metalnom elektrodom (Šimor et al., 2002). Ove osobine kao i mogućnost korišćena vazduha za dobijanje pražnjenja na atmosferi svrstava DCSBD izvor u ozbiljnog kandidata za primene u brznoj linijskoj industriji (Černák et al., 2011). Međutim, za svaki izvor pražnjenja, koji bi mogao da se koristi u primenama, je potrebno uraditi temeljna istraživanja (Čeh et al., 2009).

Kada su u pitanju plazme koje rade na niskom ili na atmosferskom pritisku od velikog je značaja poznavanje njihovog hemijskog sastava pre bilo kakve primene. U ovom radu korišćićemo Hiden HPR60 MBMS maseni spektrometar za analizu DCSBD-a za nekoliko različitih smeša azota i kiseonika gde će posebna pažnja biti usmerena na istraživanju ponašanja reaktivnih azotnih i kiseoničnih vrsta (*eng. reactive oxygen and nitrogen species* - RONS).

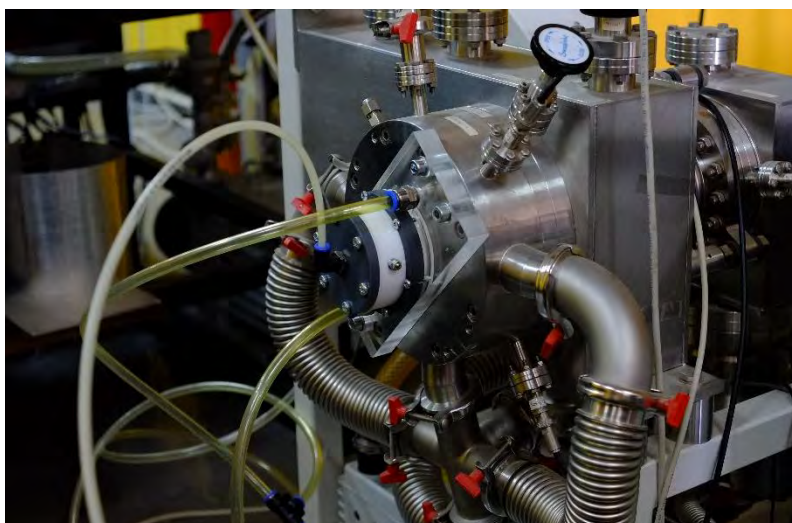
6.2 Eksperimentalna postavka

DCSBD izvor čija je masena analiza urađena u ovoj disertaciji posebno je dizajniran da bude manjih dimenzija, ukupne površine od 7.5 cm². Tri glavna dela od kojih je izvor sačinjen su ćelija za pražnjenje, uljani sistem za hlađenje i visokonaponsko napajanje. Ćelija za pražnjenje se sastoji od šupljeg polimernog cilindra koji se hladi uljem koje protiče kroz sistem za hlađenje. DCSBD element je instaliran na gornji deo cilindra i sastoji se od keramičke ploče (96% od glinice - Al₂O₃) sa sistemom elektroda koje su usađene u nju. Shema eksperimentalne postavke prikazana je na Slici 6.1 (a). Dimenzije keramičke ploče koje su korišćene za ovaj eksperiment su: površina 80×40 mm i debljine 0.2 mm. Tri paralelna (koplanarna) para elektroda dužine 45 mm, širine 1.5 mm i međusobnog rastojanja (razmaka) 1.0 mm sačinjavaju sistem elektroda (Slika 6.1 (b)). Plazma je generisana na vrhu keramičke ploče korišćenjem naizmeničnog sinusnog talasa visokog napona amplitude 11.2 kV i promenljive frekvencije (15 kHz, 30 kHz i 50 kHz). Visokonaponsko napajanje je napravljeno u laboratoriji na Masarik Univerzitetu, a monitoring električnih parametara je izveden korišćenjem osciloskopa Agilent DSO6052A i visokonaponske sonde Tektronix P6015A (na slici 1 (a) je sonda označena kao Pr_u).



Slika 6.1 (a) Shematski prikaz eksperimentalne postavke; (b) Geometrija sistema elektroda DCSBD-a. (Čeh *et al.*, 2017.)

Širina cilindrične komore u kojoj se stvaralo pražnjenje je bila 10 cm a visina 3 mm, što je obezbeđivalo kontrolisan rad u zadatom radnom gasu. U ovoj studiji korišćena je smeša azota i kiseonika (čistoće 5.0) gde je ukupan protok od 500 sccm održavan konstantnim tokom celog eksperimenta. Za kontrolu protoka korišćena su dva protokomera: OMEGA FMA5518 (za azot) i Bronckhorst MV-302 (za kiseonik). Posebno napravljena komora u kojoj se nalazila ćelija za pražnjenje bila je povezana (zaptivena uz pomoć O prstena od silikonske gume) na prednju ploču masenog spektrometra i centrirana je tačno ispred otvora za uzorkovanje (Slika 6.2). Takođe, prednja ploča masenog spektrometra (od nerđajućeg čelika) bila je preventivno zaštićena PET folijom debljine 0.3 mm da ne bi slučajno došlo do varničenja između napajane electrode i nje (Maletić *et al.*, 2012). Uloga folije kao dielektričnog lejera bila je obezbeđivanje homogenog pražnjenja između izvora i otvora za uzorkovanje. Tokom merenja neutrala dobijenih tokom rada izvora nisu primećene razlike kada je folija korišćena i tokom probnih merenja kada je nismo koristili.



Slika 6.2 Fotografija komore sa ćelijom za pražnjenje DCSBD-a koja je povezana na otvor za uzorkovanje HIDEN HPR60 masenog spektrometra.

U ovakvoj postavci bilo je moguće održavati pražnjenje u kontrolisanoj radnoj atmosferi prilikom koje smo analizirali sastav plazme. Odnosi azota i kiseonika u smeši radnog gasa su varirani na sledeći način: N₂/O₂: 100/0%, 80/20%, 60/40%, 50/50%, 40/60% i 20/80%. Dielektrična pločica DCSBD-a se nalazila na rastojanju od 2 mm u odnosu na otvor za uzorkovanje da bismo izbegli varničenje ka uzemljenoj prednjoj ploči masenog spektrometra. Konfiguracija otvora za uzorkovanje je bila sledeća: inlet = 0.3 mm, konus 1 = 0.3 mm, konus 2 = 1 mm.

Merenja masenim spektrometrom su izvedena korišćenjem tzv. Analize Rezidualnog Gasa (*eng. Residual Gas Analyzer - RGA*). RGA predstavlja tehniku merenja MBMS-a u kojoj se koristi unutrašnja jonizaciona komora pomoću koje se neutralne čestice prevode u stanje jonizovanog gasa i nakon toga bivaju detektovane. Dva posebna režima merenja su izvršena u ovom eksperimentu: 1. Snimanje integrisanih masenih spektara neutrala i 2. Konstantno praćenje selektovanih vrsta (MID tehnika u kojoj se prati vremenska sekvenca odabranih neutrala ili jona).

Analiza mase plazme na atmosferskom pritisku predstavlja veliki izazov. U principu, mogu se usvojiti dva pristupa za analizu proizvodnje radikala u proučavanom sistemu plazme i to u zavisnosti od energije elektrona korišćenih za jonizaciju analiziranog gasa. Merenja se mogu izvoditi pri a) određenoj fiksnoj energiji jonizujućih elektrona; ili b) variranjem energije jonizujućih elektrona - merenje „filtriranjem energije“. Oba pristupa nude prednosti, kao i ozbiljne nedostatke i stoga ćemo ih prodiskutovati.

Ako se koristi fiksna energija jonizujućih elektrona, informacije o poreklu otkrivenih vrsta se gube, osim ako se posebna pažnja ne posveti protokolu merenja. To je zbog toga što je energija jonizujućih elektrona obično vrednosti od 70 eV, što je dovoljan nivo za jonizaciju, ali i disocijaciju otkrivenih vrsta, uzrokuje detekciju signala masenih spektara radikala, koji se proizvode ne samo u ispitivanoj plazmi sistema, nego i u jonizatoru samog masenog spektrometra. Uprkos ovom nedostatku, ovo je izabrani režim za prikazana merenja, jer prinos jonizacije može biti znatno veći u ovom režimu što rezultuje dovoljnim odnosom signal-šum otkrivenih vrsta. U daljem tekstu biće prikazan protokol merenja i napomene o opravdanosti zaključaka zasnovanih na ovom pristupu.

Drugi pristup je usvajanje „energijski filtriranih“ merenja, pri kojima se energija jonizujućih elektrona postepeno povećava i signal određenog radikala prati u masenom spektru. Ključna prednost ovog pristupa je izbegavanje parazitskih signala masenog spektra, zadržavajući energiju udarajućih elektrona znatno ispod praga disocijacije vrsta od kojih bi određeni radikal mogao da se stvori. Rezultujući energijski-zavisan signal mogao bi se koristiti za razlikovanje radikala stvorenih u samom instrumentu od onih koji potiču iz proučavanog sistema plazme. Ali merenja filtriranjem energije imaju i ozbiljne

nedostatke, koji su nas sprečili da usvojimo ovu metodu u našim merenjima i o kojima će biti reči u daljem tekstu.

Merenja masene spektroskopije sa energijskim filtriranjem uspešno su koristili koautori za praćenje vrsta generisanih u plazmi (npr. (Maletić *et al.*, 2012; Škoro *et al.*, 2014; Spasić *et al.*, 2014)), ali ova tehnika se mora primeniti nedvosmisleno uz pažljivo uzimanje u obzir ograničenja metode. Polazeći od pragova za disocijativnu jonizaciju molekula N₂ i O₂ koji su 22 eV, odnosno 20 eV (Stoffels *et al.*, 2007; Rapp *et al.*, 1965) i od pragova za elektronsku disocijaciju (kojom bi se proizveli neutralni atomi u osnovnom stanju) koji su 9.75 eV i 5.11 eV respektivno (Frost *et al.*, 1956) vidimo da je raspon energije razuman, a ipak je prilično uzak. U tom energijskom opsegu može doći do jonizacije atoma (pragovi jonizacije su 14.5 odnosno 13.6 eV), ali poprečni presek ispod 20 eV je i dalje prilično mali, što obezbeđuje malu verovatnoću jonizacije fragmenata nastalih u jonizatoru.

Ako uzmemo u obzir molekul NO, koji je dominirajući produkt neravnotežene plazme na atmosferskom pritisku (Stoffels *et al.*, 2006), sa svojim pragovima za disocijativnu jonizaciju (22 eV, odnosno 21 eV (Stoffels *et al.*, 2007; Hogness i Lunn, 1927)), ovaj opseg bi se mogao naći. Atomi N i O, stvoreni „samo“ u pražnjenju, moraju biti između pragova jonizacije N i O i <20 eV. Uzimajući u obzir preseke za jonizaciju N i O atoma, možemo videti da smanjenjem energijskog opsega jonizujućih elektrona (na primer 15–19 eV) imamo nisku efikasnost jonizacije. Poprečni presek jonizacije pri 19 eV za oba atoma je u rasponu od $0.2\text{--}0.3 \times 10^{-20} \text{ m}^2$, što je samo oko 10% maksimuma poprečnog preseka. To ukazuje da je u ovom opsegu energija efikasnost detekcije N i O atoma stvorenih u plazmi prilično niska i sklona potcenjivanju stvarnog broja neutrala u plazmi.

Ovde se takođe može pomenuti molekul N₂O koji je stvoren u plazmi ((Stoffels *et al.*, 2006), Slika 6.5 u ovoj glavi). Pragovi disocijacije za ovaj molekul i stvaranje O i N atoma počinju već na oko 8 eV (Mason i Newell, 1989; Allcock i McConkey *et al.*, 1978). To znači da izmereni signal za N i O atome za merenja u opsegu od, na primer 15 eV do 19 eV, takođe obuhvata i atome stvorene u instrumentu disocijacijom N₂O. Isto slučaj je i sa molekulima CO₂ sa pragom disocijacije od 11 eV (McConkey *et al.*, 2008). Ovih molekula nema previše u atmosferskom pražnjenju, ali ipak mogu doprineti izmerenom signalu i izazvati određenu grešku prilikom analize. Pored toga, mora se uzeti u obzir i termalna razgradnja vrsta. Čak i ako merimo u 'sigurnom' opsegu energija elektrona, temperatura filameta koji emituje elektron je oko 2000 K, što će dovesti do termalne razgradnje molekula i doprinosa signalu neutralnih atoma. Ovo je više pitanje za O atome nego za N atome (Stoffels *et al.*, 2007). Da zaključimo, postoji nekoliko ograničenja tehnike energijskog profila najvažnije je u ovom slučaju istaći njenu lošu efikasnost. Pražnjenje proučavano u ovom radu je difuzno planarno pražnjenje sa malim protokom čestica u bilo kojoj tački, ali sa velikom ukupnom proizvodnjom radikala i atoma. Ovaj energijski pristup merenja, u slučaju ispitivanog izvora DCSBD plazme, se učinio neupotrebljivim, jer je dokazano da je signal tokom eksperimenata DCSBD sa energijskom rezolucijom suviše nizak, da bi se razlikovao od šuma pozadine. S druge strane, izvor sa svojom velikom površinom i ostalim parametrima pruža brojne prednosti u primenama ali svakako je značajno uraditi, dijagnostiku različitih vrsta proizvedenih u plazmi.

Tokom izvođenja eksperimenta energija elektrona koja je korišćena za jonizaciju neutrala bila je podešena na vrednost od 70 eV. U svim eksperimentima meren je pozadinski signal (molekularni snop je blokiran zatvaranjem tzv. SwageLok ventila) koji je kasnije oduziman od ukupne vrednosti signala, kako bi se dobila realna vrednost signala analiziranog gasa koji potiče iz pražnjenja. Maseni spektar neutrala prikazan je u opsegu od 1 amu do 100 amu. Rezultati su dobijeni za različite setove parametara koji su varirani (smeša gasova i frekvencija primenjenog napona).

Da bi se dobio bolji uvid u ponašanje vrsta koje generišu plazmu, vršena su merenja „vremenske sekvence“ visoke osetljivosti pomoću režima akvizicije (MID scan). Za sva merenja primenjen je sledeći postupak: prvo je ulaz molekularnog snopa analizatora mase zaštićen (čime je blokiran signal akvizicije pozadinskog signala plazme), zatim je otvoren ulaz MBMS-a i sistemu je omogućeno da dostigne

ravnotežu (≈ 2 min). Nakon toga, plazma je uključena i sistem je ponovo pušten da dostigne ravnotežu (≈ 2 min). Tada je pražnjenje isključeno i nakon postizanja konačne ravnoteže eksperiment je završen. Čitav postupak MID skeniranja trajao je približno. 10–12 min. Uključivanje i isključivanje plazme dovodi do promena u proizvodnji određenih radikala pri različitim eksperimentalnim uslovima. Utvrđene su ukupne varijacije za O_3 i NO radikale za unapred odabranu struju na jonizacionom filamentu od $50 \mu A$.

6.3 Rezultati masene spektrometrije

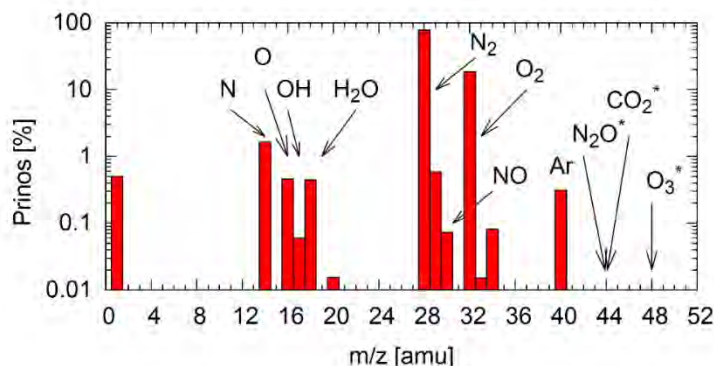
Rezultate smo podelili u dve grupe: 1. Pregled masenih spektara i 2. Merenja vremenske sekvence radikala od interesa.

Iz čitavog skupa proučenih uslova pražnjenja odabran je ograničeni podskup za prezentaciju. Za analizu spektralnih istraživanja izabrani su tipični uslovi pražnjenja koji se koriste za obradu materijala plazmom, tj. sastav radnog gasa od 80% N_2 i 20% O_2 , kao zamena ambijentalnog vazduha. Visokonaponska amplituda od 11,2 kV na frekvenciji od 15 kHz korišćena je kao tipični radni uslovi pražnjenja DCSBD. Ovi rezultati se mogu uporediti sa prethodnim merenjima izvršenim na DCSBD sistemu industrijske veličine u uslovima ambijentalnog vazduha 2008. godine (Lazović *et al.*, 2008). Za merenja vremenske sekvence odabranih radikala, dva radikala (O_3 i NO) su odabrana kao predstavnici isparljivih jedinjenja koja se moraju kontrolisati i nadgledati u industrijskom okruženju zbog njihovih bioloških efekata.

U ovoj disertaciji biće prikazani set pražnjenja za frekvenciju od 15 kHz pri konstantom visokom naponu od 11.2 kV smeše N_2/O_2 pri odnosu 80/20. Kao primer najtipičnijeg merenja obratićemo pažnju na smešu azota i kiseonika pri odnosu 80% N_2 i 20% O_2 (kao zamena za ambijentalni vazduh). Što se tiče merenja pri kojima je praćena vremenska sekvenca pratili smo sledeće radikale: N , O , OH , N_2 , NO , O_2 , NO_2 , N_2O ili CO_2 i O_3 .

6.4 Pregled masenog spektra DCSBD-a – prinosi radikala

Na Slici 6.3 prikazan je prinos masenih spektara neutrala snimljen masenim spektrometrom tokom pražnjenja DCSBD-a varirajući tri različite frekvencije i šest različitih smeša radnog gasa. Prinosi predstavljaju relativne odnose raspodela izmerenih vrsta. Za računanje prinosa korišćena je formula za prinos koja je već objašnjena u prethodnoj glavi „2.4.3 Merenje sastava atmosfere“. Ovi prinosi daju nam uvid relativnog odnosa masa u kompoziciji gasa. Korišćena je logaritamska skala da bi se uočile sve vrste čije bi praćenje bilo od značaja.

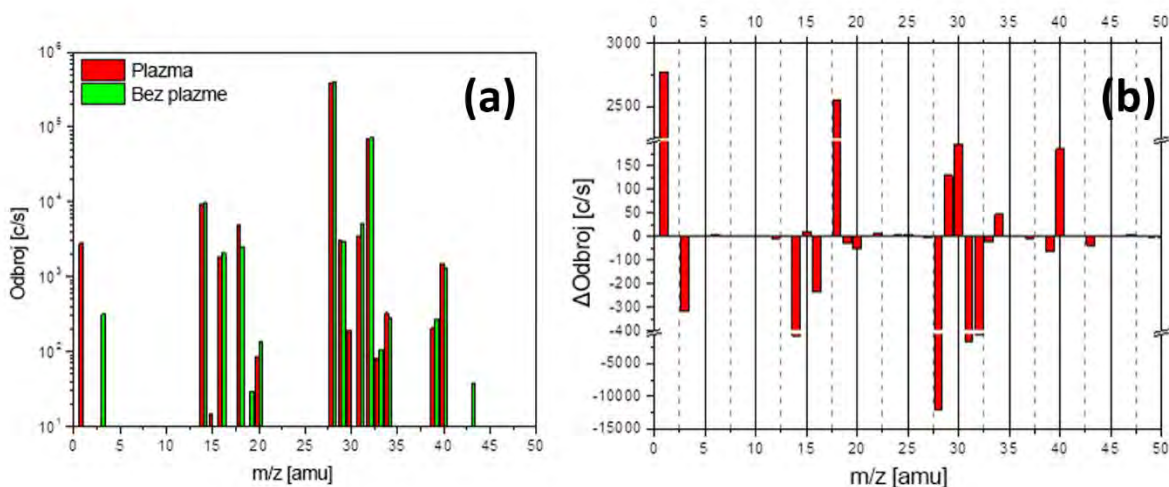


Slika 6.3 Maseni spektar radnog gasa za sledeće uslove: odnos N_2/O_2 je 80/20, pobudna frekvencija od 15 kHz i amplitudi napona 11.2 kV. Spektar je skraćen na 52 amu, zbog nedostatka signala koji bi se detektovao u datim eksperimentalnim uslovima. Vrste označene zvezdicom su blizu granice otkrivanja u datim uslovima (Čeh *et al.*, 2017).

Prinosi snimljenih masenih spektara mogu nam dati relativni sastav gasa. Iz spektra na slici 6.3 vidljivo je da su dominantne vrste u pražnjenju bili molekularni azot i molekularni kiseonik. Atomi azota i atomi kiseonika prisutni su kao rezultat reakcija plazme, kao i NO radikal. OH radikal je takođe prisutan zbog reakcija plazme, kao i disocijacije vode u masenom spektrometru. Imajući u vidu da su spektri dobijeni za elektronske energije od 70 eV, neophodno je upoređivanje masenih spektara sa i bez uključenog pražnjenja.

Na Slici 6.4 prikazan je uticaj pražnjenja na sastav gasa. Slika 6.4 (a) prikazuje poređenje sastava radnog gasa koji je pod uticajem pražnjenja (označen sa "Plazma") i gasa koji je dobijen kada je pražnjenje bilo ugašeno (označen sa "bez plazme"). Iako su prinosi vrsta kao što su N, O ili NO relativno visoki, relativni sastav gasa kod ostalih vrsta sa/bez plazme razlikuje se vrlo neznatno, obično manje od 1% relativnog sastava (videti Slika 6.4 (b)). To bi moglo najviše doprineti činjenici da je najviše detektovanih vrsta proizvedeno u jonizacionoj komori. Sistem takođe nije bio u mogućnosti da uzorkuje gas direktno iz plazma sloja DCSBD. Uzorkovanje atmosfere pražnjenja mora se realizovati održavanjem minimalnog rastojanja između pražnjenja DCSBD i uzemljene prednje ploče MBMS da bi se izbeglo varničenje ka uzemljenoj ploči masenog spektrometra. Tako se mogu javiti i post-plazma reakcije direktnih produkata DCSBD-a plazma-hemijskih reakcija sa okolnim gasom u plazma komori. Ovo ograničava bolje razumevanje reakcija direktno u pražnjenju DCSBD-a, ali ne ograničava svrhu prezentovanog istraživanja, tj. istraživanje industrijski važnih nusproizvoda dobijenih u primenama DCSBD-a.

Uprkos velikom pozadinskom signalu koji je tokom RGA analize bio prilično visok ipak se mogao steći uvid u uticaj plazme na sastav gasa.



Slika 6.4 Primer masenog spektra neutrala pri frekvenciji od 15 kHz i primenjenom naponu od 11.2 kV: (a) - kada DCSBD ima uključeno pražnjenje i kada je pražnjenje isključeno; (b) – odgovarajući odbroj razlike signala sa plazmom i bez plazme.

Oduzimanjem signala sa i bez plazme dobija se apsolutna razlika koja je prikazana na Slici 4. (b). Pozitivna razlika predstavlja povećanje signala radikala nakon proboja u plazmi. Može se primetiti da se sastav gasa značajno razlikuje za N₂ i O₂ signale, jer u pražnjenju dolazi do dekompozicije tih molekula. Povećanje NO ukazuje na proizvodnju tih radikala u plazmi.

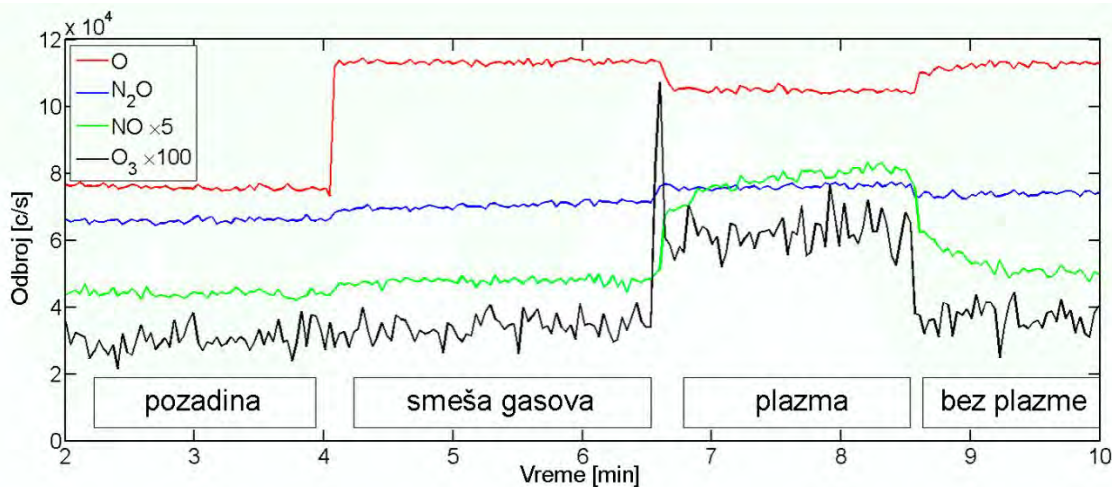
Do identičnog zaključka da se NO obilno stvara u plazmi, a da se N₂ gubi usled disocijacije molekula došli su i Stoffels i saradnici (Stoffels *et al.*, 2006; Stoffels *et al.*, 2007) s tim što su oni u svojoj studiji kao izvor koristili plazma iglu čiju dijagnostiku su radili na identičnom masenom spektrometru HIDDEN HPR60. Međutim, u masenoj analizi plazma igle oni nisu detektovali signal O₃, što je slučaj kod DCSBD-a. Zbog značajnog signala O₃ urađeno je i merenje molekula ozona korišćenjem skeniranja vremenske

sekvence (režim rada HPR-60 – eng. MID scan). Iako prisustvo O₃ molekula nije poželjno u spektru plazme koja ima potencijal za primene, pogotovo pri radu u blizini ljudi, merenja sa različitim osetljivostima vremenske sekvence će osim O₃ molekula dati uvid i ostalih RONS radikala u realnom vremenu, što i jeste cilj da se pokaže.

6.5 Merenja vremenske sekvence radikala – MID skeniranje

U prethodnom poglavlju mogli smo da steknemo uvid najzastupljenijih vrsta iz integriranih masenih spektara dobijenih usrednjavanjem tri sukcesivna ciklusa tokom RGA akvizicije. Ovi maseni spektri će nam poslužiti pri odabiru vrsta koje će biti praćene u realnom vremenu, tako što ćemo za vrste sa manjim odbrojem koristiti struju emisije elektrona 50 μA u jonizacionoj komori, dok će za vrste koje su imale dobar signal kod integriranih spektara biti korišćena struja od 5 μA. Za ovaj tip merenja odabrali smo sledeće vrste: 1. vrste merene pri struji emisije 5 μA: N₂, O₂, OH; i 2. vrste merene pri struji emisije 50 μA : N, O, NO, NO₂ (i/ili CO₂), N₂O i O₃.

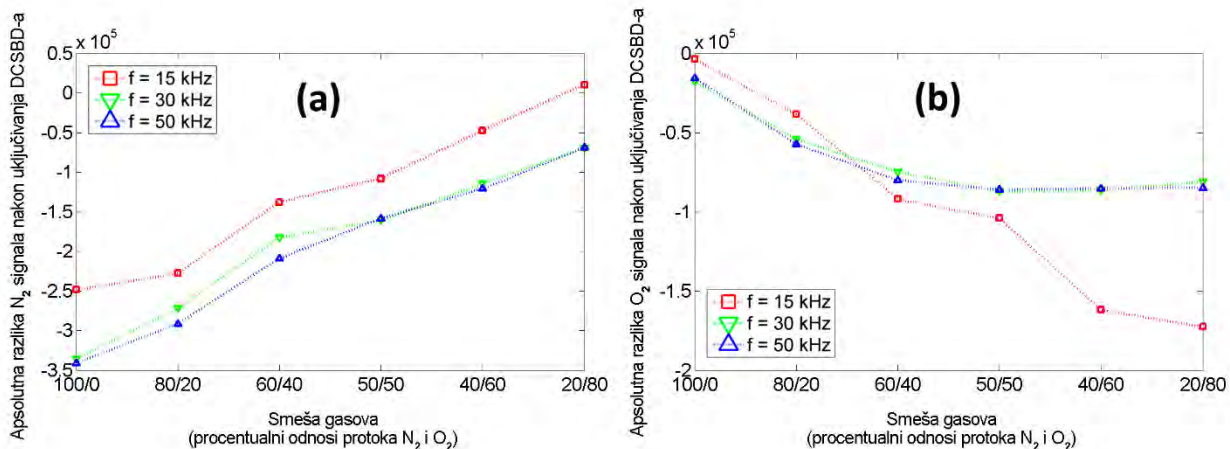
Slika 6.5 prikazuje tipični primer „MID skeniranja“, tj. merenja „vremenske sekvence“ odabranih vrsta. Mogu se identifikovati četiri etape merenja (videti pravougaonike sa natpisima na Slici 6.5): tragovi rezidualnog/zaostalog gasa unutar masenog spektrometra (označeno kao „pozadina“) i sastav radnog gasa koji je identifikovan (označen kao „smeša gasova“) i uticaj prisustva plazme (s oznakom „plazma“, odnosno „bez plazme“). Za naredna izračunavanja korišćene su apsolutne razlike usrednjenih vrednosti signala „plazma“ i „bez plazme“. Na slici 6.7 na početku i na kraju faze ‘plazme’ može se videti inercija masenog signala. Pripisivanje ovog ponašanja parametrima pražnjenja ili celog sistema merenja u ovom trenutku nije jasno i biće potrebne dalje analize.



Slika 6.5 Primer „MID skeniranje“ merenja vremenske sekvence radikala pri kojem se konstantno prate selektovane vrste u realnom vremenu, četiri etape su označene pravougaonicima sa natpisima.

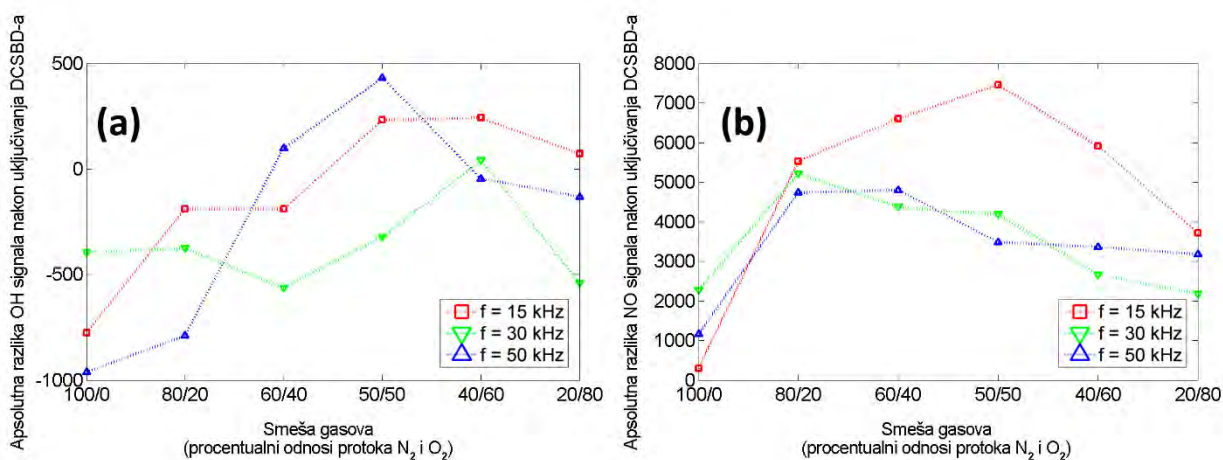
Parametri koji su korišćeni kod ovog merenja su sledeći: smeša radnog gasa 80% N₂ i 20% O₂, frekvencija 15 kHz, struja emisije elektrona 50 μA.

Rezultati procesuiranih MID skeniranja za molekule azota i kiseonika dati su na Slici 6.6 U slučaju molekula azota, Slika 6.6 (a), može se primetiti trend linearnog povećanja odbroja sa promenom smeše. Dodavanje kiseonika u smešu sa azotom smanjuje se disocijacija N₂ radikala. Najveća zastupljenost molekulskog azota se može pripisati frekvenciji od 15 kHz. Za razliku od N₂, molekul kiseonika pokazuje suprotan trend gde se sa povećanjem procenta kiseonika u smeši sa azotom smanjuje odbroj O₂. Smanjenje frekvencije na 15 kHz dovodi do bržeg pada O₂ pri smešama N₂/O₂ 40/60 i 20/80 u odnosu na druge dve frekvencije koje su prilično sličnog odbroja.



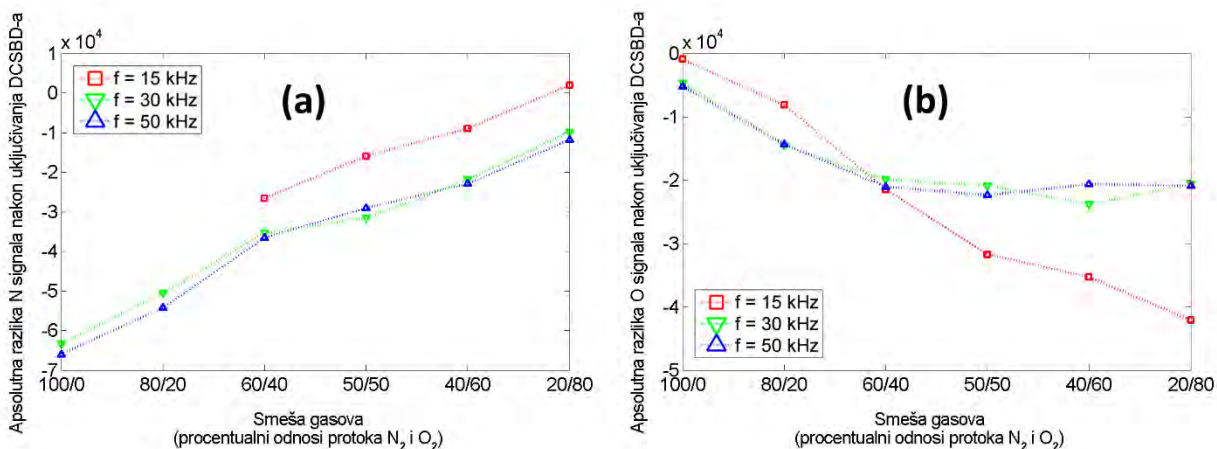
Slika 6.6 Apsolutna razlika signala nakon paljenja plazme u komori za pražnjenje za N₂ (a) i O₂ (b) kao rezultat analize signala „MID skeniranje“ (merenje „vremenske sekvence“). Za prikazane molekulske vrste data je zavisnost njihove proizvodnje od variranih smeša radnog gasa.

Slika 6.7 predstavlja molekule OH i NO snimljenih tokom MID skeniranja. Već je rečeno da proizvodnja OH koja nije velika, ali ni beznačajna, najverovatnije potiče od dekompozicije vode deponovane u komori masenog spektrometra i ovaj rezultat se mora uzeti sa rezervom. Maksimum proizvodnje OH (Slika 6.7 (a)) je dobijen pri odnosu smeše 50/50 radnog gasa i frekvenciji od 50 kHz. Što se tiče azot-monoksida njegovo ponašanje u odnosu na varijacije u frekvenciji i gasnoj smeši dato je na Slici 6.7 (b). Proizvodnja NO je značajna i mogu se primetiti dva maksimuma koji se menjaju u zavisnosti od frekvencije. Proizvodnja NO je najveća na najnižoj frekvenciji. Maksimalna proizvodnja azot-monoksida za najnižu frekvenciju je pri smeši gasova odnosa N₂/O₂ 50/50. U slučaju 30 kHz i 50 kHz, maksimalna proizvodnja NO je za gasnu smešu 80/20. Za detaljnu analizu proizvodnje RONS-a i ravnotežnih koncentracija potrebno je uraditi kompletan plazma-kinetički model, što je izvan obima ovog rada.



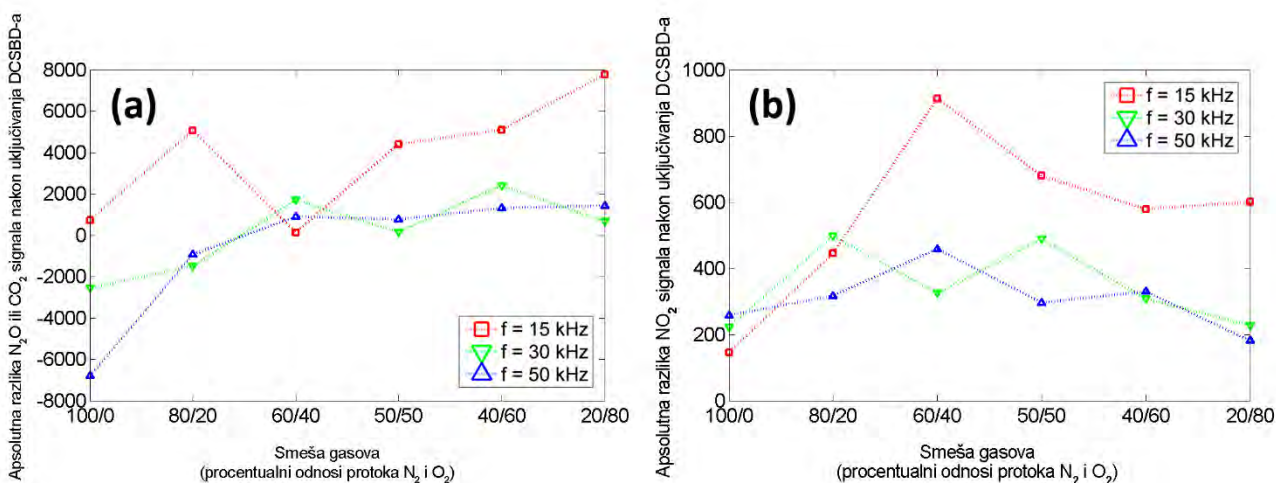
Slika 6.7 Apsolutna razlika signala nakon paljenja plazme u komori za pražnjenje za OH (a) i NO (b) kao rezultat analize signala „MID skeniranje“ (merenje „vremenske sekvence“). Za prikazane molekulske vrste data je zavisnost njihove proizvodnje od variranih smeša radnog gasa.

Na narednoj Slici 6.8 nalaze se atomske vrste azota i kiseonika. Ono što se ovde može zaključiti jeste da obe vrste pokazuju identičan trend koji je već opisan za molekulske vrste N₂ i O₂ sa bitnom razlikom gde su atomske vrste izmerene za vrednost odbroja koji je za red veličine manji od molekularnih vrsta.



Slika 6.8 Apsolutna razlika signala nakon paljenja plazme u komori za pražnjenje za N (a) i O (b) kao rezultat analize signala „MID skeniranje“ (merenje „vremenske sekvence“). Za prikazane atomske vrste data je zavisnost njihove proizvodnje od variranih smeša radnog gasa.

Kod radikala azotsuboksida N_2O (zbog identične mase koju poseduje i CO_2 moguće je da dolazi do superponiranja signala) na Slici 6.9 (a) može se primetiti značajnija produkcija pri frekvenciji od 15 kHz sa tendencijom linearnog rasta usled povećavanja molekula kiseonika u smeši sa azotom. Na Slici 6.9 (b) prikazan je radikal azotdioksida kod kojeg se maksimum proizvodnje postiže za odnos smeše gasova pri odnosu 60/40 i pri najnižoj frekvenciji od 15 kHz. Za druge dve frekvencije prikazan signal je četiri puta manji od navedenog maksimuma i prilično je uniforman za sve smeše radnog gasa koji smo koristili.

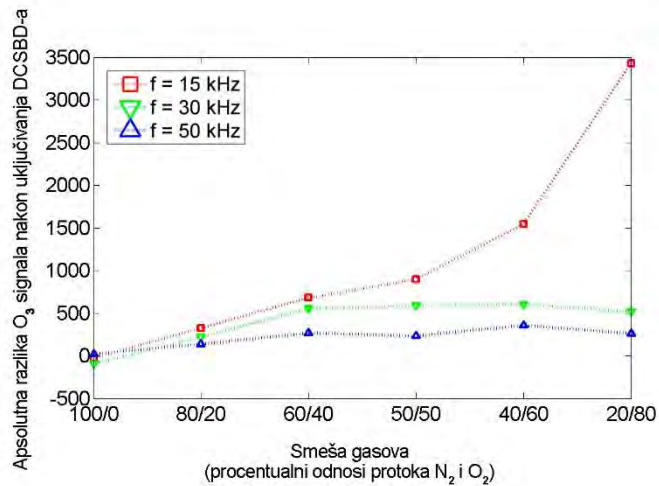


Slika 6.9 Apsolutna razlika signala nakon paljenja plazme u komori za pražnjenje za N_2O/CO_2 (a) i NO_2 (b) kao rezultat analize signala „MID skeniranje“ (merenje „vremenske sekvence“). Za prikazane molekulske vrste data je zavisnost njihove proizvodnje od variranih smeša radnog gasa.

Slika 6.10 prikazuje rezultate procesuirane vremenske sekvence pri datim uslovima za ozon. Pad proizvodnje ozona (Slika 6.10) sa povećanjem frekvencije pražnjenja jasno je vidljiv pri smešama radnog gasa bogatijih kiseonikom. Isti efekat smanjenja proizvodnje ozona sa porastom frekvencije primećen je i kod Mericam-Bourdet i saradnici (Mericam-Bourdet *et al.*, 2012), ali maksimalna frekvencija koju su oni koristili iznosila je 2 kHz i smanjenje nije bilo toliko izraženo kao u našem slučaju pri masenoj analizi DCSBD-a. Mikropražnjenje DBD-a može se tretirati kao mali reaktor u kome se dešavaju plazma-hemijske reakcije (Kogelschatz, 2003). Pod pretpostavkom da prosečna energija koja se prenosi po

pojedinačnom mikropražnjenju DBD-a delimično zagreva kanal pražnjenja (Rahel' *et al.*, 2016), dolazi do porasta frekvencije koje može dovesti do povećanja prosečne temperature kanala za pražnjenje. Pietsch i Gibalov (Pietsch i Gibalov, 1998) su ustanovili da je povećanje gustine energije povezano sa povećanjem temperature procesuiranog radnog gasa, o čemu hemijske reakcije koje proizvode ozon snažno zavise.

Najverovatniji mehanizmi pada proizvodnje ozona u ispitivanom pražnjenju DCSBD-a mogu se pripisati rastu gustine energije na višim frekvencijama, što dovodi do povećanja temperature gasa direktnim i/ili indirektnim zagrevanjem plazme i/ili kod dužih vremena ekspozicije pražnjenja pri uslovima više frekvencije za isto vreme zadržavanja gasa u komori (konstantan protok gasa).



Slika 6.10 Apsolutna razlika signala nakon paljenja plazme u komori za pražnjenje za O₃ kao rezultat analize signala „MID skeniranje“ (merenje „vremenske sekvence“). Za prikazan atom data je zavisnost njegove proizvodnje od variranih smeša radnog gasa.

6.6 Zaključak

U ovoj glavi obrađena je masena spektrometrija DCSBD pražnjenja malih dimenzija generisanog u uslovima atmosferskog pritiska. Uticaj sastava radnog gasa (šest različitih smeša kiseonika i azota) i variranje tri različite frekvencije pri konstatnom visokom naponu uticali su na proizvodnju reaktivnog kiseonika i vrsta azota (RONS).

- Posebna pažnja je bila usmerena za praćenje N, NO_x ili O₃, jer ove vrste igraju važnu ulogu u interakciji plazme sa živim organizmima i tkivima.
- Rezultati su potvrdili značajne efekte frekvencije visokog napona i odnosa smeše kiseonik/azot na proizvodnju RONS-a.
- Proizvodnja molekula ozona (O₃) i azotnog oksida (NO) bila je najveća na najnižoj frekvenciji od 15 kHz od ispitivanog raspona od 15–50 kHz.
- Ova studija je pokazala da se proizvodnja ovih vrsta može suzbiti ili pojačati izborom frekvencije visokonaponskog izvora kao i smeše gasova radnog gasa.

7 PRIMENE ELEKTRIČNIH PRAŽNENJA NA ATMOSFERSKOM PRITISKU

7.1 Uvod

Pored oblasti u kojima plazma igra nezamenljivu ulogu u industriji kao što su proizvodnja poluprovodničkih uređaja, integrisanih kola, optičkih uređaja, solarnih ćelija, njena uloga u biomedicinskim primenama dobija na svojoj važnosti iz dana u dan. Poslednjih par decenija značajno je povećan razvoj niskotemperaturnih električnih pražnjenja na atmosferskom pritisku koja se koriste u različitim biomedicinskim istraživanjima. Veliki broj kliničkih primena niskotemperaturnih plazmi je bio ispitivan i rezultati tih studija pokazuju obećavajući potencijal za jednu od naučnih oblasti koja je u ekspanziji – Plazma medicinu.

U poređenju sa konvencionalnim termalnim plazmama (Vargo, 2004; Puhlev *et al.*, 2001; Colt, 2006; Raiser, 2006), niskotemperaturne plazme su selektivne prilikom tretmana i prilično bezbedne s obzirom na činjenicu da ne mogu termalnim putem oštetiti tkivo. U ovim plazmama se generišu aktivne vrste, radikali i UV koji se koriste za različite hemijske modifikacije i katalize (Stoffels, 2006; Boagerts *et al.*, 2002) za razliku od termalnih plazmi čija proizvodnja visoke temperature izaziva isušivanje, sagorevanje i opekotine. Idealan tip niskotemperaturnih plazmi za primene u medicini jeste onaj gde je temperatura električnog pražnjenja približna sobnoj temperaturi. U ovom slučaju je termalno oštećenje tkiva svedeno na minimum ili se skroz može isključiti.

Ovde se mogućnosti novih potencijalnih primera značajno povećavaju uključujući sterilizaciju medicinskih instrumenata koja se zasniva na činjenici da je plazma u stanju da ukloni gram-pozitivne i gram-negativne bakterije, gljivice, spore i viruse (Boudam *et al.*, 2006; Fridman *et al.*, 2006; Gadri *et al.*, 2000; Cheng *et al.*, 2006; Shimizu *et al.*, 2006), primene u ćelijskoj biologiji koje su zasnovane na sposobnosti plazme da indukuje ćelijsku smrt, proliferaciju, i utiče na faze u ćelijskim ciklusima. Takođe, veoma su značajne primene u bolničkoj higijeni, tretmanu hroničnih rana, kožnih oboljenja (Fridman *et al.*, 2007; Fridman *et al.*, 2008; Fridman *et al.*, 2006; Reshetov *et al.*, 2000; Shekhter *et al.*, 2005), bolesti zuba (Arora *et al.*, 2014; Kim *et al.*, 2013), za koagulaciju krvi, u kozmetici, u preciznoj hirurgiji (Stoffels, 2006; Priglinger *et al.*, 2005; Stoffels, 2007; Stoffels *et al.*, 2006) za uklanjanje ožiljaka i precizno razdvajanje tkiva bez krvi, kao i druge primene (Laroussi i Lu, 2005; Laroussi *et al.*, 2003; Kolb *et al.*, 2006).

Pre nego što se izvor niskotemperaturne plazme implementira u primene, a posebno ako je slučaj tretiranja uzoraka biološkog porekla, od izuzetne je važnosti uraditi što kompletniju analizu samog izvora. Pre svega se tu podrazumeva bezbednost usled variranja električnih parametara koje je potrebno detaljno ispitati korišćenjem električne karakterizacije kao i ispitivanje sastava plazme što je moguće učiniti korišćenjem optičke emisije spektroskopije kao i masene spektrometrije.

Poznavajući konstituente koji sačinjavaju električno pražnjenje kao i uticanje na njihovu promenu variranjem parametara (promena radnog gasa, promena električnih karakteristika i sl.) pojednostavljuje se dalja analiza tretiranih uzoraka što ima za cilj postizanje dobrih efekata primenom plazme.

7.2 Plazma igla

7.2.1 Uvod

Plazma medicina, plazma u industriji hrane, plazma u agrikulturi, primena plazmi u biologiji, kao i u prečišćavanju zagađenih voda zbog potencijalno velikog uticaja na društvo, predstavlja glavnu pokretačku snagu razvoja, ispitivanja i primene niskotemperaturnih električnih pražnjenja na atmosferskom pritisku (Becker *et al.*, 2005; Machala *et al.*, 2011; Petrović *et al.*, 2012).

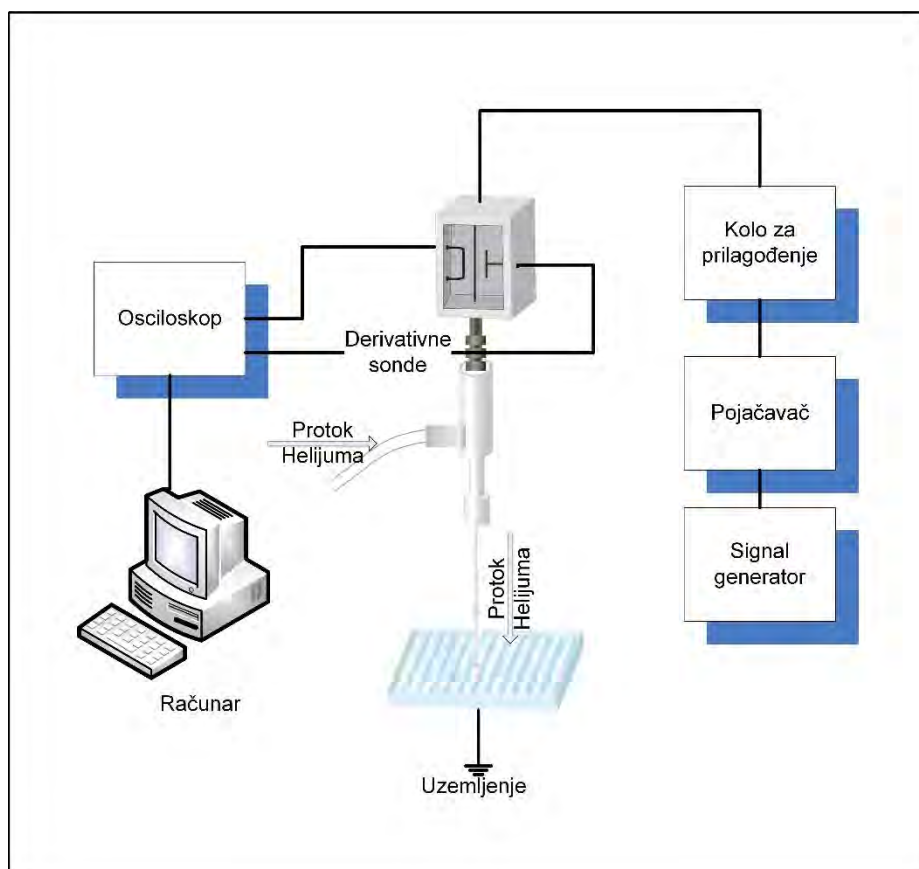
Neki od izvora plazme na atmosferskom pritisku su plazma igla, plazmena baklja, dielektrično barijerno pražnjenje - DBD, KinPen, razne vrste plazmenih mlazova i mikro plazmenog mlaza (Maletić *et al.*, 2012; Moon *et al.*, 2003; Pipa *et al.*, 2012; Fridman *et al.*, 2008; Goree *et al.*, 2006). Iako ovi izvori imaju različite konfiguracije elektroda, frekvencije pobude (jednosmerna, kHz, MHz do mikrotalasne), radni napon, zajednički im je cilj postizanje velike gustine aktivnih vrsta uz održavanje niske temperature gasa i uzoraka (ispod 40°C). Tek tada se električno pražnjenje na atmosferskom pritisku može uspešno primeniti na živo tkivo. Važni rezultati su postignuti u domenu plazma sterilizacije (Lerouge *et al.*, 2001; Hong *et al.*, 2009), zarastanja rana (Nastuta *et al.*, 2011; Isbary *et al.*, 2013; Arndt *et al.*, 2013), koagulacije krvi (Fridman *et al.*, 2005; Kalghatgi *et al.*, 2007), stomatologije (Li *et al.*, 2017; Nakagawa *et al.*, 2006) i uklanjanja raka (Gweon *et al.*, 2011; Wang *et al.*, 2013). Mnogi očigledni efekti su empirijski utemeljeni bez detaljnog i potpunog razumevanja tačnih mehanizama interakcija plazma-ćelija.

Plazma igla koja će biti korišćena u ovoj disertaciji je već pokazala odlične rezultate u primeni na uzorcima biološkog porekla kao što su slatke paprati, mezenhimske matične ćelije iz ljudske krvi, MRSA bakterije, DNK, sterilizacija bakterijskih suspenzija, dentin ljudskog zuba (Puač *et al.*, 2006; Lazović *et al.*, 2010; Miletić *et al.*, 2013; Miletić *et al.*, 2014; Lazović *et al.*, 2014; Puač *et al.*, 2015; Stašić *et al.*, 2018).

Cilj primena u ovoj disertaciji je bio da istraživanje temeljnih aspekata efekta plazme na živo tkivo poput izazivanja apoptoze i nekroze ćelija raka, smanjenja njene rezistivnosti na citostatike. Matične ćelije nisu jedine ćelije koje imaju sposobnost diferencijacije. Biljne ćelije kalusa imaju istu sposobnost i u ovoj disertaciji biće prikazan kratkoročan i dugoročan efekat tretmana niskotemperaturnom plazmom na biljne matične ćelije. Takođe biće prikazani rezultati određivanja veličine inhibicionih zona pri tretmanu meticilin rezistivnih stafilocoka za neravnotežne procese i primene plazmi korišćenjem hladne plazme. Da bismo to uradili, koristili smo plazma iglu koja je detaljno istražena u laboratoriji pomoću masene spektrometrije, derivativnih sondi i ICCD kamere (Puač *et al.*, 2006).

7.2.2 Eksperimentalna postavka

Eksperimentalna postavka plazma igle koju smo koristili u bio-medicinskim primenama, o kojima će biti reči u sledećim poglavljima, prikazana je na Slici 7.1. Telo plazma igle sačinjeno je od Teflona i staklene cevčice od Pyrexa (spoljašnjeg prečnika 6 mm i unutrašnjeg prečnika 4 mm). Električni konektori su ušrafljeni sa gornje strane tela plazma igle i oni služe za direktnu konekciju i napajanje centralne elektrode od volframa (prečnika 0.5 mm) koja se nalazi unutar keramičke cevčice koja prolazi kroz celu staklenu cevčicu do njenog ruba. Uloga keramičke cevčice jeste da spreči pojavu pražnjenja unutar staklene cevčice. Centralna elektroda izlazi izvan keramičke i staklene cevčice približno 1-2 mm i usled pražnjenja plazma se formira u sfernom obliku male zapremine na vrhu elektrode.



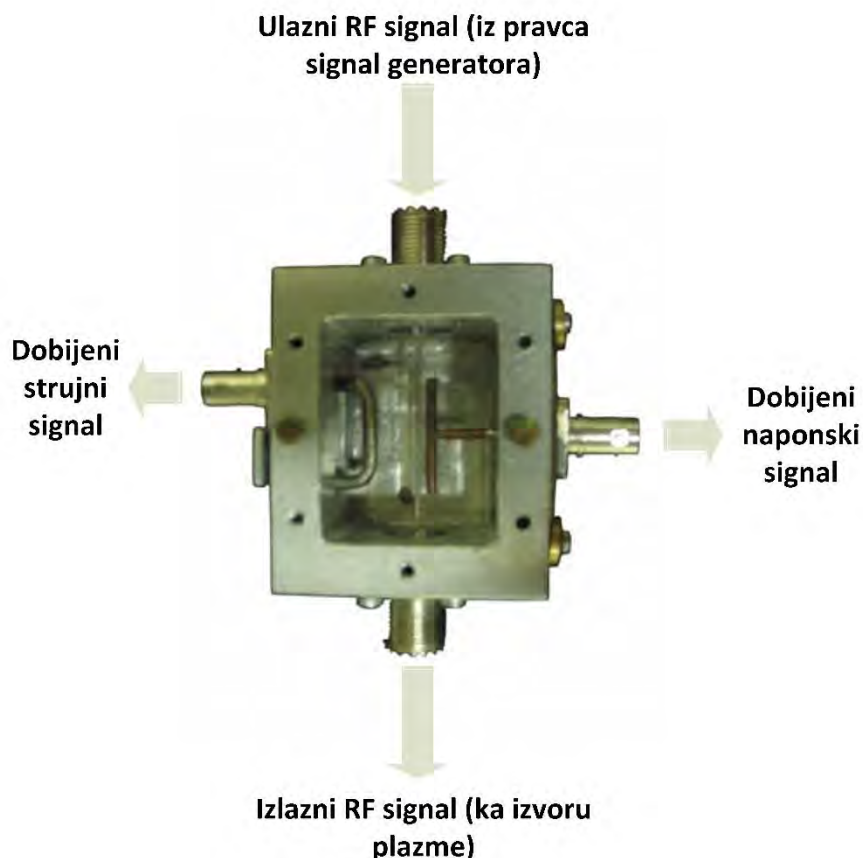
Slika 7.1 Eksperimentalna postavka električnog kola plazma igle

Plazma igla napaja se radiofrekventnom sinusoidnim signalom frekvencije 13.56 MHz koji je generisan pomoću dva različita sistema za napajanje: 1. signal generatora (Agilent N9310A), linijskog pojačala (Barthel RFA – 0,1 / 50-100 B00) i manualnog CCP kola za prilagođenje; 2. Kurt J Lesker – komercijalnog napajanja koji je takođe sačinjen od signal generatora, pojačavača i kola za prilagođenje; (u zavisnosti od primene koristili smo ili 1. i 2. sistem za napajanje plazma igle). Pre nego što signal dođe do centralne elektrode on prolazi kroz derivativne sonde koje su smeštene u kutiju od nerđajućeg čelika i njihova uloga je bila merenje srednje snage predate plazmi. Signali su snimani pomoću osciloskopa (Agilent DSO3202A) i računara. Kao radni gas korišćen je helijum sa protokom radnog gasa od 1 slm.

Uzorci su u zavisnosti od tipa primene stavljeni u mikrotitarske ploče sa 96, 48 i 24 bunarčića ili u Petri posude i rastojanje od vrha igle do uzorka je variralo u zavisnosti od primene i korišćenog uzorka o čemu će više detalja biti u narednim poglavljima.

7.2.3 Derivativne sonde

Derivativne sonde koje su korišćene u eksperimentu dizajnirane su u Laboratoriji za neravnotežne procese i primenu plazme za merenje snage predate radiofrekventnoj plazmi pri ekscitacionoj frekvenciji od 13.56 MHz (u našem slučaju izvoru električnog pražnjenja na atmosferskom pražnjenju – plazma igla). One su sačinjene od strujno naponske sonde u obliku ćiriličnog slova II i naponske sonde u obliku slova T. Sonde su smeštene u kućište od nerđajućeg čelika jedna naspram druge i što je moguće bliže napajanom vodu centralne elektrode koja se nalazi između njih (Slika 7.2).



Slika 7.2 Metalno kućište sa derivativnim sondama. (izvor: doktorska disertacija, Nevena Puač)

Trenutne vrednosti napona i struje praćene su pomoću derivativnih sondi povezanih na osciloskop kablovima identične dužine da ne bi bilo došlo do dodatne fazne razlike između struje i napona korišćenih signala. Svi talasni oblici struje i napona prikupljeni su računarnom za dalju obradu podataka.

Numerička obrada dobijenih podataka se sastoji od brze Furijeove transformacije (*eng.* fast fourier transform - FFT), kalibracije u frekventnom domenu obe amplitude, i fazna i inverzna brza Furijeova transformacija (*eng.* inverse fast fourier transform IFFT) nakon čega se dobijaju realni kalibrisani talasni oblici.

Tokom merenja vodilo se računa da reflektovana snaga ne bude veća od 1%, što je regulisano pomoću manuelnog i automatskog kola za prilagođenje (*eng.* matching network). Merenja primenom derivativnih sondi pokazala su se kao efikasna i precizna metoda za praćenje i kontrolu električnih parametara prilikom primene plazma igle u biomedicini. Detaljan opis rada sondi i njihov razvoj prikazan je u disertaciji dr Nevene Puač (Puač, doktorska disertacija, 2008; Puač *et al.*, 2006).

7.3 Tretman biljnih matičnih ćelija plazma iglom

7.3.1 Uvod

U ovom poglavlju biće opisan uticaj tretmana plazma igle na biljne matične ćelije, tačnije kalusa *lat. Daucus carota*, meristematskih ćelija šargarepe. Ovaj eksperiment je urađen u saradnji naše Laboratorije za neravnotežne procese i primenu plazme, Instituta za fiziku u Beogradu i Laboratorije za fiziologiju biljaka, Instituta za biološka istraživanja Siniša Stanković - IBISS. Rezultati ove saradnje publikovani su u vrhunskom međunarodnom časopisu (Puač *et al.*, 2014).

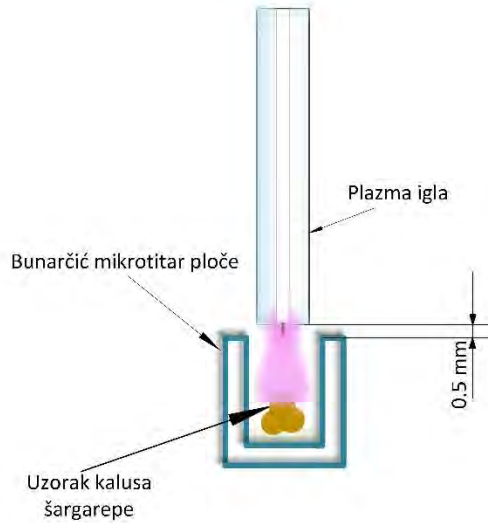
Kao što je već rečeno u prethodnim poglavljima, pražnjenje koje generiše plazma igla proizvodi reaktivne vrste kiseonika i azota (ROS i RON) tj. radikale kao što su N, O, O₃, OH i NO koji snažno utiču na metabolizam živih ćelija. Glavno pitanje predstavlja povezivanje spoljašnjih proizvoda plazme (elektrona, jona, RNS, ROS, fotona, jakog električnog polja, itd.) sa neposrednim unutrašnjim odzivom koji trigeruje (pokreće) ili indukuje efekte unutar žive ćelije. Da bismo pokušali da damo odgovor na to pitanje u ovom istraživanju posmatraćemo kinetiku dva enzima koji su tipični pokazatelji identiteta reaktivnih vrsta stvorenih u pražnjenju i koji mogu izazvati trigerovanje transdukcije signala u ćeliji i prouzrokovati ćelijsku aktivnost.

Kod biljaka dolazi do formiranja ROS-a neizbežnim atačmentom/vezivanjem elektrona za O₂ što predstavlja posledicu transporta elektrona usled aktivnosti hloroplasta, mitohondrija i plazmatskih membrana ili kao nusprodukt različitih metaboličkih puteva lokalizovanih u različitim ćelijskim odeljcima (Luis *et al.*, 2006; Blokhina i Fagerstedt, 2010; Heyno *et al.*, 2011). Ukoliko visoke koncentracije reaktivnih kiseoničnih vrsta borave u živom organizmu izuzetno su štetni i izazivaju toksične efekte. Ćelija doživljava tzv. „oksidativni stres“ u trenutku kad nivo ROS pređe odbrambene mehanizme. Da bi se izbegla šteta izazvana ROS-om, biljke su razvile molekularne odbrambene sisteme koji ograničavaju stvaranje ROS-a i promovišu njegovo uklanjanje (Alscher *et al.*, 2002). Biljne enzimske odbrane uključuju antioksidativne enzime, kao što su fenol peroksidaza (POKS), askorbat peroksidaza (APKS), glutation peroksidaza, (GPKS), superoksid dismutaza (SOD) i katalaza (CAT), koji zajedno sa ostalim enzimima ciklusa askorbat – glutation promovišu uklanjanje (scavenging) ROS-a.

U narednom poglavlju Tretman i rezultati biće prikazane aktivnosti SOD i CAT u ćelijama kalusa *Daucus carota* neposredno nakon tretmana plazmom i dve nedelje nakon tretmana. SOD se smatra prvom linijom odbrane od ROS-a (Gomez *et al.*, 2004). On dovodi do katalizovanja dismutacije O₂ u H₂O₂ i molekularni kiseonik. CAT je prisutan u peroksidomima gotovo svih aerobnih ćelija i, u manjoj meri, u mitohondrijima (Shigeoka *et al.*, 2002). Katalaza služi kao zaštita ćelije od H₂O₂ katalizujući njeno razlaganje na O₂ i H₂O (Foyer i Graham, 2000). Dakle, i jedna i druga aktivnost služe kao dobri markeri unutrašnjih hemijskih efekata spoljašnjih proizvoda pražnjenja dobijenog u plazma igli i takođe mogu inspirisati slične procedure kod životinjskih i ljudskih ćelija, kao i kod bakterija.

7.3.2 Eksperimentalna postavka i priprema uzoraka za tretman plazma iglom

Embriogene kulture kalusa šargarepe (*Daucus carota*) su izdvojene iz eksplantata korena te su zatim uskladištene i održavane na čvrstom MS medijumu (Murashige i Skoog, 1962), dopunjenom sa 2,4-dihlorfenoksiocetenom kiselinom (2.4-D, 1.0 mg l⁻¹). Uzorci kalusa, mase 10–30 mg, su postavljani pojedinačno na dno bunarčića mikrotitarske pločice (96 bunarčića). Razmak između ivice bunara i ruba staklene cevčice iznosio je 0.5 mm prilikom svih tretmana. Prečnik bunara bio je veći od prečnika staklene cevi za 0.5 mm. Takođe, vrh igle nalazi se 1.5 mm izvan staklene cevi, tako stvorena plazma je bila u mešavini helijuma i ambijentalnog vazduha (Slika 7.3). Tokom svih eksperimenata, plazma je pokrivala celu površinu uzoraka, a prosečna udaljenost između igle i površine kulture bila je 4 mm. Kultura je bila prikazana za gelsku sredinu postavljenu na dno mikrotitarskih ploča.

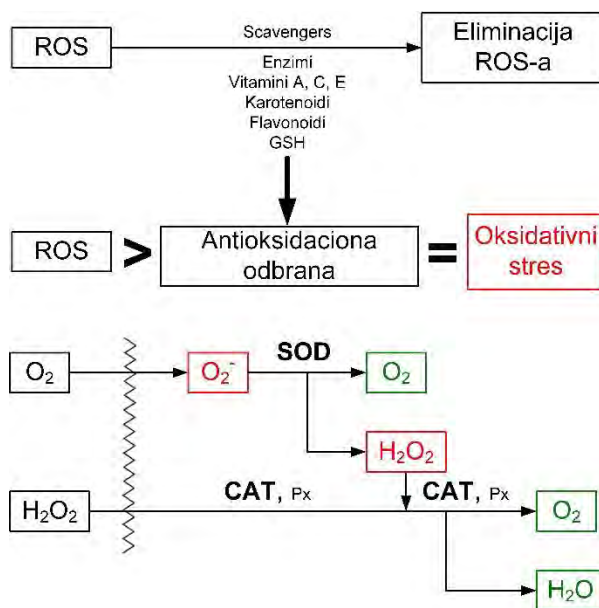


Slika 7.3 Shema pozicije plazma igle u odnosu na uzorak tokom tretmana

Kod plazma igle su tokom tretmana varirani sledeći parametri: vreme ekspozicije iznosilo je 10 s, 30 s, 60 s i 120 s; snaga predata plazmi iznosila je 0.4 W i 1.6 W. Kontrolni uzorci kalusa su tokom eksperimentalnog postupka držani u mikrotitarskoj ploči kako bi se obezbedili jednaki uslovi tretmana. Uzorci su takođe tretirani samo protokom helijuma bez pražnjenja sa vremenom ekspozicije od 120 s kako bi se utvrdilo da li inertni radni gas utiče na njihovu modifikaciju. Odmah nakon tretmana kalusi su zamrzavani u tečnom azotu i držani na konstantnoj temperaturi od 70°C do upotrebe, ili kontinuirano kultivisani na svežem bazalnom MS medijumu bez regulatora rasta biljaka 2 nedelje pre upotrebe. Biljno tkivo je mleveno u fini prah, a proteini su ekstrahovani u 100 mM kalijum-fosfatnom puferu (pH6.5), dopunjenom koktelom koji inhibira proteazu za ekstrakte biljnog tkiva i 5 mM askorbata. Sadržaj proteina određen je prema Bradford (1976) koristeći goveđi serumski albumin kao standard (Bradford, 1976) i odvojen prirodnim PAGE gelom. Količine proteina koje su primenjivane u svakom bunarčiću za merenje aktivnosti enzima iznosile su 30 µg. Gelovi su obojeni za CAT aktivnost kao u (Woodbury *et al.*, 1971). Aktivnost SOD-a (u jedinicama enzimske aktivnosti mg⁻¹ rastvorljivog proteina) određena je kao u (Beyer i Fridovich, 1987).

7.3.3 Aktivnost SOD i CAT enzima usled tretmana plazma iglom

Tokom normalnog funkcionisanja ćelija dolazi do proizvodnje ROS koji uključuju hidroksilne radikale ($\text{OH}\cdot$), superoksid (O_2^-) i vodonik-peroksid (H_2O_2). Antioksidacioni sistem, kada su standardni uslovi u pitanju, uspeva da svede perturbacije izazvane ROS-om na minimum. Međutim, ukoliko se proizvodnja ROS-a poveća značajno, bilo da je to povećanje izazvano spoljašnjim uzrocima ili unutar ćelije, zbog neravnoteže unutar ćelijskog sistema može doći do oksidativnog stresa (Mates, 2000; Graves, 2012; Francm *et al.*, 2007).

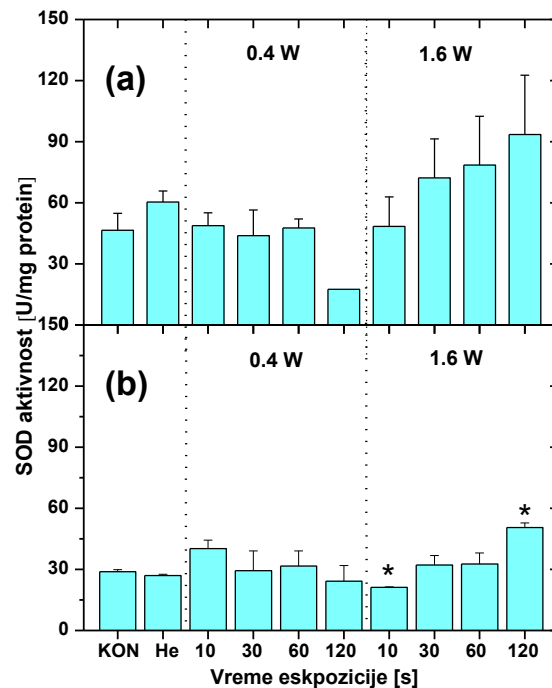


Slika 7.4 Reakcioni putevi u ćeliji za SOD i CAT (Puač *et al.*, 2014.).

Zbog štetnosti ROS-a, a takođe i zbog njenog učestvovanja u signalnim događajima, biljnoj ćeliji su potrebna najmanje dva različita mehanizma za regulisanje unutarćelijske koncentracije uklanjanjem ROS: jedan koji će omogućiti finu modulaciju niskog nivoa ROS u signalne svrhe i jedan koji će omogućiti detoksikaciju viška ROS-a, posebno tokom stresa (Mittler, 2002). Glavni mehanizmi uklanjanja ROS-a kod biljaka podrazumevaju SOD, CAT i različite vrste peroksidaza. Njihova uloga je direktno uklanjanje suvišnih količina ROS-a stvorenih unutar ćelije ili koje u njih dolaze iz spoljnog izvora. SOD uklanja super-oksidni anion katalizujući njegovu dismutaciju u vodonik-peroksid, dok CAT smanjuje vodonik-peroksid konvertujući ga u vodu (Willekens *et al.*, 1997). Na slici 7.4 može se videti stvaranje oksidanata kao rezultat normalnog unutarćelijskog metabolizma u mitohondrijima i hloroplastu, kao i iz enzimskih sistema. Sofisticirani enzimski i neenzimski antioksidativni odbrambeni sistem, uključujući CAT, SOD, peroksidaze (Px) i glutation (GSH), suprotstavlja se i reguliše ukupni nivo ROS-a da bi se održala fiziološka homeostaza. Kada proizvodnja ROS premaši antioksidativnu odbranu, javlja se oksidativni stres.

Odmah nakon tretmana uzoraka plazmom izmerena je aktivnost SOD i CAT kao i dve nedelje nakon tretmana. Analiza aktivnosti SOD izvršena neposredno nakon tretmana u plazmi (slika 7.5 (a)) pokazala je da se enzimski aktivnost kalusa šargarepe za tretmane sa plazmom od 0.4 W nije značajno promenila u poređenju sa kontrolnim uzorcima. S druge strane, aktivnost enzima se postepeno povećavala sa vremenom tretmana za veću snagu od 1.6 W. Najveća aktivnost SOD primećena je za vreme tretmana od 120 s. Povećanje aktivnosti enzima podrazumeva da je više ćelijskih anjona prisutno u ćeliji i da su ćelije kalusa pod oksidativnim stresom. Da bi se izborili sa povišenim nivoom ROS antioksidanasa, pokrenuti su mehanizmi u kalusu šargarepe i aktivnost enzima je povezana sa nivoom stresa.

SOD aktivnost u kalusu šargarepe nakon neprekidnog dvonedeljnog rasta pokazala je mnogo niže vrednosti (dvostruko), što potvrđuje da u nestresnim uslovima antioksidativni odbrambeni sistem pruža adekvatnu zaštitu od ROS-a (slika 7.5 (b)). Ipak, za dve nedelje rasta kalusa aktivnost SOD-a zadržala je više nivoe u poređenju sa kontrolom sa jasnom zavisnošću od snage plazme. Poređenje aktivnosti SOD u ćelijama kalusa neposredno nakon tretmana plazmom i dve nedelje nakon tretmana ilustruje značaj SOD za antioksidativnu odbranu biljaka u uslovima prekomernog stresa.



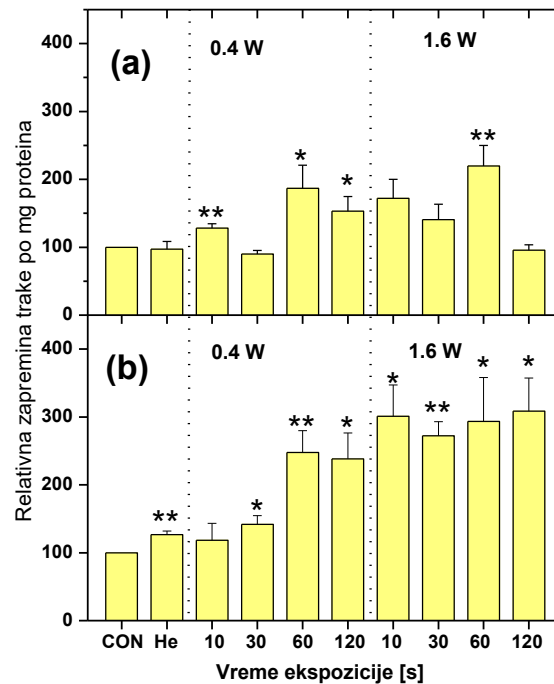
Slika 7.5 SOD aktivnost kalusa šargarepe izmerena odmah (a) ili dve nedelje nakon tretmana plazma iglom (b). Vrednosti predstavljaju usrednjene vrednosti (sa standardnim greškama) dobijene iz dva nezavisna eksperimenta. Zvezdica predstavlja statističku značajnost u poređenju sa kontrolnom vrednošću (* $p < 0.05$ i ** $p < 0.01$) (Puač *et al.* 2014).

Objašnjenje za povišene nivoe aktivnosti SOD neposredno nakon tretmana u plazmi može se naći u prisustvu radikala i jona koji se usled tretmana plazmom isporučuju u ćelijsku membranu. Oksigenacija tkiva je već primećena u lečenju ćelije carcinoma (Collet *et al.*, 2014).

U ovoj studiji, CAT aktivnost je izmerena neposredno nakon tretmana plazmom (videti sliku 7.6 (a)) i u kalusu šargarepe koji je rastao dve nedelje nakon tretmana (slika 7.6 (b)). CAT aktivnost se povećala u poređenju sa kontrolnim uzorcima, ali nismo mogli dobiti jasnu sliku o njenoj zavisnosti od primenjene snage ili vremena tretmana. U našim eksperimentima merili smo aktivnost enzima ubrzo nakon tretmana u plazmi. 1-2 minuta nakon tretmana uzorci su zamrznuti u tečnom azotu i pripremljeni za merenja. Pretpostavljamo da ovo vreme nije bilo dovoljno da enzim reaguje adekvatno. Naročito u slučaju CAT-a koji je drugi po redu i aktivira se nakon enzima SOD. Očigledno je neophodno proceniti aktivnost ovih enzima u periodu od nekoliko sati nakon tretmana u plazmi.

Međutim, CAT aktivnost u uzorcima kalusa koji su gajeni dve nedelje povećavala se postepeno sa povećanjem snage plazme i vremena tretmana pokazujući 1.5 puta veće vrednosti u poređenju sa CAT

aktivnošću merenom u uzorcima kalusa neposredno nakon tretmana u plazmi. To znači da su u tretiranim ćelijama, čak dve nedelje kasnije, nivoi H₂O₂ povišeni u poređenju sa kontrolnim uzorcima. Ovaj dugoročni efekat plazme mogao bi se objasniti povećanjem morfogenog potencijala direktno tretiranih ćelija (Bajaj *et al.*, 1996; Mihai *et al.*, 2009; Tan *et al.*, 2010).



Slika 7.6 Aktivnost katalaze kalusa šargarepe izmerena odmah (a) ili dve nedelje nakon tretmana plazma iglom(b). Zapremina opsega je zabeležena pomoću denzitometrijske analize. Podaci su normalizovani pomoću kontrolnog signala. Vrednosti predstavljaju usrednjene vrednosti (sa standardnim greškama) dobijene iz tri nezavisna eksperimenta. Zvezdica predstavlja statističku značajnost u poređenju sa kontrolom (*p<0,05 i **p<0.01) (Puač *et al.* 2014).

Do povećanja morfogenog potencijala može doći aktiviranjem nekog dela u DNK odgovornog za proliferaciju i/ili somatsku embriogenezu (SE) ćelija. Sve veći broj publikacija korelira ROS i SE. Kairong *et al.* sugerisali su da je biljka SE poseban proces diferencijacije ćelija i uspostavili su vezu ili „delimično preklapanje“ ROS-a i ćelijske diferencijacije (Kairong *et al.*, 2002).

7.3.4 Zaključak – tretman biljnih ćelija kalusa

U ovom poglavlju opisan je tretman plazma iglom biljnih ćelija kalusa *Daucus carota*. Posmatrani su efekti na uzorcima odmah nakon tretmana kao i dve nedelje kasnije. Reaktivne kiseonične vrste – ROS koje se stvaraju u ćelijama i u električnom pražnjenju plazma igle izazivaju aktivnost enzima unutar ćelija. Enzimi svojim delovanjem predstavljaju odbrambeni mehanizam od oksidativnog stresa do kojeg dolazi ukoliko je vrednost ROS-a visoke koncentracije. Iz dobijenih rezultata mogu se izvući sledeći zaključci:

- Uočljivi efekti na enzime postignuti su odmah nakon tretmana plazmom, a takođe i nakon perioda od dve nedelje.

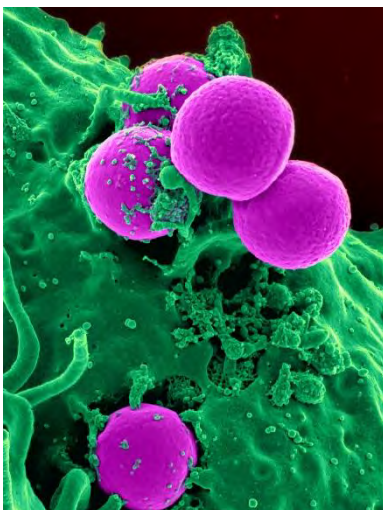
- SOD aktivnost, iako je i dalje bila znatno veća od aktivnosti u netretiranim ćelijama, smanjila se posle dve nedelje u poređenju sa aktivnošću neposredno posle tretmana.
- Prisustvo povećane gustine ROS može dovesti do povećanja ili smanjenja enzima. Smanjenje se dešava kada odgovor žive ćelije ne može pratiti povećan unos ROS-a i u tom slučaju je ugrožen opstanak ćelije.
- Povećanje može značiti da je, podstaknuto ROS-om, povećana proizvodnja enzima i konačna koncentracija nije pod kontrolom enzima.
- CAT aktivnost je značajno povećana u uzorcima u periodu od dve nedelje nakon tretmana.
- Treba imati na umu da su primećena povećanja nakon dužeg perioda značajna, jer su zabeležene vrednosti uvek normalizuju na masu rastućih kalusa (po mg proteina izolovanog iz uzoraka).

7.4 Tretman MRSA bakterija

7.4.1 Uvod

U ovom poglavlju biće prikazan tretman plazma iglom meticilin rezistentnih stafilokokus aureus (MRSA) bakterija. *In vitro* eksperiment je izveden u saradnji naše Laboratorije za neravnotežne procese i primenu plazme sa kolegama sa Instituta za imunologiju i mikrobiologiju i Stomatološkog fakulteta.

MRSA bakterije su posebna vrsta gram pozitivnih bakterija iz grupe stafilokoka koje su otporne na antibiotike i zbog toga je teško izlečiti pacijenta koji je njime inficiran. Kao i sve stafilokoke, MRSA je loptastog oblika i gradi jata, grozdove, skupove (Slika 7.7). One se mogu naći u sluznici nosa kod čoveka ili na koži. Bakterija je bezopasna sve dok ne uđe u telo preko posekotina ili rana (najčešće su to u pitanju postoperativne hirurške rane koje lako mogu da se inficiraju).



Slika 7.7 Mikrografski prikaz MRSA bakterija.

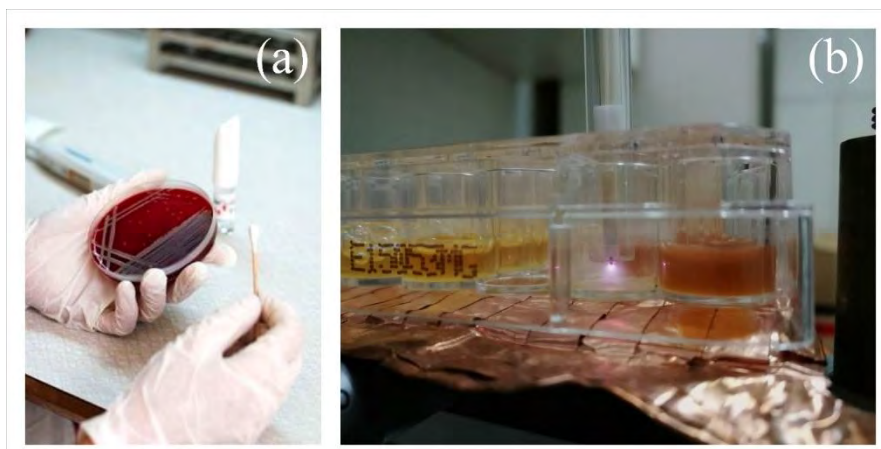
(https://commons.wikimedia.org/wiki/File:Human_neutrophil_ingesting_MRSA.jpg)

Zbog sposobnosti MRSA da razvija rezistentnost na antibiotike koji se primenjuju u lečenju pristupa se novim metodama koje bi mogle da sterilisu ove bakterije kao što je već do sada i pokazano korišćenjem plazma igle (Miletić *et al.*, 2014) koju ćemo koristiti i u ovom eksperimentu.

Efekti koji su dobijeni na MRSA bakterijama posmatrani su nakon tretmana plazma iglom na uzorcima koji su pripremljeni na dva različita načina: 1. bakterije uzgajane na čvrstom medijumu; 2. bakterije u formi biofilma;. U sledećem poglavlju biće prikazani rezultati MTT testa, metode kojom je proverena vijabilnost bakterija i metoda određivanja veličine inhibicionih zona dobijenih usled tretmana plazma iglom.

7.4.2 Eksperimentalna postavka i priprema MRSA bakterija za tretman plazma iglom

U ovom eksperimentu kao uzorak korišćene su MRSA bakterije izolovane iz hirurške rane i fenotipski su identifikovane od strane uređaja BD PhoenixAutomated Microbiology System (Becton Dickinson Diagnostic Systems, Sparks, MD), a zatim potvrđene detekcijom *nuc* i *mecA* gena (evaluacija BD). Kapacitet formiranog biofilma ocenjen je kao jak. Biofilm MRSA je prenet iz smrznute stočne kulture na triptični sojin agar sa kojeg su se uzimale kolonije bakterija (Slika 7.8 (a)) i inkubirane aerobno tokom 24 sata na 35-37°C. Nekoliko kolonija bakterija suspendovano je u fiziološkom rastvoru soli i zamućenost suspenzije je prilagođena na 0.5 McFarland standarda ($\approx 10^8$ CFU/ml) (eng. CFU – colony forming unit) pomoću Densimat fotometra (BioMerieux, Francuska). Suspenzija bakterija je ravnomerno raspoređena na površinu Mueller-Hinton agar ploče za određivanje rasta zone inhibicije, dok je za rast biofilma suspenzija serijski razređena kako bi se dobio konačni ispitni inokulum od 10^4 CFU po bunarčiću sterilne polistirenske mikrotitarske pločice koja sadrži 48 bunarčića sa ravnim dnom. Ispitivanje biofilma u mikrotitarskim pločicama sprovedeno je u skladu sa protokolom koji su opisali Stepanovic i saradnici (Stepanović *et al.*, 2007). Svaki bunarčić sadržao je 450 μ l podloge za kultivaciju Brain Heart Infusion (BHI) tečnosti, dopunjenu sa 1% glukoze i 50 μ l prethodno razblažene bakterijske suspenzije. Karakteristike negativne kontrole sadržale su 500 μ l tečnosti medijuma. Inokulirane pločice su inkubirane 24 sata na 35-37°C pre tretmana plazma iglom.

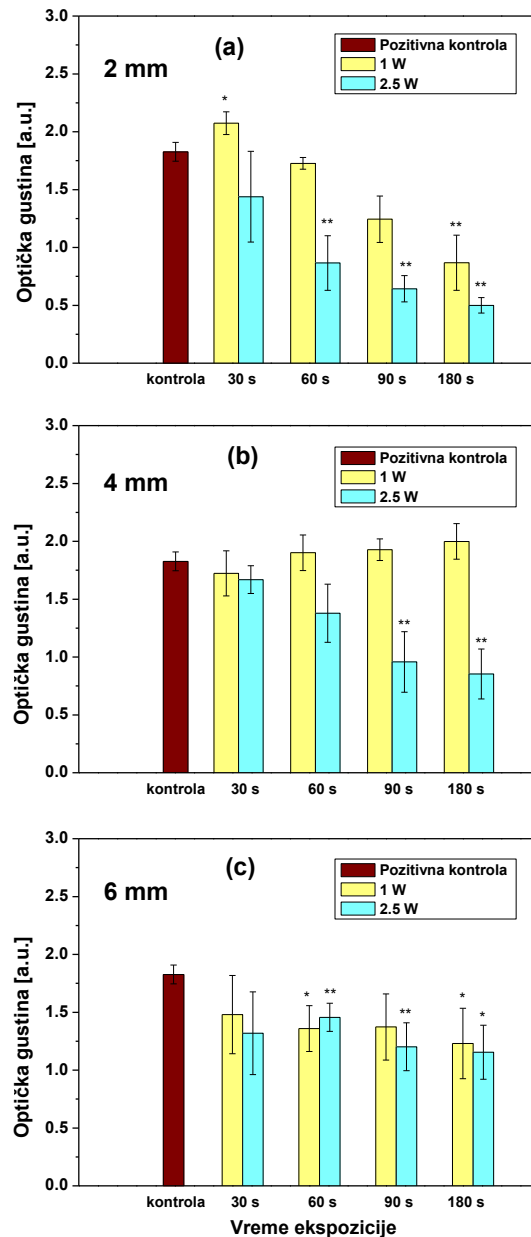


Slika 7.8 (a) Uzorkovanje bakterija sa podloge za gajenje, (b) Tretman bakterija plazma iglom unutar bunarčića mikrotitar pločice (48 bunarčića sa ravnim dnom). (Selaković *et al.*, publikacija u pripremi)

Pripremljene su dve vrste uzoraka bakterija. Prva je postavljena u Petrijevu posudu u sa agaroznim filmom na koji je ravnomerno nanet sloj bakterijske suspenzije ($1.5 \cdot 10^8$ CFU/ml), dok je druga bakterijska suspenzija (10^4 CFU po bunarčiću) smeštena u mikrotitarsku pločicu sa 48 bunarčića sa ravnim dnom. Plazma igla bila je usmerena normalno na pripremljene uzorke (Slika 7.8 (b)). Za tretman plazma igle varirana su tri eksperimentalna parametra: snaga (1 W i 2.5 W), udaljenost od vrha centralne elektrode do površine uzorka (2 mm, 4 mm i 6 mm) i vreme ekspozicije (30 s, 60 s, 90 s i 180 s). Protok radnog gasa (helijuma) bio je konstantan 1 slm (standardni litar u minuti) tokom svih tretmana.

3.4.3 MTT test

Odmah nakon tretmana plazma iglom urađen je MTT test. Ova metoda predstavlja kolorimetrijski test za procenu metaboličke aktivnosti bakterija (njeno preživljavanje iliti vijabilnost). Enzimi ćelijske (bakterijske) oksidoreduktaze koje zavise od NAD(P)H (Nikotinamid adenin dinukleotid fosfat - koristi se u anaboličkim reakcijama, kao što su sinteze lipida i nukleinskih kiselina, za koje je NADPH neophodan kao redukujući agens) mogu pod definisanim uslovima da održavaju vijabilnost prisutnih ćelija.



Slika 7.9 MTT test za tretman plazmom iglom MRSA biofilma. Varirani parametri: rastojanja od 2, 4 i 6 mm (slike a, b i c respektivno), dve različite primenjene snage (1 W i 2.5 W) i četiri vremena ekspozicije (30, 60 s, 90 s i 180 s). Protok gasa je održavan konstantnim na 1 slm. Kontrolni uzorci su bile netretirane bakterije. * $p < 0,05$ i ** $p < 0,01$ u poređenju sa netretiranim kontrolnim bakterijama. (Upareni uzorak T-testa) (Selaković *et al.*, publikacija u pripremi)

Na Slici 7.9 prikazani su rezultati MTT testa za tri različite udaljenosti od vrha plazma igle u odnosu na tretirani uzorak (2 mm, 4 mm i 6 mm) gde su primenjene 2 različite snage (1 W i 2.5 W). Rastojanja su pažljivo odabrana zbog ograničenog prostiranja plazme izvan cevčice. Tokom eksperimenta, moglo se primetiti da pri većoj udaljenosti od 2 mm plazma ne dodiruje uzorak pri manjoj snazi od 1 W.

Može se primetiti, (Slika 7.9. (a)), da plazma igla smanjuje koncentraciju bakterija, izuzev pri snazi od 1 W i najkraćeg vremena ekspozicije od 30 s gde je koncentracija bakterija porasla. Kako se vreme ekspozicije, tj. vreme izlaganju uzorka električnom pražnjenju povećava, koncentracija bakterija se postepeno smanjuje. Konzistentniji rezultat postiže se za snagu od 2.5 W. U ovom slučaju plazma značajno smanjuje koncentraciju bakterija tokom najdužeg vremena izlaganja od 180 s.

Za rastojanje od 4 mm rezultati su prikazani na Slici 7.9 (b). Ovde se vide varijacije koncentracija za različita vremena izlaganja plazmi. Do njih dolazi zbog većeg rastojanja između površine uzorka i vrha plazma igle. Kao što je i ranije rečeno, snaga od 1 W nije bila dovoljna da obezbedi prostiranje električnog pražnjenja do samog uzorka (bar se to nije moglo potvrditi golim okom!). Do smanjenja gustine bakterija nije došlo najverovatnije ne samo zbog nedostatka kontakta plazme sa uzorkom, već i zbog efekta disperzije reaktivnih vrsta pod uticajem protoka helijuma od 1 slm. Pri većoj snazi od 2.5 W može se primetiti postepeni pad koncentracije usled intenzivnijeg električnog pražnjenja tamo gde je plazma bila u kontaktu sa površinom uzorka.

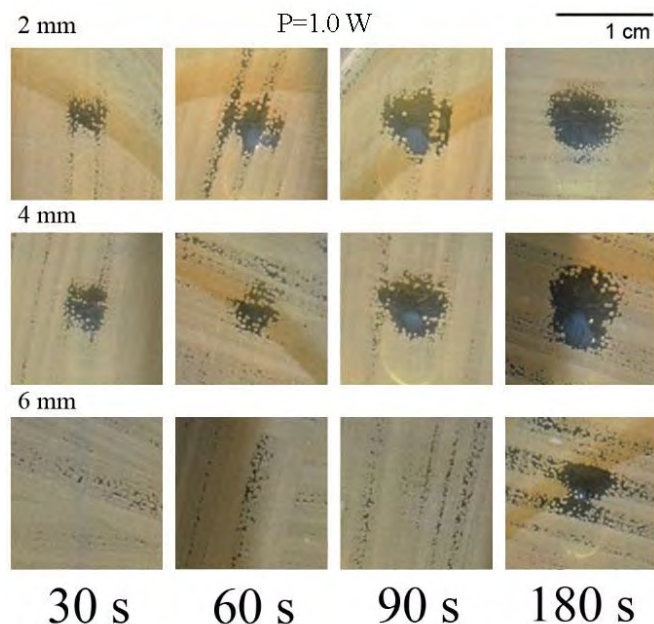
Rezultati MTT testa za najveće rastojanje plazma igle od uzorka, Slika 7.9 (c), prikazuju smanjenje za obe primenjene snage, ali u svim vremenima ekspozicije ne dolazi do pada ispod 1 a.u. optičke gustine. Kao i za prethodni slučaj sa rastojanjem od 4 mm, ovde takođe imamo nedostatak kontakta plazme sa površinom uzorka, ali za razliku od njega, ovde nemamo efekat disperzije, tako da reaktivne vrste mogu nesmetano doći do tretiranog uzorka. vrste koje sterilisu bakterije, očigledno ne mogu dati znatno veći efekat na ovoj udaljenosti.

7.4.4 Zone inhibicije

Naredna metoda koju smo koristili za posmatranje efekata tretmana plazma igle na MRSA bakterije bila je određivanje površine zona inhibicije. Eksperiment je pokazao da električno pražnjenje na atmosferskom pritisku može u potpunosti da sterilise površinu na kojoj su zasađene bakterije. Na Slici 7.10 i Slici 7.11 prikazane su fotografije zona inhibicije MRSA bakterija nakon tretmana plazma iglom. Mereni su dijametri inhibicionih zona pomoću laboratorijskog lenjira. Bakterije su bile zasejane u Petri posudama a korišćen je rastvor koncentracije od 0.5 McFarland-a, a ta vrednost je odgovarala koncentraciji od $1.5 \cdot 10^8$ CFU/ml. Takođe je urađen uticaj protoka radnog gasa (helijuma) od 1 slm bez električnog pražnjenja na zasađene bakterije i pri tom tretmanu nije došlo do bilo kakve promene kod MRSA.

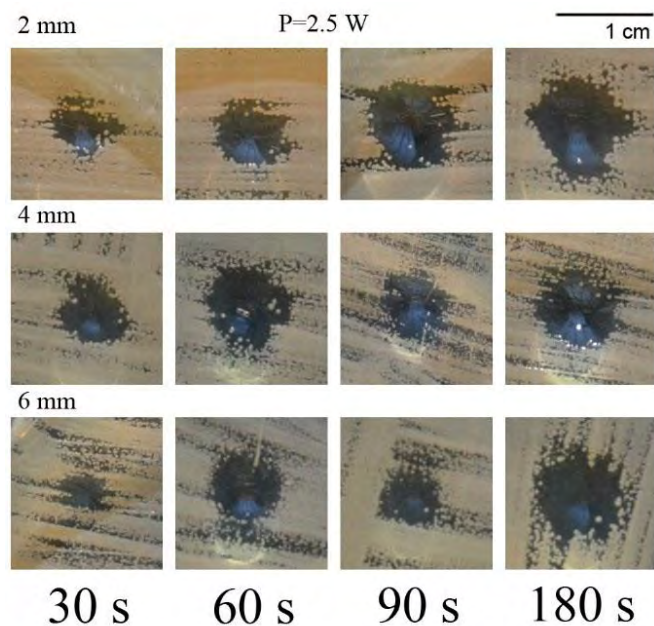
Parametri koje smo varirali prilikom ovog dela eksperimenta su bili sledeći: rastojanje od ruba cevčice do površine uzorka je iznosilo 2 mm, 4 mm i 6 mm, ispitivan je uticaj dve različite primenjene snage (1 W i 2.5 W) kao i četiri vremena ekspozicije (30 s, 60 s, 90 s i 180 s). Protok helijuma je održavan konstantnim na 1 slm.

Zone inhibicije dobijene tretmanom plazma iglom za rastojanje od 2 mm od površine uzorka prikazane su na Slici 7.10, a grafičko poređenje je prikazano na Slici 7.12 (a). Na slici se mogu uočiti nepravilni kružni oblici inhibicionih zona čiji dijametri rastu sa primenjenom snagom i vremenom ekspozicije. Za snagu od 1 W izmerene zone su veličine od 3 mm do 5 mm u prečniku, a za 2.5 W od 4.5 mm do 9 mm. Najveći dijametar zone inhibicije za ove parametre tretmana plazma iglom iznosi 9 mm. Na rastojanju od 2 mm imamo maksimalnu zonu inhibicije za tretman plazma iglom.

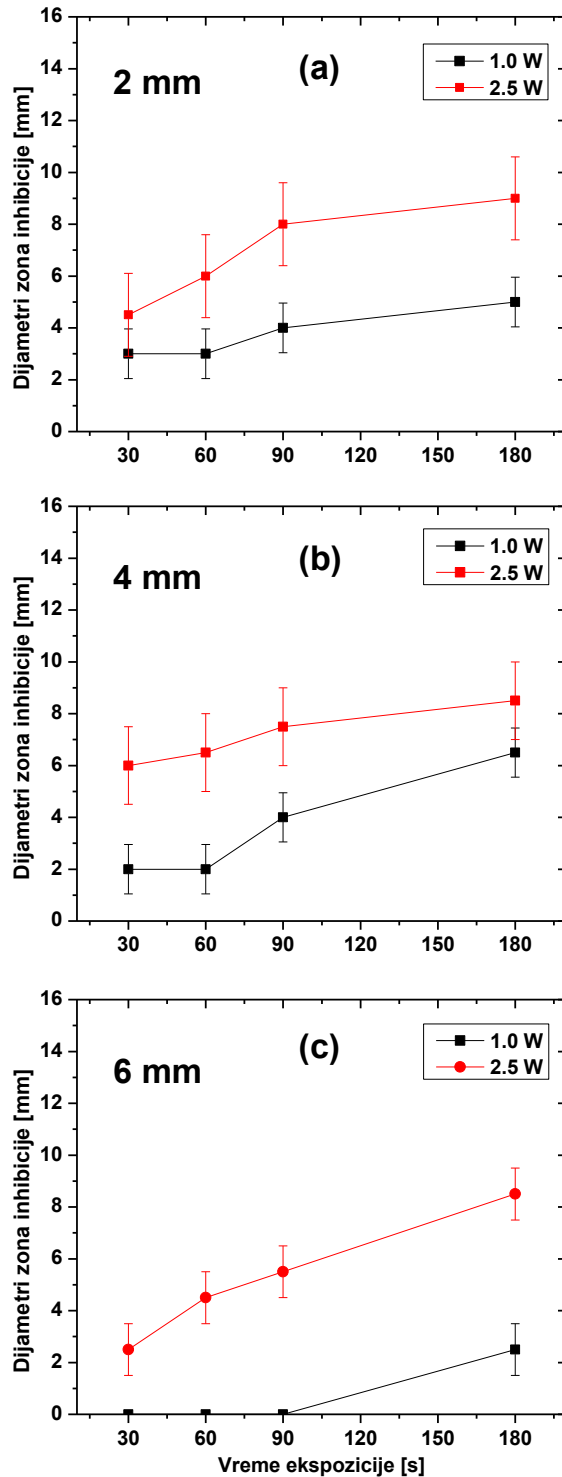


Slika 7.10 Površine zona inhibicije MRSA bakterija dobijene nakon tretmana plazma iglom. Varirani parametri: rastojanja od 2, 4 i 6 mm, za primenjenu snagu 1.0 W i četiri vremena ekspozicije (30, 60 s, 90 s i 180 s). Protok gasa je održavan konstantnim na 1 slm

Sa povećanjem udaljenosti plazma igle sa 2 mm na 4 mm od površine uzorka, zone inhibicije su nešto manje pri snazi od 1.0 W 2-6.5 mm (Slika 7.10) i 2.5 W 6-8.5 mm (Slika 7.11). Manje zone inhibicije posledica su manjeg fluksa reaktivnih čestica koje udaraju o površinu. Povećavanje rastojanja na 6 mm, plazma ne stvara vidljivu zonu inhibicije za snagu od 1.0 W pri 30 s, 60 s i 90 s vremenima ekspozicije (Slika 7.10). Samo za veću primenjenu snagu od 2.5 W plazma proizvodi zonu inhibicije od 2.5 mm do 8.5 mm (Slika 7.11).



Slika 7.11 Površine zona inhibicije MRSA bakterija dobijene nakon tretmana plazma iglom. Varirani parametri: rastojanja od 2, 4 i 6 mm, za primenjenu snagu 2.5 W i četiri vremena ekspozicije (30, 60 s, 90 s i 180 s). Protok gasa je održavan konstantnim na 1 slm (Selaković *et al.*, publikacija u pripremi)



Slika 7.12 Dijametri zona inhibicije tretiranih MRSA bakterija. Varirani parametri: rastojanja od 2 mm, 4 mm i 6 mm (slike a, b i c respektivno), dve različite primenjene snage (1 W i 2.5 W) i četiri vremena ekspozicije (30, 60 s, 90 s i 180 s). Protok gasa je održavan konstantnim na 1 slm. (Selaković *et al.*, publikacija u pripremi)

Grafički prikaz dijametara zona inhibicije u zavisnosti od vremena ekspozicije (Slika 5.) u sva tri slučaja implicira da veća snaga i duže vreme ekspozicije daje najefikasnije rezultate prilikom sterilizacija bakterija.

7.4.5. Zaključak

U ovom poglavlju prikazani su dobijeni efekti na MRSA bakterijama nakon tretmana plazma iglom gde su varirani sledeći eksperimentalni uslovi: rastojanje plazma igle od površine uzorka (2 mm, 4 mm i 6 mm), snaga plazme (1.0 W i 2.5 W), vreme ekspozicije, tj. vreme izlaganja uzorka električnom pražnjenju (30 s, 60 s, 90 s i 180 s). Korišćena su dva tipa pripreme MRSA bakterija: bakterije na hranljivoj podlozi u Petri posudi i biofilm bakterija u mikrotitar pločicama.

Zaključci dobijeni iz rezultata su sledeći:

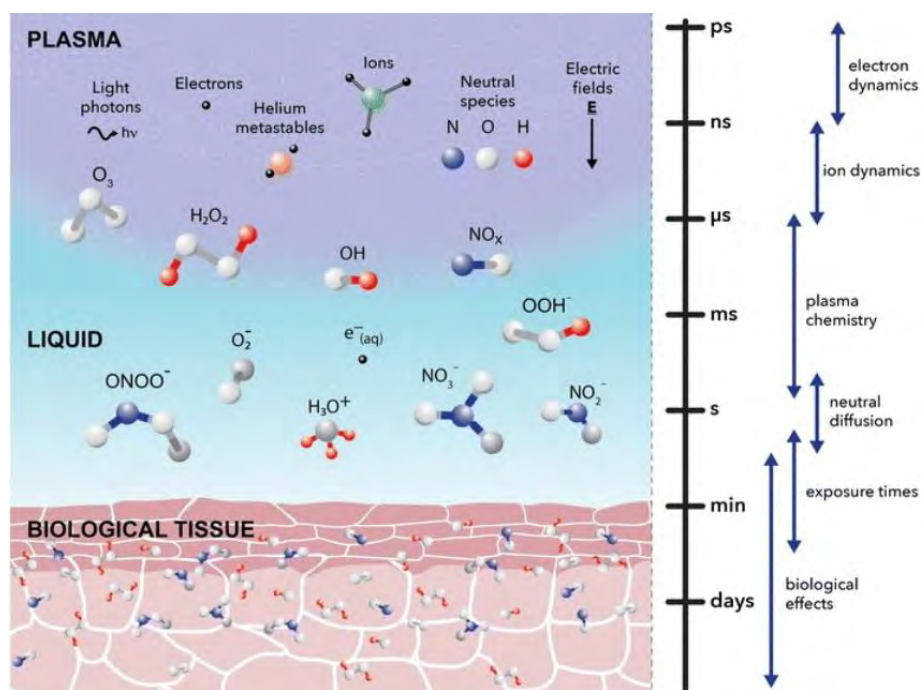
- Najduža vremena ekspozicije i najmanje rastojanje od plazma igle u odnosu na uzorak prilikom tretmana predstavljaju i najefikasnije parametre za primenu pri sterilizaciji MRSA bakterija kako kod tretiranja uzoraka na hranljivoj površini tako i pri tretmanu biofilma u mikrotitar pločicama.
- Plazma igla bez obzira što formira vidljivo pražnjenje dijametra 3-4 mm za određeni set parametara svojim delovanjem formira inhibicione zone MRSA bakterija značajno većeg dijametara (za najduže vreme ekspozicije 180 s i snagu plazme od 2.5 W je ona iznosila 9 mm) što implicira širenje reaktivnih vrsta pod uticajem protoka helijuma.

7.5 Tretman kancerogenih ćelija

7.5.1 Uvod

Veliki broj različitih izvora niskotemperaturnih plazmi korišćeno je do sada u primenama na velikom broju malignih kancerogenih ćelijskih linija u laboratoriji pri kontrolisanim uslovima. Dobijeni brojni rezultati su vrlo obećavajući i publikovani su u naučnim časopisima (Hirst *et al.*, 2016; Gümbel *et al.*, 2017).

Prilikom tretmana, u zavisnosti od korišćene vrste izvora plazme, može se dobiti niz ćelijskih odgovora, uključujući oštećenje DNK (Han *et al.*, 2013), smanjena vijabilnost/održivost ćelija i klonogenost (Wende *et al.*, 2015; Guerrero-Preston *et al.*, 2014), smanjena proliferacija i zaustavljanje ćelijskog ciklusa (Nakai *et al.*, 2014). Brojne studije su pokazale da se mehanizmi ćelijske smrti nakon tretmana plazmom razlikuju kako u zavisnosti od tipa ćelije tako i od samog izvora. Većina proučavanja dala je rezultate o apoptozi; međutim, takođe su predstavljeni starenje ćelija i ne-apoptotična ćelijska smrt (Arndt *et al.*, 2013).



Slika 7.13 Ilustrativni prikaz višefaznog prenosa vrsta iz plazme ka biološkom uzorku. (A. M. Hirst *et al.* 2016.)

Na Slici 7.13 prikazane su glavne komponente jonizovanog gasa, uključujući jone, fotone i neutrane, „plazma-aktivirana-tečnost“ i njihovo difuziono širenje kroz proizvoljan sloj tkiva. Pored toga, prikazani su približni vremenski okviri koji upravljaju različitim procesima u sve 3 oblasti prikazanim na slici.. (A. M. Hirst *et al.* 2016.)

Postoji niz objavljenih preglednih radova koji razmatraju primenu niskotemperaturnih plazmi na lečenje raka (Barekzi *et al.*, 2013; Keidar *et al.*, 2013; Schlegel *et al.*, 2013; Ratovitski *et al.*, 2014; Keidar *et al.*, 2015; Hirst *et al.*, 2016). Od prvog izveštaja o efektu DBD tretmana na ubijanje melanoma 2007. godine, područje primene niskotemperaturnih plazmi u lečenju karcinoma doživelo je brz rast. Sveobuhvatnim istraživanjem svih publikacija do septembra 2016. godine utvrđeno je da je oko 75% radova iz ove oblasti objavljeno u multidisciplinarnim časopisima i fizičarskim časopisima. S druge strane, samo oko 25% radova je objavljeno u časopisima vezanim za medicinu. Kao rezultat, istraživanje u ovoj oblasti je uglavnom usmereno na opisivanje antikancerogenog efekta lečenja niskotemperaturnom plazmom na različitim ćelijskim linijama karcinoma (Keidar *et al.*, 2013; Schlegel *et al.*, 2013; Schlegel *et al.*, 2013; Ratovitski *et al.*, 2014; Keidar *et al.*, 2015) i tumorima na životinjskim modelima (Vandamme *et al.*, 2011; Brulle *et al.*, 2012; Keidar *et al.*, 2011). Do danas je tretman atmosferskom plazmom pokazao svoj značajan antikancerogeni kapacitet u približno 20 vrsta karcinoma *in vitro*. Među ovim ćelijskim linijama raka, rak mozga (Vandamme *et al.*, 2012; Kaushik *et al.*, 2013), rak kože (Lee *et al.*, 2009; Fridman *et al.*, 2007), rak dojke (Kim *et al.*, 2010; Ninomiya *et al.*, 2013; Wang *et al.*, 2013), rak debelog creva (Ishaq *et al.*, 2014; Plewa *et al.*, 2014), rak pluća (Keidar *et al.*, 2011; Kim *et al.*, 2011), rak grlića materice (Ahn *et al.*, 2014; Kim *et al.*, 2014; Tan *et al.*, 2014) i dr. Štaviše, oko 70% publikacija koristilo je plazmeni mlaz kao sredstvo protiv raka. Pored toga što se odupire rastu ćelija karcinoma, niskotemperaturna plazma je takođe u stanju da povрати osetljivost hemorezistentnih ćelija karcinoma na određene lekove.].

Mnoštvo reaktivnih vrsta kiseonika i azota (RONS) koje se generišu u električnim pražnjenjima bi mogle da pruže značajnu prednost u odnosu na druge terapije raka, jer npr. radioterapija i fotodinamička terapija uglavnom proizvode samo reaktivne vrste kiseonika ROS, Slika 7.13.

Različite tolerancije normalnih ljudskih ćelija i ćelija karcinoma na povišeni nivo ROS (25) i mnoštvo RONS koje generiše plazma mogu predstavljati terapijski prozor za plazmu kao potencijalnu terapiju karcinoma.

Ova primena proistekla je iz saradnje naše Laboratorije za gasnu elektroniku Instituta za fiziku i Eksperimentalne laboratorije Instituta za onkologiju i radiologiju. Kolege biohemičari i molekularni biolozi pripremili su nam uzorke koje smo tretirali u njihovoj laboratoriji koristeći naš izvor plazme.

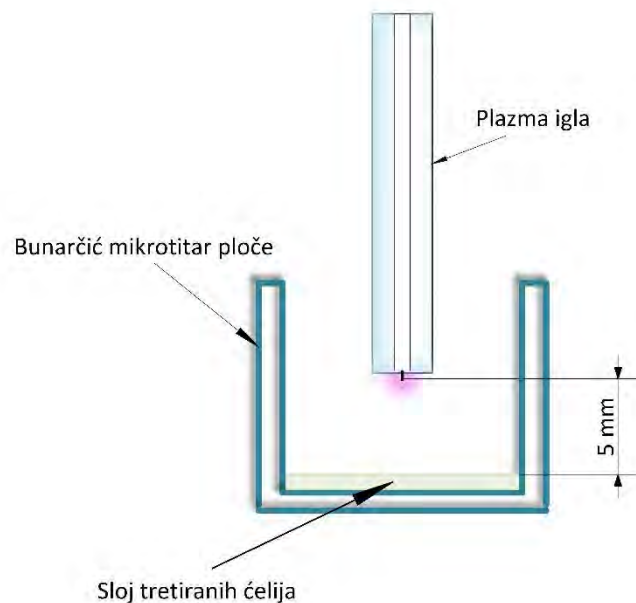
Kod ove studije korišćena je plazma igla koja je već detaljno opisana u prethodnim poglavljima. Da bismo odredili njen potencijal usled interferencije sa različitim ćelijskim procesima u ćeliji tumora, istraživali smo njen antiproliferativni potencijal u 2D i 3D modelu sistema, apoptotički potencijal, potencijal modulacije ćelijskog ciklusa i nivoa ROS u A549 ćelijama. Takođe je istražen kombinovani uticaj našeg izvora plazme na ćelijski opstanak tumorskih ćelija posle tretmana sa PARP inhibitorom.

7.5.2 Eksperimentalna postavka i priprema uzoraka za tretman plazma iglom

U prikazanoj studiji koristili smo sledeće ćelijske linije: adenokarcinom pluća čoveka (A549), ćelije karcinoma humanog grlića materice (HeLa) i ćelije bronhijalnog epitelijskog porekla bez raka (BEAS-2B) koje su održavane kao jednoslojne kulture u hranljivom medijumu Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chemicals Co, SAD). Hranljivi medijum RPMI 1640 pripremljen je u sterilnoj jonizovanoj vodi, dopunjenog penicilinom (100 U/ml), gentamicinom (50 µg/ml), 4-(2-hidroksietil) piperazin-1-etansulfonskom kiselinom (HEPES) (25 mM), L-glutamin (3 mM) i 10% toplotno inaktiviranog fetalnog telećeg seruma (FCS) (pH 7,2). Ćelije su gajene na 37°C u vlažnoj atmosferi od 95% vazduha i 5% CO₂.

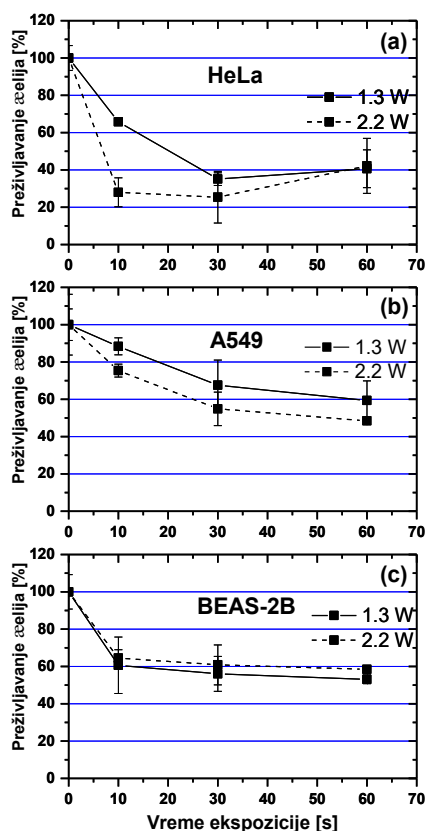
7.5.3 Analiza vijabilnosti ćelija (MTT test)

MTT test je test koji se koristi za potrebe određivanja sposobnosti održavanja ćelija u životu, tzv. vijabilnosti. Efekat citotoksičnosti tretmana niskotemperaturne plazma igle određen je pomoću 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazolium bromidom (MTT, Sigma) kao što je prethodno opisano (Supino, 1995). Ćelije su posejane u mikrotitar pločice sa 96 bunarčića (Thermo Scientific Nunc™), sa odgovarajućom gustinom ćelija za svaku ćelijsku liniju. Nakon 24 sata rasta, ćelije su bile izložene tretmanu plazma igle. Svi eksperimenti su izvršeni u sterilnoj sobi unutar komore sa laminarnim protokom u kojoj se nalazio i izvor plazme. Tokom eksperimenta varirana su dva eksperimentalna parametra: vreme ekspozicije (10 s, 30 s i 60 s) i snaga isporučena plazmi (1.3 W i 2.2 W). Svi tretmani su rađeni u tri primerka. Pred svaki tretman medijum je pipetom vađen iz bunarčića tako da je plazma dolazila u direktan kontakt sa slojem tretiranih ćelija. Nakon tretmana medijum je vraćan u bunarčiće. Udaljenost između vrha plazma igle i površine uzorka bila je 5 mm i tokom svih eksperimenata je održavana konstantnom (Slika 7.14).



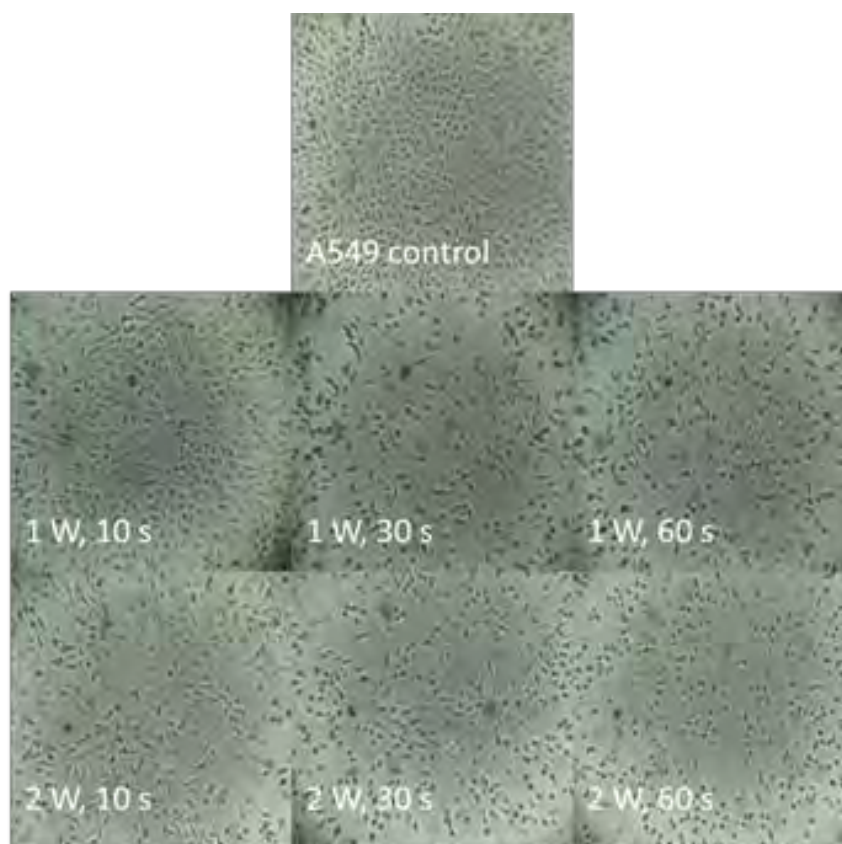
Slika 7.14 Shema pozicije plazma igle u odnosu na tretirani uzorak. (Gligorijević *et al.*, publikacija u pripremi)

Nakon 24 sata, u svaki bunarčić je dodato 20 μL rastvora MTT (5 mg/ml u fosfatnom baferu, pH 7.2). Uzorci su inkubirani 4 sata na 37°C u vlažnoj atmosferi od 5% CO_2 , a zatim je dodato 100 μL 10% natrijum dodecil sulfata (SDS). Apsorbancija je zabeležena nakon 24 sata, na čitaču enzimski imunosorbentnog testa (ELISA) (Thermo Labsystems Multiskan EKS 200-240 V), na talasnoj dužini od 570 nm.



Slika 7.15 Zavisnost preživljavanja ćelija u odnosu na vreme izlaganja tretmanu plazma iglom određena MTT testom 24 sata nakon tretmana (a) HeLa, (b) A549 i (c) BEAS-2B ćelijske linije sa tretmanom iglom u plazmi. Primenjene su dve različite snage snage 1.3 i 2.2 W i tri različita vremena ekspozicije (10, 30, 60 s). Udaljenost plazme do uzorka bila je 5 mm. (Gligorijević *et al.*, publikacija u pripremi)

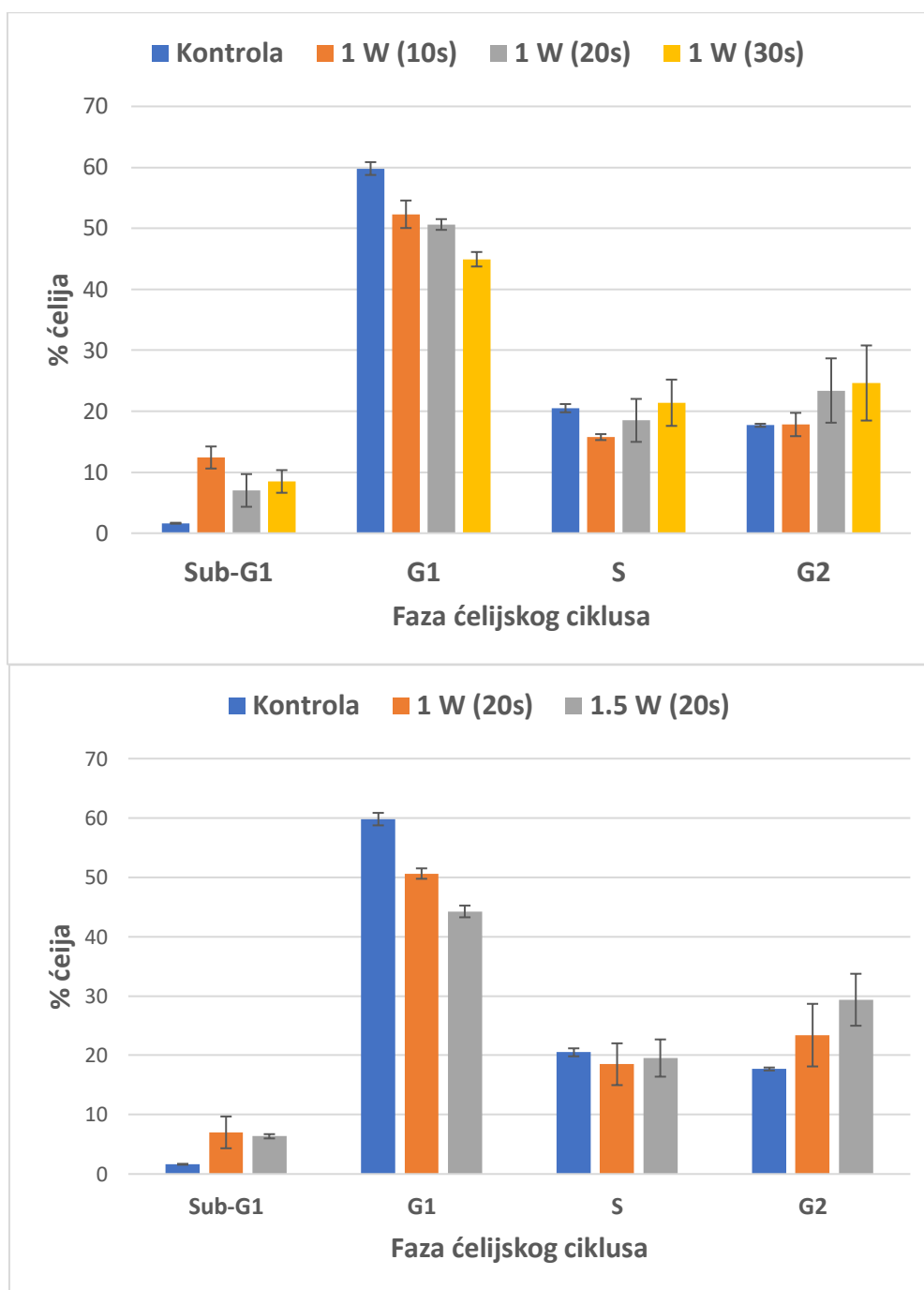
Uticaj tretmana plazme na održivost ćelija različitog porekla utvrđen je i predstavljen na Slici 7.15 kao zavisnost preživljavanja ćelija u odnosu na vreme izlaganja tretmanu plazma iglom određene MTT analizom i fotomikrografima na Slici 7.16. Dve ćelijske linije tumora A549 (humane ćelije karcinoma pluća) i HeLa (ćelije karcinoma humanog grlića materice) i jedna normalna ćelijska linija BEAS-2B (ćelije humanog bronhijalnog epitela) bile su izložene niskotemperaturnoj plazmi sa dve različite snage 1.3 i 2.2 W i tri različita vremena izlaganja (10, 30, 60 s). Rezultati prikazani na slici 7.15 pokazuju da su HeLa ćelije pokazale najveću ranjivost na tretman plazmom među ispitivanim ćelijskim linijama. Razlika između dve primenjene snage bila je najveća na HeLa ćelijama. Ćelije A549 kao primer otpornije ćelijske linije ljudskog ne malog ćelijskog karcinoma pluća (NSCLC) pokazuju manju osetljivost na lečenje. Otkriveno je određeno smanjenje preživljavanja ćelija za normalnu ćelijsku liniju, BEAS-2B, ali nije dostiglo 50% smanjenja preživljavanja ćelija nakon lečenja. Antiproliferativni i citotoksični potencijal mnogih različitih niskotemperaturnih sistema plazme primećen je i potvrđen kod mnogih vrsta tumora, uključujući ćelijske linije korišćene u našim eksperimentima (Arjunan *et al.*, 2015). Eksperimenti su potvrdili potencijal našeg izvora plazme za netermičko lečenje gde se smanjuje održivost ćelija karcinoma.



Slika 7.16 Fotografije snimljene mikroskopom za netretirane ćelije A549 (kontrola) i tretirane ćelije A549 za različite snage plazme i vreme izlaganja. (Gligorijević *et al.*, publikacija u pripremi)

7.5.4 Analiza ćelijskog ciklusa

Analiza fazne raspodele ćelijskog ciklusa A549 ćelija nakon tretmana plazma iglom u zavisnosti od vremena ekspozicije i snage izvršena je bojanjem propidijum jodidom (PI) i protočno-citometrijskom analizom (Ormerod, 1994). Ćelije su posejane gustinom od 1.5×10^4 ćelije po bunarčiću, u mikrotitar pločici sa 48 bunarčića (Thermo Fisher Scientific), u hranljivom medijumu. Nakon tretmana, ćelije su inkubirane u vremenskom periodu od 24 sata, a zatim su sakupljene tripsinizacijom i fiksirane u 70% etanolu na niskoj temperaturi. Ćelije su ostavljene na temperaturi od -20°C do dalje analize. Za analizu, ćelije su sakupljene centrifugiranjem i resuspendovane u 800 μL PBS. Zatim su tretirani sa 100 μL RNaze (1 mg/ml) i 100 μL propidijum jodida (400 $\mu\text{g}/\text{ml}$) i inkubirani na 37°C tokom 30 minuta. Obojene ćelije su analizirane pomoću fluorescentno aktiviranog sortirnika ćelija (FACS) na protočnom citometru Calibur Becton Dickinson, na pobudnoj liniji od 488 nm (argon-jonski laser). Podaci su analizirani pomoću računarskog softvera Cell Kuest.



Slika 7.17 Vremena ekspozicije (10 s, 20 s, 30 s) (gore) i perturbacije ćelijskog ciklusa ćelija A549 ćelije zavisne od snage (1 i 1.5 W) (dole) 24 sata nakon tretmana. Prikazan je reprezentativni eksperiment, svaki uzorak je analiziran u duplikatu. (Gligorijević *et al.*, publikacija u pripremi)

Na Slici 7.17 prikazani rezultati analize ćelijskog ciklusa. pokazuju da lećenje plazma iglom ima potencijal da indukuje poremećaje ćelijskog ciklusa A549 ćelija. Posle samo 10 s tretmana sa 1 W, procenat ćelija u G1 fazi opada. Produženje tretmana na 20 s i 30 s indukovalo je izraženiji pad procenta ćelija u G1 fazi do 45% (za 30 s tretmana) u poređenju sa kontrolom koja je imala 60% ćelija u G1 fazi. Tretman plazmom je takođe indukovao povećanje procenta ćelija u G2 fazi, dok se procenat ćelija u Sub-G1 fazi (koja predstavlja fragmentiranu DNK) nije značajno povećao (do 12% u poređenju sa 2% u kontroli). Promene u ćelijskom ciklusu pokazale su isti trend, jer smanjenje procenta ćelija u G1 fazi i

povećanje procenta ćelija u G2 fazi zavise od upotrebljene snage. Zastoj G2 faze primećen je u nekoliko drugih ćelija karcinoma lečenih hladnom plazmom (Dayun *et al.*, 2017), bez jasnog objašnjenja mogućeg uzročnog puta.

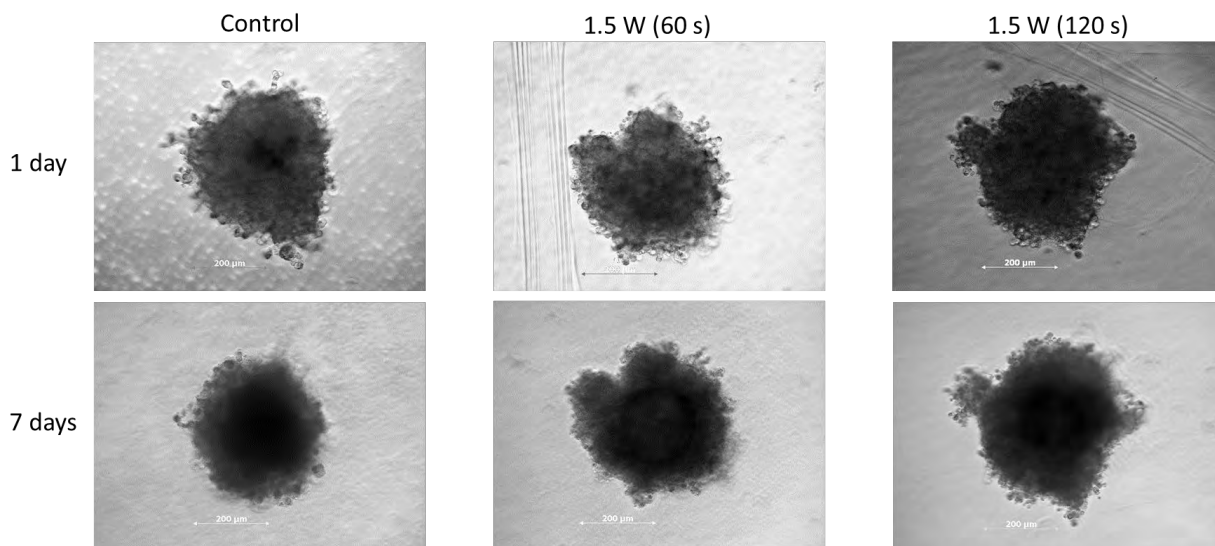
7.5.5 Istraživanje inhibicije rasta u 3D modelu kulture

3D model kulture višecelijskih tumorskih sferoida (*eng. MultiCellular Tumor Spheroides - MCTS*) ćelija A549 pripremljen je korišćenjem pločica sa dnom za pričvršćivanje ćelija U96 sa 96 bunarčića Thermo Scientific Nunclon Sphera (Nunclon Sphera pločica od 96 bunarčića sa dnom U-oblika). Ćelije A549 u eksponencijalnoj fazi rasta razdvojene su rastvorom tripsin / EDTA da bi se dobile suspenzije jednoćelija. Izvestan broj od 5000 ćelija/bunarčiću je prebačen na ploče sa 96 bunarčića zapremine od 200 μ L RPMI koji sadrži 10% seruma. Pojedinačne ćelije su formirale MCTS agregate prečnika približno 400 μ m nakon tri dana. Stvaranje i rast sferoida ispitivani su pomoću fluorescentnog mikroskopa Akio Observer Z1, koristeći softver za obradu slika AkioVision (Carl Zeiss MicroImaging GmbH). Sferoidi A549, prethodno izabrani za homogenu zapreminu i oblik, tretirani su plazma iglom sa 1 W tokom 60 s i 120 s u tri primerka. Inhibicija rasta praćena je prečnikom mernih sferoida nakon 1 dana i 7 dana lečenja. Slike svetlog polja dobijene korišćenjem fluorescentnog mikroskopa Akio Observer Z1, (objektiv 10k/0.5) analizirani su pomoću AkioVision Rel 4.7 Imaging Sistem interaktivnim merenjem prečnika MCTS-a.

7.5.6 Rezultati ispitivanja inhibicije rasta u 3D modelu kulture

Konačno, 3D model kulture višecelijskih tumorskih sferoida (MCTS) je korišćen za predviđanje *in vivo* antitumorskih efekata našeg sistema za tretman niskotemperaturnom plazmom u ćelijama A549. MCTS pružaju uvid u metabolička svojstva slična profilima solidnih tumora kao što su gradijenti hranljivih sastojaka i kiseonika, hipoksični/nekrotični regioni, interakcije matriksa ćelijskih ćelija i ekspresija gena (Sritama i Devi, 2016).

Rezultati prikazani na Slici 7.18 pokazuju da nije bilo smanjenja rasta MCTS ćelija A549 nakon tretmana plazmom vremenima ekspozicije od 60 s i 120 s za ispitivane eksperimentalne uslove. Zbog složenosti sistema 3D modela potrebna su dalja opsežna ispitivanja, različitog trajanja, kao i drugi eksperimentalni uslovi, za sveobuhvatnije istraživanje efekta plazma igle.



Slika 7.18 Praćenje inhibicije rasta tretiranih uzoraka A549 MCTS. (Gligorijević *et al.*, publikacija u pripremi)

Ćelije A549 posejane su sa gustinom od 5000 ćelija po bunarčiću, u pločici Thermo Scientific Nunclon Sphera sa dnom za pričvršćivanje ćelija U96. Nakon 3 dana kulture (vreme sferoidizacije), A549 MCTS prečnika manjeg od 400 μm , prethodno odabrani za homogenu zapreminu i oblik, tretirani su plazma iglom snage 1.5W tokom 60 s i 120 s. Slike svetlog polja su dobijene korišćenjem Akio Observer Z1, (objektiv 10k/0.5), skala: 200 μm , koristeći softver za obradu fotografija AkioVision (Carl Zeiss MicroImaging GmbH).

7.5.7 Zaključak

U prikazanim eksperimentima primenom plazma igle tretirani su uzorci biološkog porekla, kancerogene ćelije:

- adenokarcinom pluća čoveka (A549),
- ćelije karcinoma humanog grlića materice (HeLa),
- 3D model kulture višećelijskih tumorskih sferoida (A549)

kao i normalne ljudske ćelije:

- ćelije bronhijalnog epitela ljudskog porekla bez raka (BEAS-2B).

Urađene su metode vijabilnosti ćelija (MTT test), analiza ćelijskog ciklusa kao i inhibicija rasta u 3D modelu kulture. Dobijeni su sledeći zaključci:

- plazma igla smanjuje vijabilnost ćelija karcinoma (u većoj meri kod HeLa u odnosu na A549 koje spadaju u grupu otpornih ćelija mada se i kod njih primeti pad vijabilnosti korišćenjem tretmana plazma iglom),
- plazma igla takođe smanjuje vijabilnost normalnih ljudskih ćelija ali u manjoj meri i te ćelije su sklonije bržem oporavku nakon stresa doživljenog usled tretmana plazma iglom,
- plazma igla ima potencijal da izazove poremećaj u fazama ćelijskog ciklusa nakon kratkog tretmana od 10 s, duža vremena i veće snage pospešuju efekat u smanjenju procenta ćelija,
- prvi put su niskotemperaturnom plazmom tretirani 3D modeli („imitacija“ kancerogenog tkiva koji služi za simuliranje *in vivo* eksperimenta) kancerogenih ćelija međutim nije došlo do smanjenja njihovog rasta i za ovaj složen sistem su potrebna dalja istraživanja.

8. ZAKLJUČAK

Glavni fokus ove disertacije bio je na masenoj spektrometriji sledećih izvora električnih pražnjenja na atmosferskom pritisku:

- **plazmenog mlaza,**
- **mногоstrukog plazmenog mlaza,**
- **koplanarnog dielektričnog barijernog pražnjenja DCSBD-a.**

Pored navedenih izvora, za potrebe biomedicinskih primena koje su prikazane, korišćena je **plazma igla**, izvor plazme za koji ranije urađena detaljna analiza.

Pored glavne dijagnostičke metode, masene spektrometrije, korišćene su i ICCD optička emisiona spektroskopija za praćenje svetlosnih intenziteta plazmenih mlazova pri praćenju slobodnog prostiranja električnog pražnjenja u okolnu sredinu kao i električna karakterizacija svakog izvora plazme radi lakšeg praćenja i kontrolisanja prilikom analize i tretmana.

Što se tiče plazmenog mlaza i ispitivanja prostiranja PAPS-a moramo istaći uticaj vodene pare na njegovo formiranje pri određenim uslovima koje do sada nije objavljeno u literaturi kao i određivanje masenog sastava PAPS-a korišćenjem vremenske evolucije pozitivnih jona.

U trećoj glavi opisano je ispitivanje uticaja vlažnosti unutar protočnog sistema radnog gasa na formiranje PAPS-a. Urađena su merenja u eksperimentalnoj postavci gde je korišćena ispiralica za veštačko dodavanje vlažnosti u sistem i varijanta bez nje. Ustanovljeno je da bez vlažnosti unutar protočnog sistema, tj. kada je on skroz prosušen, PAPS gubi svoj oblik i pražnjenje ima formu razmrljanog traga u obliku difuznog režima. Ustanovljeno je da veštački dodata vodena para vraća formu PAPS-a ali mu smanjuje domet i menja hemijski sastav električnog pražnjenja. Pretpostavka je da veštački dodata vlažnost ima homogeniju strukturu molekula vode u poprečnom preseku protočnog sistema, dok se vodena para koja nije veštački dodata prosušuje pod uticajem protoka helijuma sa zidova cevi na kojima je adsorbovana najverovatnije u obliku prstena i da zato ne utiče toliko na hemijski sastav i na skraćanja prostiranja PAPS-a.

U četvrtoj glavi opisana je studija vremenske evolucije PAPS-a korišćenjem sinhronizovanih merenja masenim spektrometrom, ICCD kamerom i naponskim sondama. Iz dobijenih merenja je pored praćenja razloženih snimaka intenziteta svetlosti plazmenog mlaza određen i maseni sastav najzastupljenijih vrsta koje sačinjavaju pražnjenje a samim tim i PAPS. Urađeno je poređenje za dva različita protoka radnog gasa na fiksnom rastojanju plazmenog mlaza u odnosu na maseni spektrometar i pri fiksnom primenjenom naponu. Ustanovljeno je da promena protoka ne utiče značajno na prostiranje težih vrsta koja se kreću prilično sinhronizovano dok su lakše atomske vrste poput vodonika fazno pomerene. Određivanje i variranje sastava PAPS-a ovom metodom može biti od velikog značaja za potencijalne buduće primene.

U petoj glavi prikazana je masena spektrometrija i ICCD optička emisiona spektroskopija intenziteta svetlosti mnogostrukog plazmenog mlaza - MPM. Ispitivana je kompleksna međusobna interakcija mlaznica mnogostrukog mlaza. Varijacijom brzine protoka radnog gasa moguće kompletno promeniti prirodu pražnjenja ovog izvora. Pravim izborom protoka moguće je dobiti spojeni ili nespojeni režim koji imaju međusobno različit broj jonizacionih frontova, različitu vremensku evoluciju i maseni sastav. Masena spektrometrija je pokazala bogatu hemiju pri različitim varijacijama mešanja helijuma sa okolnim vazduhom. Primećena je i pojava višestrukih sekundarnih strimera koji se kreću duž ponavljajućih jonizacionih kanala tokom rada MPM-a. Iz električne karakterizacije MPM u spojenom režimu iskazuje nelinearnost tokom pražnjenja usled povećanja inicijalnog napona. Nakon određene

vrednosti inicijalnog napona dolazi do pojave tinjavog pražnjenja koji karakteriše struja visokog intenziteta i stabilan napon na napajanoj elektrodi.

Šesta glava se bavi opisom masene spektrometrije na atmosferskom pritisku DCSBD pražnjenja malih dimenzija. Za šest različitih smeša kiseonika i azota (najveća pažnja je posvećena odnosu 80/20 što predstavlja odnos koji je u atmosferi) i tri različite frekvencije (15 kHz, 30 kHz i 50 kHz) pri konstatnom visokom naponu (11.2 kV) posmatrana je produkcija RONS-a (N, O, OH, N₂, NO, O₂, NO₂, N₂O ili CO₂ i O₃) u električnom pražnjenju. Posebna pažnja je posvećena vrstama od značaja kao što su N, NO_x ili O₃, jer ove vrste igraju važnu ulogu u interakciji plazme sa živim organizmima i tkivima. Variranje eksperimentalnih uslova je prikazalo značajne razlike. Pri višim frekvencijama od 30 kHz i 50 kHz primećen je maksimum odbroja za NO radikal u uslovima 80/20 odnosa azota i kiseonika. Za najnižu frekvenciju od 15 kHz proizvodnja molekula O₃ i NO bila je najveća u ispitivanom rasponu od 15kHz–50 kHz. U zavisnosti od tipa primene produkcija ovih vrsta se variranjem parametara može smanjiti ili povećati.

U glavi sedam prikazani su rezultati tretmana plazma igle na biljne ćelije kalusa *Daucus carota*. Značajni efekti na uzorcima su dobijeni odmah nakon tretmana kao i dve nedelje kasnije. Za ovo su zaslužne reaktivne kiseonične vrste – ROS koje se stvaraju u ćelijama i u pražnjenju plazma igle i oni izazivaju aktivnost enzima unutar ćelija. Enzimi su deo odbrambenog mehanizma od oksidativnog stresa do kojeg dolazi ukoliko je vrednost ROS-a visoke koncentracije. Aktivnost SOD enzima, iako je i dalje bila znatno veća od aktivnosti u netretiranim ćelijama, smanjila se posle dve nedelje u poređenju sa aktivnošću neposredno posle tretmana. Aktivnost CAT enzima se značajno povećava nakon dve nedelje tretmana.

U istoj glavi opisani su efekti nakon tretmana MRSA bakterija plazma iglom gde su varirani sledeći parametri: rastojanje plazma igle od površine uzorka (2 mm, 4 mm i 6 mm), snaga plazme (1.0 W i 2.5 W), vreme ekspozicije, tj. vreme izlaganja uzorka električnom pražnjenju (30 s, 60 s, 90 s i 180 s). Korišćena su dva tipa pripreme MRSA bakterija: bakterije na hranljivoj podlozi u Petri posudi i biofilm bakterija u miktortitar pločicama. Dobijeni rezultati tretmana impliciraju da je najefikasniji set parametara najduže vreme ekspozicije i najmanje rastojanje od plazma igle u odnosu na uzorak. Plazma igla bez obzira što formira vidljivo pražnjenje dijametra 3-4 mm za određeni set parametara svojim delovanjem formira inhibicione zone MRSA bakterija značajno većeg dijametra (za najduže vreme ekspozicije 180 s i snagu plazme od 2.5 W je ona iznosila 9 mm). Pretpostavka je da se širenje dešava pod uticajem reaktivnih vrsta generisanih u električnom pražnjenju na atmosferskom pritisku.

Poslednja primena opisana u sedmoj glavi predstavlja ispitivanje tretmana gde su primenom plazma igle tretirani uzorci biološkog porekla, kancerogene ćelije: adenokarcinom pluća čoveka (A549), ćelije karcinoma humanog grlića materice (HeLa), 3D model kulture viševićijskih tumorskih sferoida (A549) kao i normalne ljudske ćelije: ćelije bronhijalnog epitelijskog ljudskog porekla bez raka (BEAS-2B). Pokazano je da u većoj ili manjoj meri plazma igla svakako smanjuje vijabilnost kod kancerogenih ćelija. U slučaju ljudskih nekancerogenih ćelija taj efekat je slabiji i te ćelije se brže oporavljaju. Ovaj tip pražnjenja pri lečenju kancerogenih ćelija može da izazove poremećaj u fazama ćelijskog ciklusa i da smanji njihovu produkciju. Prikazani su i preliminarni rezultati tretmana 3D modela, tj. klastera kancerogenih ćelija koji za ispitivanje predstavljaju veliki korak ka in vivo tretmanima jer se ponašaju kao tkiva kancera. Za sad se nisu dobili veći efekti u pokušaju njihovog smanjenja nakon tretmana i potrebna su dodatna istraživanja.

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Nenad Selaković je predlog teme uspešno odbranio pred Kolegijumom doktorskih studija na Fizičkom fakultetu 24.10.2018. godine. Na sednici Nastavno–naučnog veća Fizičkog fakulteta održanoj 30.1.2019. godine usvojen je Izveštaj Komisije za ocenu ispunjenosti uslova i opravdanost predložene teme za izradu doktorske disertacije pod nazivom *Masena spektrometrija plazmenog mlaza i primene električnih pražnjenja na atmosferskom pritisku u biomedicini*, pod mentorstvom dr Nevene Puač naučnog savetnika u Institutu za fiziku.

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Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду**.

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

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Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

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Effects of non-thermal atmospheric plasma treatment on dentin wetting and surface free energy for application of universal adhesives

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Abstract

Objectives The study aims to evaluate the effects of non-thermal atmospheric plasma (NTAP) treatments on dentin wetting and surface free energy (SFE) and compare the effects of NTAP treatment, etch-and-rinse, and self-etch protocols for application of universal adhesives.

Materials and methods Mid-coronal dentin of intact third molars was used to measure contact angles of distilled water, ethylene-glycol, and diiodomethane and calculate SFE following different NTAP preset treatments (feeding gas consisting of pure He, He + 1% O₂, He + 1.5% O₂), power input (1 or 3 W), and tip-to-surface distance (2, 4, or 8 mm). Contact angles of reference liquids and SFE of dentin following He + 1.5% O₂ at 3-W and 4-mm treatment was compared to phosphoric acid etching. Contact angles of Single Bond Universal (SBU; 3M ESPE) and Clearfil Universal Bond (CUB; Kuraray Noritake) were measured following NTAP, etch-and-rinse, and self-etch protocols.

Results NTAP significantly reduced contact angles of reference liquids and increased dentin SFE compared to untreated dentin ($p < 0.05$). O₂ intensified the effect of He NTAP ($p < 0.05$). NTAP and phosphoric acid increased dentin polarity and Lewis base surface characteristics. Phosphoric acid increased contact angles of adhesives compared to the self-etch protocol ($p < 0.05$). NTAP resulted in lower adhesive contact angles than phosphoric acid, the difference being statistically significant for CUB ($p < 0.05$). Compared to the self-etch protocol, NTAP slightly reduced CUB contact angle but not that of SBU ($p > 0.05$).

Conclusions He NTAP with and without O₂ increased dentin wetting and SFE, surpassing the effect of phosphoric acid and lowering adhesive contact angles. NTAP produced no apparent micro-morphological changes on dentin surface comparable to acid etching.

Clinical significance NTAP treatment of dentin prior to adhesive application increases dentin wetting and surface free energy facilitating better adhesive distribution on dentin surface compared to phosphoric acid etching and similar to the “self-etch” application protocol.

Keywords Contact angle · Dentin · Non-thermal atmospheric plasma · Surface free energy · Universal adhesive · Wetting

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Introduction

Current concepts of dentin adhesion include “etch-and-rinse” (ER) or “self-etch” (SE) approaches. An ER approach consists of etching dentin with 32–37% phosphoric acid to remove the smear layer, demineralize superficial dentin, expose the ultra-fine collagen mesh, and open dentinal tubules for subsequent adhesive penetration [1]. An SE approach excludes phosphoric acid step and instead relies on acidic monomers in dental adhesive formulation to partially demineralize dentin surface [2].

Universal adhesives, a recently marketed group of dental adhesives, are recommended for adhesion to dental tissues following either approach as well as to materials for indirect

restorations without separate priming, hence the term “universal.” Despite reports favoring the SE approach [3, 4], the manufacturers’ recommendations leave it up to the practicing dentist to choose a preferable application strategy for universal adhesives, suggesting that both ER and SE are likely used in current dental practice.

Surface modification by phosphoric acid etching has long been the preferred method of choice for optimizing dentin adhesion, though more complex than that on enamel. The effects of phosphoric acid on dentin are well known from the micro-morphological point of view [1]. However, the reported effects of acid etching on physico-chemical surface characteristics of dentin are contradictory. Attal et al. [5] concluded that acid etching results in a hydrophobic dentin surface while other authors reported increased hydrophilicity of dentin surface [6, 7].

Non-thermal atmospheric plasma (NTAP) is another powerful surface modification tool recently gaining momentum in adhesive dentistry research. NTAP is widely used in material engineering, production of semiconductor devices, integrated circuits, solar cells, etc. [8–11]. In case of biomedical applications, NTAP may be used for treatment of heat-sensitive samples that cannot withstand vacuum. Also, NTAP or plasma liquid interaction is becoming great interdisciplinary area of research [12]. NTAPs are known for high amounts of reactive oxygen and nitrogen species (RONS) responsible for plasma interactions with biological samples [13–15]. In this paper, as an NTAP source, we used a plasma needle constructed and developed in Laboratory for gaseous electronics, Institute of Physics Belgrade, Serbia. The plasma needle was previously used to enable an osteogenic differentiation of human periodontal ligament mesenchymal stem cells (hPDL-MSCs) that can help in cell-based treatment of periodontal defects [16], decontamination of bacteria in planktonic samples and biofilms [17–19], and the damage effect of plasma treatment was compared to the effects of ionizing radiation through induced DNA damage [20].

Literature review on NTAP in adhesive dentistry shows that NTAP was previously used to modify surfaces of dental tissues and restorative materials, induce resin polymerization, and facilitate resin penetration and grafting onto collagen [21]. More specifically, NTAP was shown to substantially decrease water contact angles on dentin [22–24] with atomic compositional changes in the form of decreased percentage of C and N and increased O, Ca, and P [22]. Similar effects of NTAP on water contact angles were observed on enamel [22, 25], composites [22], and ceramics [26–28], suggesting increased hydrophilicity of all these substrates. Argon NTAP increased surface free energy and wettability of root dentin following sodium hypochlorite irrigation [29]. A recent paper [30] found no significant changes in dentin-surface topography after NTAP treatment for clinically relevant times. However, more numerous resin tags and a thinner dentin

hybrid layer were visible after NTAP treatment. Enzymatic activity of matrix metalloproteinases was dependent on the NTAP treatment time [30]. Previous studies offered inconsistent evidence on the effects of NTAP on adhesive bond strength to dentin warranting further research [23, 24, 30–34]. An insight into previous studies reveals a non-standardized approach to the use of NTAP with differences regarding carrier gas, power, time, and distance between the plasma source and the treated substrate.

Wetting (wettability) indicates the ability of an adhesive to spread over dentin as a result of the balancing adhesive and cohesive forces. It further contributes to adhesive penetration into collagen interfibrillary spaces and dentinal tubules by capillary forces, thereby improving dentin hybridization as the primary mechanism of adhesion [5]. In dental research, wetting characteristics of a substrate (e.g., dentin) are commonly determined by measuring contact angles of water. Conclusions on adhesive-dentin interaction based only on water contact angles may underestimate the effects of a complex adhesive mixture of polar and apolar, hydrophilic, and hydrophobic components. Taking into account polar and apolar interactions between the liquid and solid, surface free energy (SFE) allows better understanding of the nature of surface interactions between adhesives and dentin.

The aims of this study were to (1) compare the effects of a range of NTAP treatments on wetting and SFE of dentin and (2) compare a selected, most efficient NTAP treatment with ER and SE application protocols for universal adhesives in terms of wetting and SFE of dentin. The following working hypotheses were tested: (1) reduced distance, increased power, and O₂ feed in NTAP reduce the contact angles of reference liquids and increase SFE of dentin and (2) compared to ER and SE application protocols, an NTAP treatment results in lower contact angles of reference liquids and universal adhesives and higher SFE of dentin.

Materials and methods

Sample preparation

A total of 278 human, intact, third molars extracted for orthodontic reasons were used in this study. Ethics Committee of the University of Belgrade, School of Dental Medicine approved (Approval No. 36/16) of the use of such teeth in research purposes and teeth were collected with patients’ consent. Following extraction, the teeth were cleaned of debris, stored in 0.2% thymol in a refrigerator at +4 °C, and used within 3 months of extraction.

Each tooth was embedded in super-hard gypsum up to the enamel-cementum junction. The cusps were cut off using a slow-speed diamond saw (Isomet 4000, Buehler, Lake Bluff, IL, USA) to expose flat dentin mid-coronally. A second cut

was made 1 mm below the exposed dentin surface, producing a 1-mm-thick dentin disk, one from each crown/tooth. Dentin disks were wet-polished manually with a 600-grit SiC abrasive paper for 30 s to produce a smear layer.

All dentin disks were allocated to groups and subgroups according to the subsequent treatment and reference liquids used for contact angle measurement, as shown in Table 1. Based on the results for 100% He NTAP, 8-mm tip-to-surface distance was not tested with NTAP with O₂ feed to reduce the overall number of groups. Additional disks were prepared for scanning electron microscopy (SEM) analysis.

NTAP treatment

An NTAP source used in this work was the so-called plasma needle [35] which generated non-thermal plasma at 13.56-MHz frequency (Fig. 1). The powered electrode of plasma needle was wolfram wire (outer diameter (o.d.) 0.5 mm) enclosed in a ceramic tube. Both were placed within a glass tube with an inner diameter of 4 mm (outer diameter (o.d.) 6 mm). The glass tube was held in Teflon support. In all experiments, we used helium or a mixture of helium and oxygen as the carrier gas and gas flow was kept constant at 1 slm (standard liter per minute). We used pure He, 99% He + 1% O₂, or 98.5% He + 1.5% O₂ mixtures. The distances between the treated dentin surface and the tip of the plasma needle were 2, 4, or 8 mm. The applied powers of plasma needle in this experiment were 1 or 3 W. NTAP treatment time was 30 s in all groups. In order to determine the power delivered to the plasma, we used derivative probes developed at the Laboratory for gaseous electronics, Institute of Physics Belgrade, Serbia [36].

Acid etching and adhesive application

To simulate the ER adhesive application protocol, the upper surface of each dentin disk was etched with 32% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE, St. Paul, MN, USA) for 15 s, rinsed under tap water for 15 s, and blot-dried using a cotton pellet (Agava, Prokuplje, Serbia).

To simulate the SE application protocol, dentin disks with the smear layer were blot-dried and used without any additional treatment. Table 2 provides details on the materials used in this study.

Contact angle measurements and surface free energy calculation

The sessile drop technique was used to measure contact angles of three reference liquids: distilled water, ethylene glycol (Sigma-Aldrich, St. Louis, MO, USA), and diiodomethane (Acros Organics, Fairlawn, NJ, USA). Following the ER and SE protocols, universal adhesives SBU and CUB were also used for contact angle measurements.

Table 1 Allocation of teeth to groups and subgroups according to treatments and reference liquids

Group	Surface treatment parameters	Reference liquid	Number of teeth	
NTAP 100% He	1 W, 2 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	1 W, 4 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	1 W, 8 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	3 W, 2 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
		Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	3 W, 4 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
3 W, 8 mm	Distilled water	5		
	Ethylene-glycol	5		
	Diiodomethane	5		
NTAP 99% He + 1% O ₂	1 W, 2 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	1 W, 4 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	3 W, 2 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	3 W, 4 mm	Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
	NTAP 98.5% He + 1.5% O ₂	1 W, 2 mm	Distilled water	5
			Ethylene-glycol	5
			Diiodomethane	5
		1 W, 4 mm	Distilled water	5
			Ethylene-glycol	5
			Diiodomethane	5
3 W, 2 mm		Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
3 W, 4 mm		Distilled water	5	
		Ethylene-glycol	5	
		Diiodomethane	5	
Phosphoric acid		15 s	Distilled water	5
			Ethylene-glycol	5
			Diiodomethane	5
Control group		No treatment	Distilled water	5
			Ethylene-glycol	5

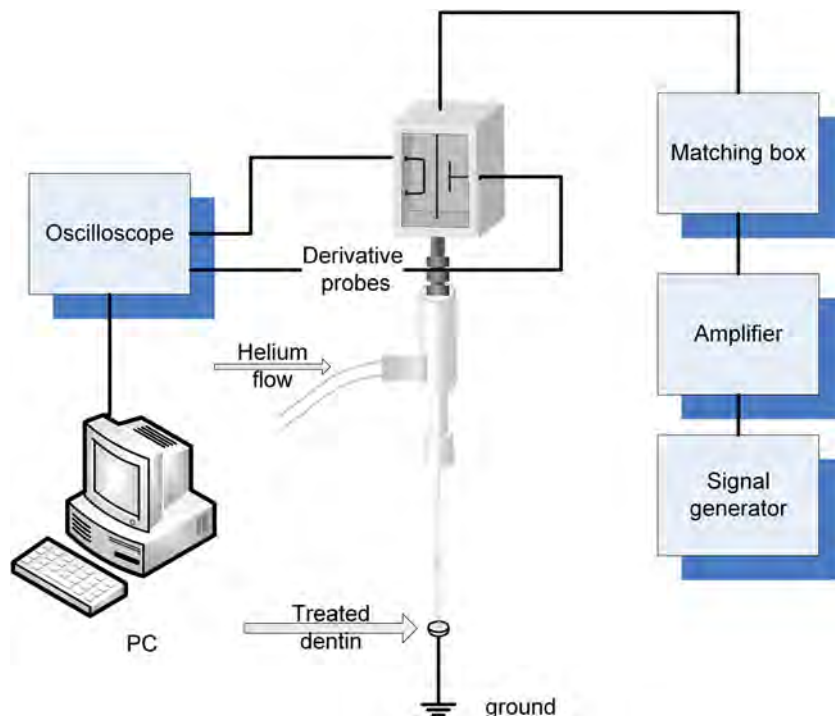
Table 1 (continued)

Group	Surface treatment parameters	Reference liquid	Number of teeth
		Diiodomethane	5
ER_adhesive	ER application protocol	SBU	5
		CUB	5
SE_adhesive	SE application protocol	SBU	5
		CUB	5
NTAP_adhesive	98.5% He + 1.5% O ₂ 3 W, 4 mm	SBU	5
NTAP_adhesive	98.5% He + 1.5% O ₂ 3 W, 4 mm	CUB	5
SEM (control × 1 teeth, phosphoric acid × 1 teeth, 100% He NTAP × 2 teeth, 99% He+1% O ₂ NTAP × 2 teeth, 98.5% He+1.5% O ₂ NTAP × 2 teeth)			8

NTAP non-thermal atmospheric plasma, SBU Single Bond Universal, CUB Clearfil Universal Bond, SEM scanning electron microscopy

The experiment was so designed that NTAP treatment or acid etching and contact angle measurement were performed under the same temperature (23.3 ± 0.3 °C) and humidity ($34.1\% \pm 1.5\%$) conditions. Immediately after NTAP or acid etching treatment, the samples were transferred onto the contact angle measurement bench, so they remained still partially wet. A pre-set amount of liquid (2 μ l) was dispensed from a micropipette (BIOHIT, BiohitOyj, Helsinki, Finland) onto dentin surface at a 90° angle and 4-mm distance. The contact angles (θ) of reference liquids and universal adhesives were recorded using a contact angle analyzer 1 s after the drop touched dentin surface and measured using ImageJ software

Fig. 1 Experimental setup of NTAP plasma needle for dentin treatment. (Signal generator, amplifier, and matching box are main components of the electrical circuit that supplies power to the NTAP. The oscilloscope and PC are used to control the power delivered to the NTAP)



(version 1.42, National Institute of Health, USA). Contact angle analyzer was constructed at the Institute of Physics to allow the abovementioned standard conditions (Fig. 2). The setup included a DSLR Nikon D7100 camera with mounted Nikkor Macro lens 105 mm f2.8 D, Nikon SB910 flashlight, and position stand. Camera in relation to the sample was adjusted using a tripod with built-in level so it was always in the horizontal plane with the sample holder.

SFE (γ) calculation was based on van Oss-Chaudhury-Good thermodynamic approach for solids and the three-liquid phase method. According to this theory, both apolar and polar interactions occur at the interface between liquid and solid materials [37]. Apolar interactions occurring between two completely apolar compounds are known as Lifshitz-van der Waals interactions. A component of SFE of compound i based on strictly apolar interactions is expressed as Lifshitz-van der Waals component or γ_i^{LW} . Polar interactions are largely based on hydrogen-bonding or electron acceptor-electron donor (Lewis acid-base) interactions between polar moieties. These Lewis acid-base interactions are asymmetrical, so electron acceptor and electron donor parameters of the polar component of SFE of compound i are expressed as γ_i^+ and γ_i^- [37]. SFE of a solid (γ_S) and its components (apolar γ_S^{LW} , Lewis acid γ_S^+ , and Lewis base γ_S^-) are calculated using the contact angles (θ) of three reference liquids with their known SFE parameters (γ_L^{LW} , γ_L^+ , and γ_L^-) according to the following formula:

$$(1 + \cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+\gamma_L^-} + \sqrt{\gamma_S^-\gamma_L^+}\right)$$

Table 2 Materials used in this study

Material (code/batch)	Manufacturer	Composition ^a	Application protocol
Single Bond Universal (SBU/595822)	3M ESPE, St. Paul, MN, USA	BisGMA (15–25%), HEMA (15–25%), DMDMA (5–15%), ethanol (10–15%), water (10–15%), silane treated silica (5–15%), 1,10-decanediol methacrylated phosphates (1–10%), copolymer of acrylic and itaconic acid (1–5%), dimethylaminobenzoat (<2%), camphorquinone (<2%), DMAEMA (<2%), methylethylketone (<0.5%)	Etch-and-rinse: apply etchant for 15 s, rinse for 15 s, blot-dry with a cotton pellet, apply SBU to dentin using a disposable applicator and rub it in for 20 s, air dry with a gentle stream of air for about 5 s Self-etch: rinse and dry apply SBU to dentin using a disposable applicator and rub it in for 20 s, air dry with a gentle stream of air for about 5 s
Clearfil Universal Bond (CUB/BN0010)	Kuraray Noritake Dental Inc., Okayama, Japan	BisGMA (15–35%), HEMA (10–35%), ethanol (<20%), 10-MDP, hydrophilic aliphatic dimethacrylate, colloidal silica, camphorquinone, silane, accelerators, initiators, water	Etch-and-rinse: apply etchant for 15 s, rinse for 15 s, blot-dry with a cotton pellet, apply CUB, dry the entire cavity by blowing mild air more than 5 s Self-etch: rinse and dry, apply CUB with a rubbing motion to the entire cavity wall with an applicator brush, dry the entire cavity by blowing mild air more than 5 s
Scotchbond Universal Etchant (Acid/603390)	3M ESPE, St. Paul, MN, USA	Water, phosphoric acid, synthetic amorphous silica, fumed, crystalline free, polyethylene glycol, aluminum oxide	Apply etchant for 15 s, rinse for 15 s

BisGMA bisphenol A diglycidylmethacrylate, *HEMA* 2-hydroxyethyl methacrylate, *DMDMA* decamethylenedimethacrylate, *DMAEMA* dimethylaminoethyl-dimethacrylate, *10-MDP* 10-methacryloyloxydecyl dihydrogen phosphate

^a Manufacturer’s data

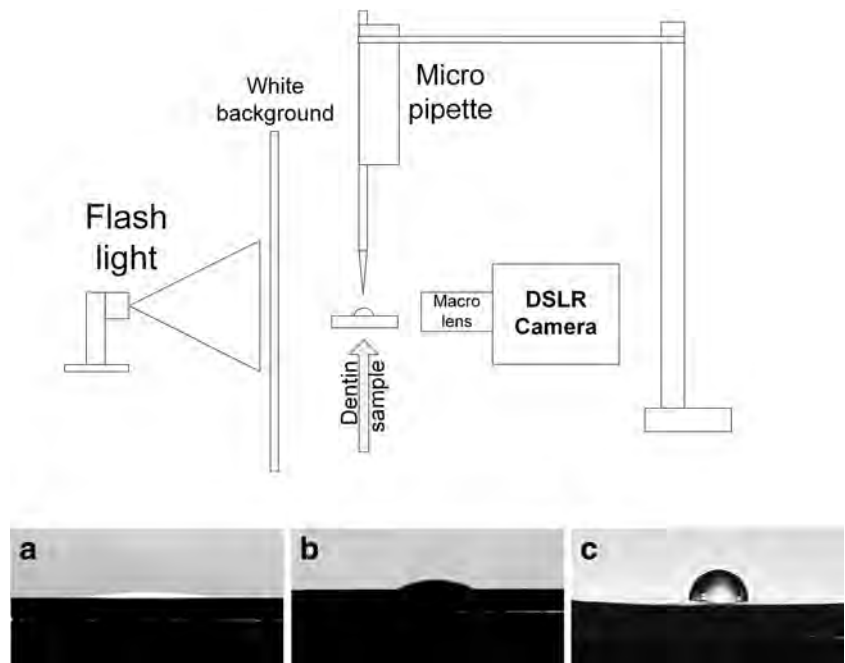
where the SFE of liquid equals the sum of SFE components (apolar Lifshitz-van der Waals and polar acid-base) according to the rule of additivity:

$$\gamma_L = \gamma_L^{LW} + \gamma_L^{AB}$$

(with) $\gamma_L^{AB} = 2\sqrt{\gamma_L^+ \gamma_L^-}$

By solving the van Oss-Chaudhury-Good equation three times for three reference liquids, it is possible to differentiate between polar and apolar surface interactions and between Lewis acid and Lewis base polar interactions of a tested solid. Though reference liquids may vary, two of them must be polar [37]. In the present study, water and ethylene glycol were used

Fig. 2 Schematic of the contact angle analyzer setup and representative images of dentin disks with reference liquid drops. **a** Water on NTAP-treated dentin. **b** Water on phosphoric acid-treated dentin. **c** Water on untreated dentin



as polar and diiodomethane as apolar liquid with known SFE parameters [38, 39].

SEM

Representative samples from NTAP, acid-etched, and control groups (eight teeth in total) were subjected to SEM to analyze the differences in surface micro-morphology. Without prior dehydration treatment, the samples were mounted on aluminum stubs, fixed with graphite conductive tape, subjected to vacuum in a sputter coater (POLARON SC502, Fisions Instruments, Ipswich, UK), and then coated with a thin film of Au alloy. The samples were analyzed using TESCAN FE-SEM (Mira 3 XMU, TESCAN a.s., Brno, the Czech Republic) operating at 10 keV.

Statistical analysis

All data were statistically analyzed in Minitab 16 software package (Minitab Inc., State College, PA, USA) with the level of significance set at 0.05. To test the differences in contact angles for each reference liquid, general linear model (GLM) was applied for factors “plasma,” “power,” and “distance” as well as their interactions. To test the differences in dentin SFE, GLM was applied using the same factors. Where the interaction was significant, further one-way analysis of variance (ANOVA) with Tukey’s post hoc test and Bonferroni correction was used. Differences in contact angles and SFE between the selected NTAP treatment, ER, and SE protocols were tested using one-way ANOVA with Tukey’s post hoc test. Equal variances were tested using Bartlett’s and Levene’s tests, and where necessary, data transformation was applied to stabilize the differences in variances. Quadratic, logarithmic, or sqrt functions were used for data transformation.

Results

Screening of NTAP treatment regimes

Figure 3 presents the box-and-whisker plots of contact angles of reference liquids following an array of NTAP treatments. GLM analyses showed that all NTAP treatments significantly reduced the contact angles of all three reference liquids compared to the untreated control group ($p \leq 0.001$). The results were most prominent for water with contact angles decreasing from $84.2^\circ \pm 2.6^\circ$ in the control group to the range of $6\text{--}30^\circ$ in the NTAP-treated group with the exception of 100% He_1W_8mm group (where the post-treatment contact angle was $69.1^\circ \pm 5.6^\circ$). Though contact angles for ethylene-glycol and diiodomethane were similar in the control group ($36.6^\circ \pm 5.3^\circ$ and $37.9^\circ \pm 1.9^\circ$,

respectively), NTAP treatment decreased the ethylene glycol contact angle ($5\text{--}12^\circ$ range) to a greater extent than that of diiodomethane ($10\text{--}30^\circ$ range).

GLM analyses for factors plasma, power, and distance showed that O₂ feed further decreased contact angles of water compared to pure He NTAP ($p \leq 0.001$). There was no difference between 1 and 1.5% O₂ in the resulting contact angles of water. Such effects of NTAP with O₂ feed were not observed with ethylene-glycol and diiodomethane ($p = 0.183$ and $p = 0.094$, respectively).

NTAP power of 3 W significantly reduced contact angles of water and diiodomethane as compared to 1 W power ($p \leq 0.001$), but this effect was not found for ethylene glycol ($p = 0.08$). Tip-to-surface distances of 2 and 4 mm did not produce any significant differences in contact angles of reference liquids (water $p = 0.068$, ethylene glycol $p = 0.109$, and diiodomethane $p = 0.454$). For 8-mm distance, 100% He NTAP at 1 W resulted in significantly higher water contact angles than other groups ($p \leq 0.001$). Therefore, we excluded 8-mm tip-to-surface distance from the NTAP treatments with O₂ feed. Within-group differences were detected using one-way ANOVAs statistical test and are marked with letters and symbols in Fig. 3.

Regarding SFE, significantly higher SFE of NTAP-treated dentin was found than that of the untreated group (Fig. 4). Moreover, NTAP with O₂ feed further increased the SFE of dentin compared to 100% He NTAP ($p \leq 0.001$). No differences were found between NTAPs with 1 and 1.5% O₂ ($p = 0.357$).

For the same NTAP source, higher SFE of dentin was associated higher power for pure He and He + 1% O₂ NTAPs, i.e., 3 W, resulting in higher SFE than 1-W treatment ($p \leq 0.001$). Conversely, different powers of NTAP with 1.5% O₂ feed had no effect on the SFE of dentin ($p = 0.09$), except for 1W_4mm group which resulted in slightly lower SFE of dentin compared to 3W_4mm ($p = 0.013$).

In general, the tip-to-surface distance did not affect the SFE of NTAP-treated dentin ($p = 0.514$), with exceptions being 100% He_1W_8mm and He+1.5%O₂_1W_4mm, which showed significantly lower SFE than their corresponding groups ($p \leq 0.001$ and $p = 0.007$, respectively) (Fig. 4).

Component analysis of SFE is presented in Fig. 5. In the control group (untreated dentin), apolar component of SFE appeared more prominent than the polar component, of which Lewis acid was predominant. NTAP treatments, except for the less effective 1W_8mm of pure He NTAP, all showed rather similar effects; i.e., polar component substantially increased compared to the control, and apolar component also slightly increased. Within the polar component of SFE in NTAP-treated dentin, Lewis base surpassed Lewis acid component. Compared to the control untreated dentin, Lewis base and apolar components increased whereas Lewis acid component decreased.

Fig. 3 Summary of the contact angle data for the three reference liquids on NTAP-treated dentin. (Same-type letters or symbols indicate statistical significance within each respective group ($p > 0.05$); $N = 5$ samples/group)

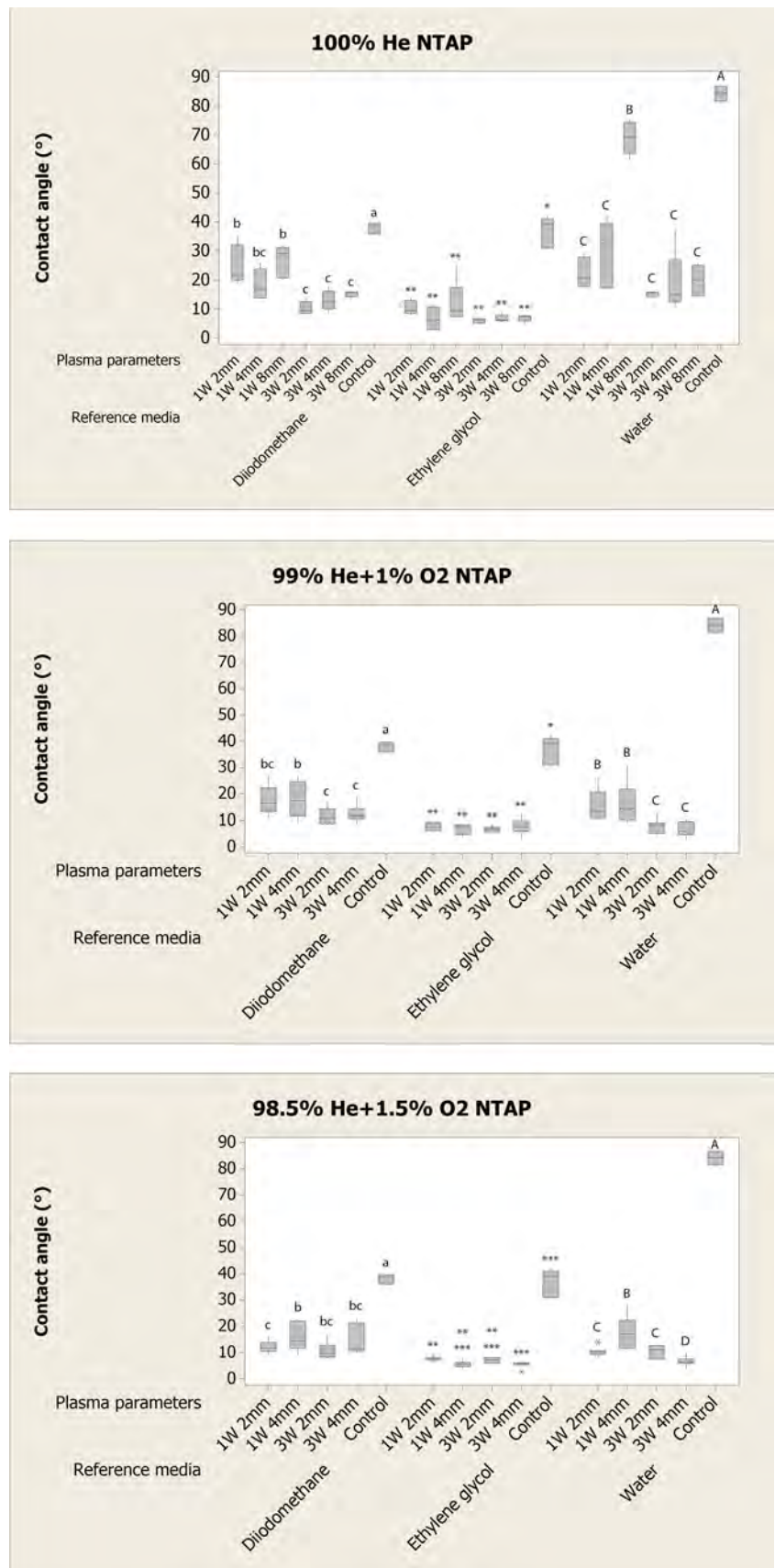
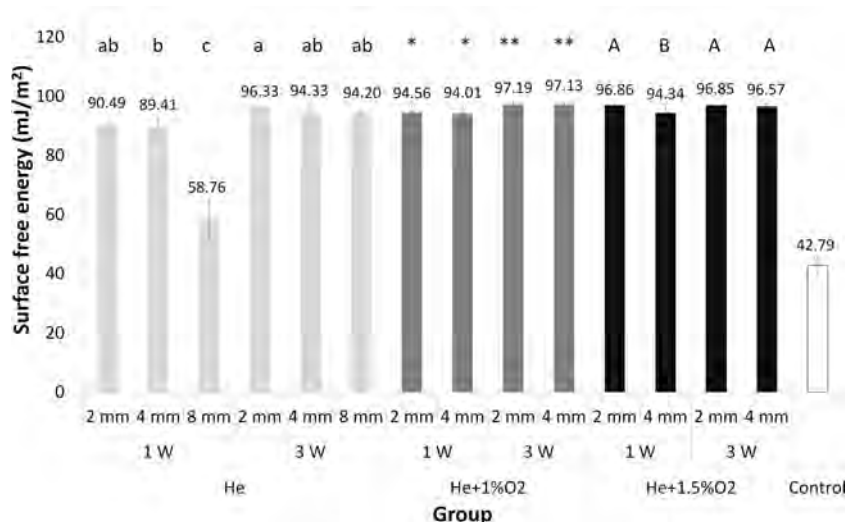


Fig. 4 Surface free energy (SFE) of dentin following various treatments (mean and standard deviation). (Same-type letters or symbols indicate statistical significance within each respective group ($p > 0.05$))



Comparison of NTAP with ER and SE protocols for universal adhesives

Based on the previous screening, the following NTAP treatment was chosen for the next part of the study: He+1.5%O₂_3W_4mm. Contact angle measurements for three reference liquids and the subsequent SFE calculation were performed for NTAP-treated, acid-treated, and untreated dentin. Universal adhesives SBU and CUB were used only to measure their contact angles on NTAP-treated, acid-treated, and untreated dentin. It was not possible to calculate the SFE of dentin using adhesives as SFE may only be calculated using reference liquids (water, ethylene glycol, and diiodomethane).

Contact angles of all reference liquids were lower on NTAP-treated than on acid-treated or untreated dentin ($p \leq 0.001$) (Fig. 6). Phosphoric acid etching produced significantly lower water contact angles compared to the untreated,

control group ($p \leq 0.001$), but the same effect was not found for ethylene-glycol and diiodomethane ($p = 0.069$ and $p = 0.120$, respectively).

As for universal adhesives, higher contact angles of SBU and CUB ($44.13 \pm 8.45^\circ$ and $33.04 \pm 6.04^\circ$, respectively) were found on acid-treated than untreated dentin ($29.94 \pm 1.86^\circ$ and $26.56 \pm 2.33^\circ$, respectively) (SBU $p = 0.003$ and CUB $p = 0.009$, respectively). Slightly lower contact angles of SBU and CUB were measured on NTAP-treated dentin ($35.68 \pm 2.35^\circ$ and $19.96 \pm 6.89^\circ$, respectively) compared to acid-etched dentin, but the differences reached statistical significance only for CUB ($p = 0.015$). NTAP-treated and untreated dentin showed no significant differences in contact angles of universal adhesives ($p = 0.808$).

The SFE of dentin was highest on NTAP-treated and lowest on untreated dentin (Fig. 7). NTAP and acid etching increased polar, Lewis base, and decreased Lewis acid component as compared to the untreated dentin. NTAP increased and

Fig. 5 Polar (Lewis base and Lewis acid) and apolar components of surface free energy on NTAP-treated and untreated, control dentin. The tested groups are presented on the x axis (100% He NTAP at 1 W, 2 mm; 100% He NTAP at 1 W, 4 mm; 100% He NTAP at 1 W, 8 mm; etc.)

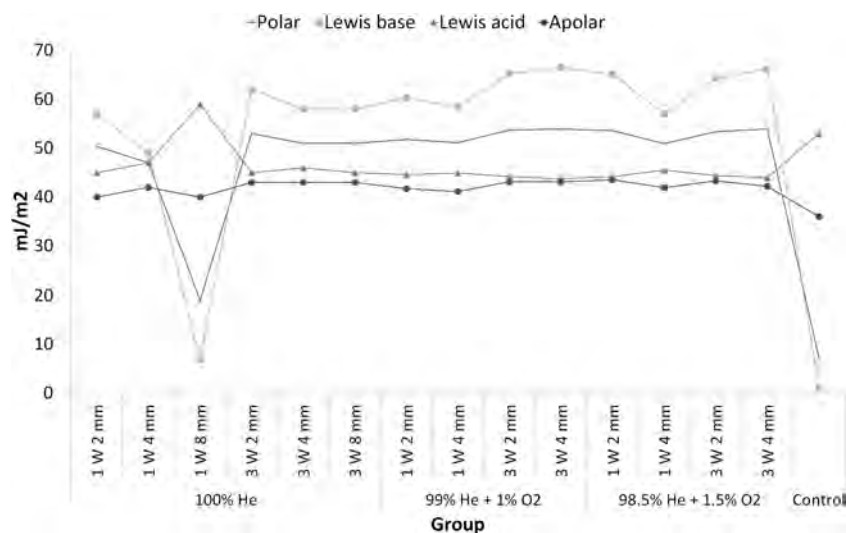
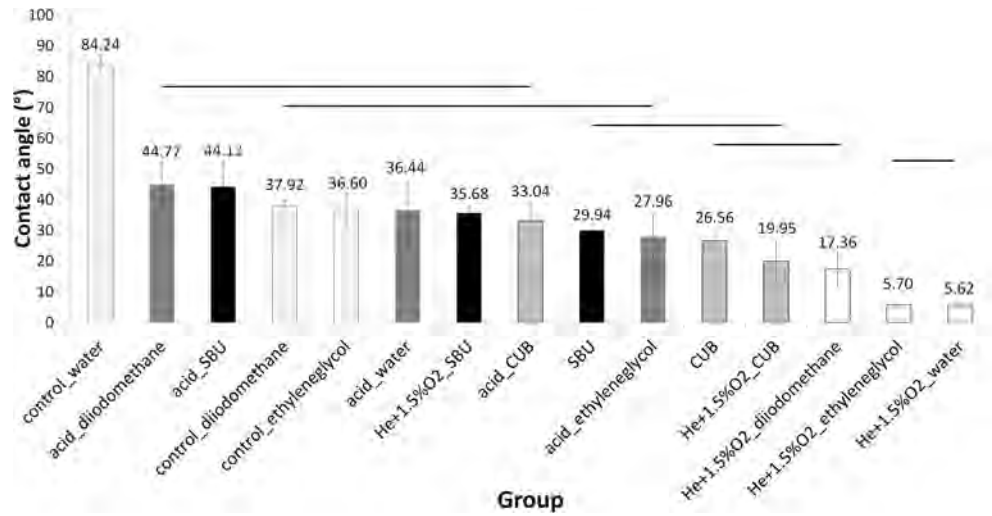


Fig. 6 Contact angle values (mean and standard deviation) for reference liquids and universal adhesives following different dentin treatments (group name: treatment_liquid). “Control” denotes untreated dentin. Groups “SBU” (Single Bond Universal) and “CUB” (Clearfil Universal Bond) present contact angle values of the respective adhesive following the self-etch application protocol. (Groups connected with horizontal bars are not significantly different ($p > 0.05$))



acid etching decreased apolar component of SFE compared to that of untreated dentin.

SEM micro-morphological analysis

Representative SEM micrographs are presented in Fig. 8. Phosphoric acid etching removed the smear layer, exposed the collagen mesh, and opened dentinal tubules. No visible micro-morphological differences were found between NTAP-treated and untreated dentin (control group), which was only ground with 600-grit SiC abrasive paper to produce the smear layer.

Discussion

The first working hypothesis was upheld; i.e., all NTAP regimes reduced contact angles of reference liquids and increased SFE of dentin compared to the untreated dentin with

further detectable differences relative to the tip-to-surface distance, power, and O₂ feed.

He and Ar are commonly used carrier gases in NTAP studies on dental tissues with powers generally higher (5–10 W) than in the present study (1 and 3 W). A comparable power of 2–3 W was used in the study by Zhang et al. [40], who used Ar-based NTAP to study adhesive penetration into demineralized dentin. Our intention was to optimize He-based NTAP regimes with lower powers for use on dentin in order to minimize the effect of heating and potential dentin cracks observable with higher-powered NTAPs. Furthermore, the chosen time and tip-to-surface distances are clinically relevant and manageable. Up to 10% O₂ was added to NTAPs used for microbial disinfection [41], whereas lower, up to 1%, O₂ was added to NTAP for dentin and root canal treatment [42]. We used 1 and 1.5% O₂ feed to investigate if slightly higher O₂ feed would intensify the effect of NTAP. The results indicated that while O₂ generally intensified the effects of NTAP on wetting and SFE of dentin, there was little or no difference between 1 and 1.5% O₂, suggesting that there

Fig. 7 Surface free energy (SFE) and its polar (Lewis acid and Lewis base) and apolar components

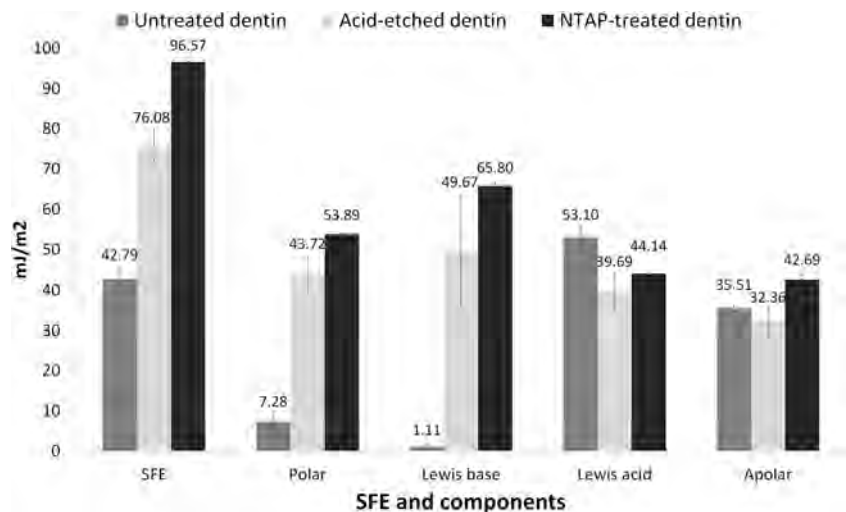
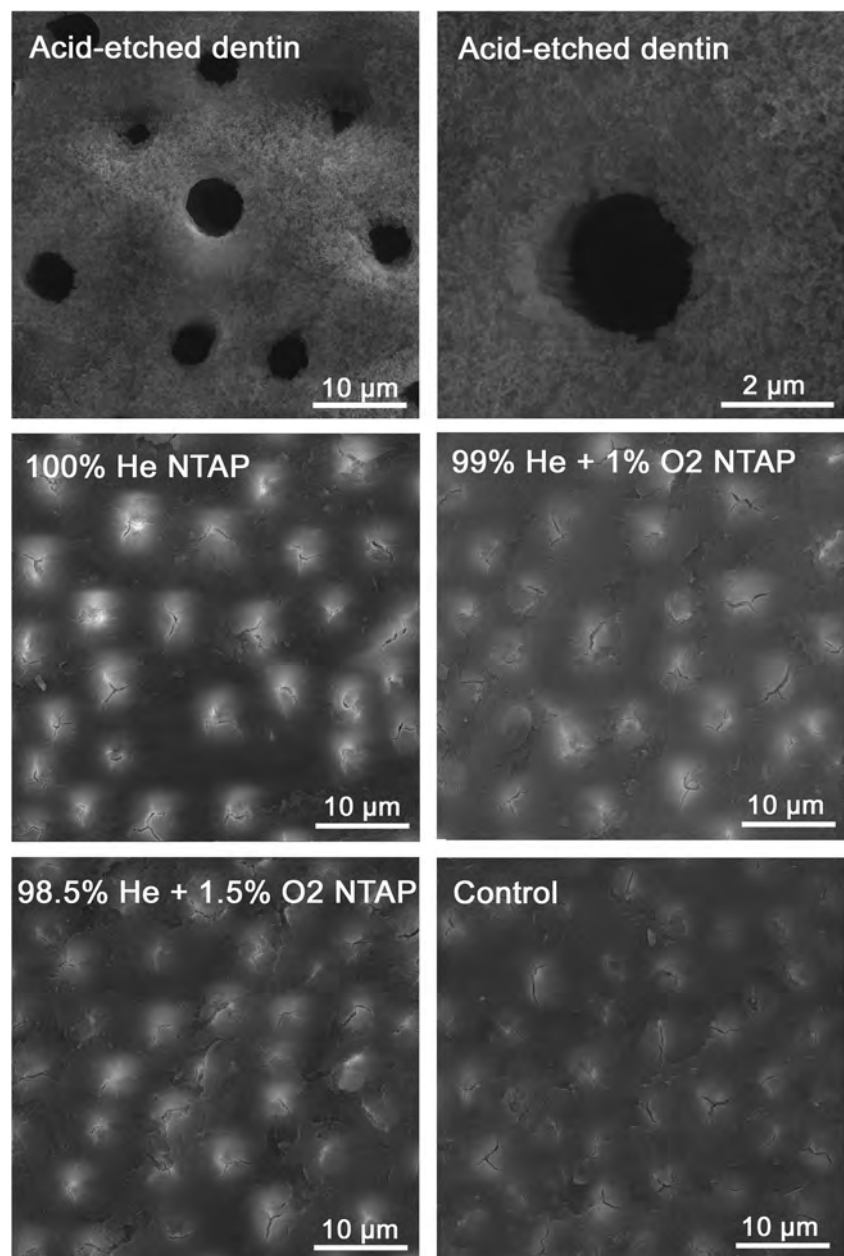


Fig. 8 SEM micrographs of dentin following different treatments. All three NTAP treatments had the same parameters (3 W and 4 mm) but differed in O₂ feed. Dentin was acid etched for 15 s. No treatment was performed in the control group



may be a “saturation” effect of O₂ feed in NTAP. In the study of Koban et al. [42], significant differences in water contact angles were found between Ar NTAP with 0.2 and 1% O₂. These findings indicate that O₂ feed as low as 0.2% may be disregarded and pure carrier gas used for plasma treatment. If O₂ is to be added to NTAP, the present results suggest that 1–1.5% O₂ may be recommended.

All NTAP regimes significantly reduced the contact angles of reference liquids, especially water, and increased SFE of dentin compared to the untreated dentin, indicating a dramatic increase in dentin wetting. The finding of lower contact angles of reference liquids was expected and is in accordance with previous studies [22–25]. Moreover, the same effects were achieved in the present study with lower-powered NTAPs

(1 W) at clinically relevant distances that allow focusing the NTAP needle on small cavities, e.g., “slot,” class III and V and minimally invasive class I cavities.

A significant decrease in water contact angle was coupled with a greater decrease in the contact angle of ethylene glycol than diiodomethane. NTAP induced a greater change with polar substances (water and ethylene glycol) than apolar diiodomethane, suggesting that a major change on dentin surface was related to hydrophilic and polar interactions. This finding was corroborated by the SFE component analysis, in which polar component increased substantially following the NTAP treatment. A change in acid-base interaction was also detected following the NTAP treatment with an increase in Lewis base component. This further suggests that more

negatively charged sites appear on the surface of the NTAP-treated dentin for potential interactions with electron-accepting molecules, such as acidic monomers from adhesives.

Our SFE component analysis is in accordance with previous spectroscopic (XPS and FTIR) studies which reported grafting polar, oxygen-containing moieties on NTAP-treated surfaces as a result of an intense attack of reactive oxygen species. Namely, Chen et al. [22] found an increased percentage of O in dentin and enamel, Ritts et al. [32] found increased carbonyl C=O groups on collagen, and Lehmann et al. [25] reported on increased carboxylate CO_3^{2-} and carbonate CO_3^{2-} ions on the surface of enamel. Beside negatively charged ions with clear affinity for reaction with Lewis acids (electron acceptors), C=O group also contributes to surface reactivity. C=O group is moderately polar, considering the differences in electronegativities of C and O, with affinity for interactions between O and Lewis acids/electron acceptors (e.g., H^+) or C and Lewis bases/electron donors (e.g., NH_3 or OH^-). The present results suggest that O_2 feed in NTAP further increases polar, specifically Lewis base, interaction sites compared to pure He NTAP. Higher-powered NTAPs further intensified this effect of O_2 feed.

Based on the screening of an array of NTAP treatments, we chose He + 1.5% O_2 NTAP at 3-W power and 4-mm tip-to-surface distance as the NTAP treatment in the second part of the study. Here, we compared the effects of the said NTAP treatment with phosphoric acid etching representing the ER protocol and no treatment representing the SE protocol of adhesive application. Two universal adhesives were chosen, SBU and CUB. According to manufacturers' technical data sheets, both SBU and CUB are ethanol- and water-based adhesives, containing BisGMA, HEMA, and 10-MDP monomers. CUB is claimed to contain a highly hydrophilic acidic amide monomer for improved wetting and adhesive penetration, yet the manufacturer did not disclose its name and chemical structure.

The second working hypothesis was partially upheld as NTAP induced greater SFE of dentin and lower contact angles of reference liquids compared to phosphoric acid etching (ER protocol) but had a limited effect on universal adhesives in relation to the SE application protocol.

The results showed that both NTAP and phosphoric acid significantly increase wetting of the treated dentin surface, this effect being more pronounced with NTAP. While NTAP significantly reduced the contact angles of all three reference liquids, phosphoric acid did so only with water whereas ethylene glycol and diiodomethane contact angles were not significantly different from those on untreated dentin. Increased wetting of dentin surface following phosphoric acid etching is in accordance with previous studies [6, 7]. Furthermore, our SFE component analysis revealed that untreated dentin exhibited both apolar and polar sites with predominant Lewis acid

electrostatic component. As is customary in dental studies, the "untreated" dentin refers to mid-coronal dentin cut with a diamond saw and ground with 600-grit SiC abrasive paper to produce a smear layer of various thicknesses. Thus, surface characteristics relate to the smear layer which is essentially an amorphous layer of inorganic-organic debris containing hydroxyapatite, collagen, and microbial remnants [43]. Electrostatic Lewis acidity of the dentin smear layer is likely associated with the abundance of Ca^{2+} ions from hydroxyapatite and contributes to the interaction of phosphate or carboxylate ions with the smear layer and dentin itself during acid etching with phosphoric acid or acidic monomers in adhesives.

Despite its effect on water, phosphoric acid etching significantly increased the contact angles of universal adhesives, SBU and CUB, compared to the untreated dentin where SBU and CUB were applied following the SE protocol. This finding highlights the importance of the present approach based on three reference liquids and SFE component analysis to elucidate the true interaction between adhesives and dentin. Though phosphoric acid etching increased surface hydrophilicity, it did not contribute to wider adhesive distribution over etched dentin compared to adhesive distribution on un-etched dentin. This could be due to the presence of hydrophobic monomers in both adhesives, namely, BisGMA, and the subsequent phase separation between hydrophobic and hydrophilic monomers within the hybrid layer [44, 45]. Phosphoric acid etching decreased the apolar component of dentin SFE, further contributing to the depleted interaction between apolar adhesive components and dentin surface. Though ether (R-O-R) and hydroxyl (R-OH) linkages in monomers exert some polarity, the majority of spacer groups in hydrophobic monomers contribute to low polarity or apolarity [46]. Slightly decreased apolar component of SFE could explain why the only apolar reference liquid, diiodomethane, exerted increased contact angle on acid-etched dentin.

Both NTAP and phosphoric acid etching resulted in an increase of electrostatic Lewis base component. Increased grafting of polar, oxygen-containing groups was previously discussed for NTAP treatment. As for acid etching, Lehmann et al. [25] detected C=O groups and CO_3^{2-} and CO_3^{2-} ions at enamel surface following acid etching using XPS analysis. A similar increase in polar, oxygen-containing moieties at dentin following acid etching could be the reason for increased Lewis base sites as found in the present study. This may favor the subsequent interaction of electron acceptors, or Lewis acids, and contribute to hydrogen bonding between adhesive resins and dentin substrate.

NTAP treatment of dentin prior to adhesive application resulted in lower contact angles of SBU and CUB adhesives compared to those on the acid etched dentin, suggesting better adhesive distribution following NTAP treatment. A more

favorable effect of NTAP than phosphoric acid in terms of adhesive distribution could be due to the increased surface reactivity of dentin as indicated by increased both apolar and polar (Lewis acid and Lewis base) interaction sites.

Increased surface reactivity and hydrophilicity are likely reasons for lower contact angles of CUB adhesive on NTAP-treated than untreated dentin. The presence of a hydrophilic amide dimethacrylate monomer in CUB, in addition to hydrophilic HEMA, may have contributed to this finding. The effect of NTAP on adhesive contact angle appeared to be material-dependent as NTAP did not facilitate the distribution of SBU adhesive compared to the untreated dentin as it did with CUB. Further research is therefore required to elucidate the adhesive-dentin interactions following NTAP treatment.

Though positive effects of NTAP on adhesive penetration were documented, adhesive phase separation was found in NTAP-treated dentin in a previous study [40]. NTAP increased the penetration of hydrophilic but not hydrophobic monomers within the hybrid layer, limiting the positive effects of NTAP. Phase separation within the hybrid layer due to increased penetration of hydrophilic monomers increases the risk of creating areas more susceptible to water sorption, as hydrophilicity was found to be the prime factor determining the extent of water sorption [47, 48]. In turn, water sorption promotes faster biodegradation of adhesive itself [48] as well as adhesive-dentin bond [49, 50]. This could be the reason for the lack of long-term effect of NTAP on adhesive bond strength despite positive effects on immediate bond strength [23, 24, 33, 34, 51, 52].

The present qualitative SEM analysis revealed no micro-morphological differences between untreated and NTAP-treated dentin. Previous studies also reported no structural differences in the appearance of NTAP-treated dentin/enamel, hence no destructive effects of NTAP [22, 25, 42]. NTAP does have a potential not only to chemically interact with the surface but also to increase surface roughness of dentin by etching [21]. However, this roughening effect was not as notable as with phosphoric acid [25] due to the selective etching of the organic phase by NTAP and not the mineral phase, which was confirmed by elemental analysis [22]. Under the present experimental conditions, no etching effect of NTAP occurred on dentin surface.

Conclusion

He NTAP with and without O₂ substantially increased dentin wetting and SFE, surpassing the effect of phosphoric acid etching. O₂ intensified the effect of He NTAP at 1- and 3-W powers and clinically relevant distances of 2 and 4 mm. Surface modification of dentin following NTAP treatment included increased hydrophilicity, polarity, and electrostatic Lewis-base interaction sites. Phosphoric acid etching did not

favor adhesive distribution on dentin surface as indicated by increased contact angles of universal adhesives, SBU and CUB, despite decreasing water contact angle compared to the untreated dentin. NTAP treatment of dentin prior to adhesive application facilitated better adhesive distribution compared to acid-etched dentin. However, NTAP did not show an improvement regarding the adhesive distribution on dentin compared to the SE protocol of application. Under the present conditions, the effects of NTAP were mainly related to surface modification, with no etching ability but also no structural damage on the dentin surface.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

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PAPER

Characterisation of a multijet plasma device by means of mass spectrometric detection and iCCD imaging

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Characterisation of a multijet plasma device by means of mass spectrometric detection and iCCD imaging

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Abstract

Atmospheric pressure plasma jets (APPJs) have been one of the most studied nonthermal discharges in the past decade. Recently, the use of multiple jets in order to cover larger areas has become desirable. However, the interaction between neighboring jets is a common phenomenon that can greatly modify plasma characteristics up to the point of merging several jets into a single one. The present study focus on bringing new insight on this phenomenon, named jet-to-jet coupling, investigating the discharge modes (coupled and uncoupled) of a plasma source composed of an array of seven plasma jets arranged adjacent to one another and driven by a sinusoidal excitation. The experimental results achieved by means of mass spectrometry, show a considerable increase in ion concentrations in the plasma, up to some orders of magnitude in the case of negative ions, as an effect of jet-to-jet coupling. Temporally resolved imaging of the discharge also shows how the evolution and intensity of the discharge are greatly affected by jet-to-jet interaction. Air/helium mole fraction and negative ions concentration are regarded as the main possible parameters affecting the coupling phenomenon. Moreover, experimental results suggest that the presence of remnant conductive channels behind the ionization fronts governs the propagation of the next ionization fronts, independently from the direction of propagation (from the source toward the target or vice versa). Furthermore, a parametrical investigation of the coupled mode discharge showed the presence of a critical value of the imposed voltage for which a drastic change in the electrical characteristics of the discharge is observed.

Keywords: cold atmospheric pressure plasma jet, negative ions, jet-to-jet coupling, mass spectrometry, iCCD, ionized channel, counter propagating streamer

(Some figures may appear in colour only in the online journal)

1. Introduction

Nowadays, cold atmospheric plasmas are investigated with growing interest because of their great potential in a broad range of applications. In the past years, cold atmospheric plasmas have shown promising results in many different

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fields, among which, the decontamination of surfaces [1], thin film deposition [2–4], surface modification of polymers [5], endodontic procedure [6], promotion of tumor cell death [7–9] and induced antioxidant enzymes activity of meristematic plant cells [10]. Along with experimentation there has been a rapid development of the technology related to cold atmospheric plasma sources. Among the many possible configurations, atmospheric pressure plasma jet (APPJ) devices

probably offer the most versatile and simple structure. At atmospheric pressure, the high breakdown voltage imposes discharge gaps normally in the range of few millimetres, therefore limiting the size of the samples that can be treated between plasma source electrodes. The main advantage of APPJ is the possibility to use a gas flow to bring the plasma outside of the interelectrode gap and enable the treatment of samples with no limitations on their size. On the other hand, the plasma plume generated by most of the common plasma jet devices has a diameter within the range of few millimetres, preventing its use for large area treatments.

In recent years, different research groups have tried to overcome this issue presenting new plasma sources based on an array of multiple plasma jets both for the treatment of large planar surfaces [11–16] and objects with complex geometry [17–21]. While partially overcoming the limitation of single plasma jets, the upscaling of plasma jet arrays has presented a unique challenge due to the tendency of individual plasma jets to interact with each other, which can lead to the merging of some plasma plumes when posed in close proximity. This phenomenon, usually referred to as *jet-to-jet coupling* or *interaction*, has also been shown to be able to generate plasma plumes with unique characteristics [22–24] that may open new fields of applications.

For the development of new plasma sources, it is of the utmost importance to increase our understanding of jet-to-jet coupling phenomenon to take full advantage of it. While extensive studies and considerable advancements have been made with individual plasma jets [25–32], multijet arrays and, in general, the interaction between plasma jets are far less studied, although the number of publications on the subject is increasing [15–18, 19, 22, 23, 33–37]. These works highlight the effects of the jet-to-jet coupling phenomenon mainly by means of data collected by high speed imaging (especially iCCD imaging), Schlieren photography and optical emission spectroscopy (OES). A considerably smaller number of papers presents also information on the substrate temperature [24], analysis on the treated substrate [24, 38], bacteria inactivation potential [38] or bi-directional electric field measurements [39].

Similarly, mass spectrometry was extensively used for the characterization of atmospheric plasma systems [40–47]. The choice of the main species to be investigated mainly depended on the type of the plasma system and its applications. In case of plasma jets, the investigations were carried out on single-jet configurations as integral or time-resolved measurements [48], while no work based on the use of this technique on a multijet plasma array has yet been published.

Kushner and Babaeva presented results from a computational investigation of the properties of small arrays of micro-plasma jets [34]. They found that the jets interact through electrostatic, hydrodynamic and photolytic forces and showed that jet–jet interactions primarily depend on how densely the capillaries are packed and on their number. Plasma ionization waves from two jets, though electrostatically repelling, are confined within the merged He flows where E/N is above the self-sustaining value. With a three jets array, the external plumes tend to converge towards the

central channel and the associated ionization wave becomes stronger.

In this paper, we try to bring new insights on the jet-to-jet coupling phenomenon, investigating a plasma jet array similar to some of those already presented in literature [23, 24, 33, 38, 49–53] by means of iCCD imaging, electrical characterisation and mass spectrometry analysis of the jet plumes.

2. Experimental setup

2.1. Multijet plasma source

The plasma source adopted in this study, already presented in [38], is an array of seven plasma jets adjacent to one another, that can rely on jet-to-jet coupling phenomenon to merge plasma plumes into a single combined intense jet (figure 1).

As it is possible to observe in figure 1, each single plasma jet is composed of a PTFE capillary (\varnothing_{ext} 1.6 mm, \varnothing_{int} 1 mm) and a high voltage electrode, made of aluminium foil (8 mm width, 70 μm thickness), wrapped around it. The electrodes of the seven jets are electrically connected to each other and simultaneously powered by a pulse generator. In this work the multijet source was driven by high voltage sinusoidal waveforms with a frequency of 80 kHz and a peak to peak voltage at the high voltage electrode up to 6.4 kV. In the result section, for every case, is reported also the imposed voltage as this revealed to be interesting for the discussion. This voltage value refers to the peak to peak voltage of the driving sinusoid before being amplified by the step-up transformer of the power supply. Helium was used as a working gas and the same flow rate was supplied to all the seven jet capillaries. The total flowrate (sum of the flows through all the capillaries) was varied between 2 slm and 7 slm according to the chosen operating conditions. A grounded electrode, consisting of a stainless steel plate, was placed 9 mm apart from the outlet of the capillaries in order to enable a more stable plasma generation and a geometrical and electrical configuration comparable with the one achieved during mass spectrometry analysis.

The *multijet* plasma source can be operated in two different modes (figure 2), namely:

- **Uncoupled mode**, when the seven plasma jets are well defined and separated from each other;
- **Coupled mode**, when through jet-to-jet coupling the plumes merge into a single combined intense jet.

In this work, the shift from one mode of operation to the other was achieved by varying the multijet gas flow rate (ex. at 9 mm from grounded target: 2 slm coupled mode; 4.5 slm uncoupled mode).

To better understand the effects of the jet-to-jet coupling phenomenon, two modified versions of the plasma source were also used in this work (figure 3). The modified versions are based on the same honey comb capillary configuration of the first version but differ as described in the following. A multijet source where only three of the capillaries were powered (figure 3(a), the other capillaries were still fluxed with the He flow) was adopted to investigate the effect of the number of coupled jets; in this case, the gap distance between the plasma

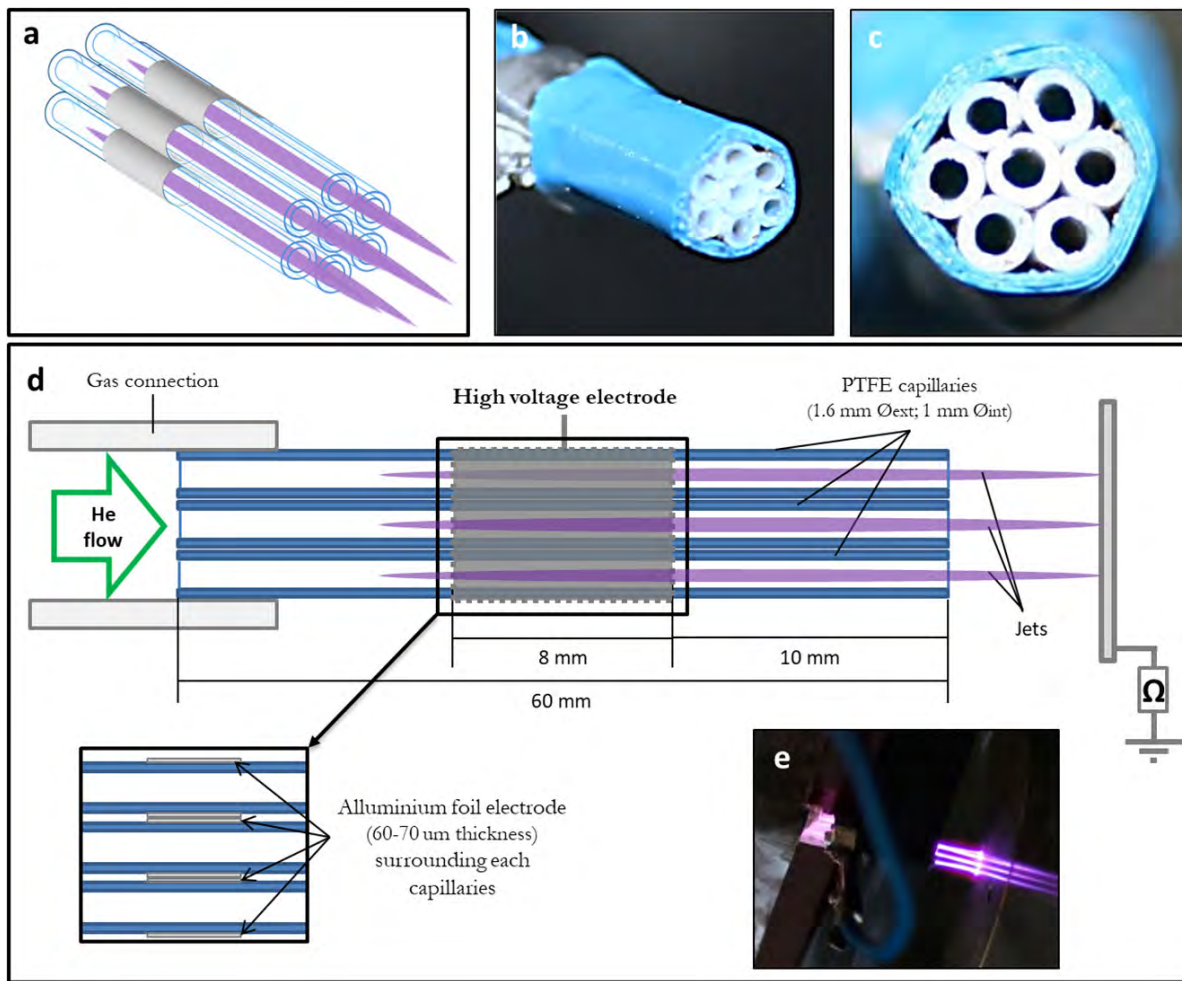


Figure 1. Schematics and pictures of the multijet plasma source: (a) schematic of the seven plasma jets array; (b) picture of the multijet; (c) frontal view of the multijet outlet; (d) longitudinal section schematic of the multijet plasma source; (e) picture of the multijet during operation.

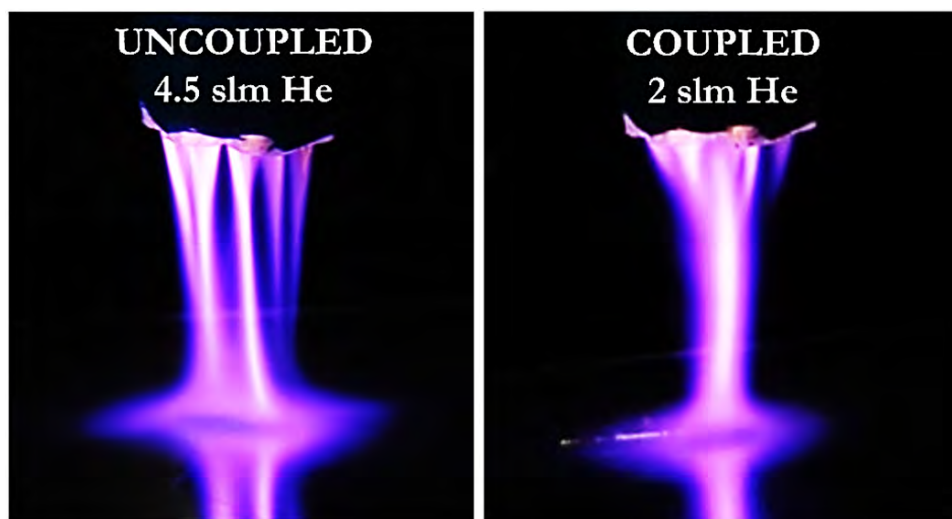


Figure 2. Pictures of the multijet plasma source while operating in uncoupled (left) and coupled modes (right). © (2014) IEEE. Reprinted, with permission, from [38].

source and the grounded plate was reduced to 7 mm and the total gas flow rate increased (4 slm, coupled; 7 slm, uncoupled) to achieve a more stable operation (no plasma plumes oscillations and repeatable electrical values) of the plasma

source. A second modified plasma source, single jet version (figure 3(b)), with only one powered electrode on the central capillary (the other capillaries were still fluxed with the He flow), was also studied to compare the multijet source with a

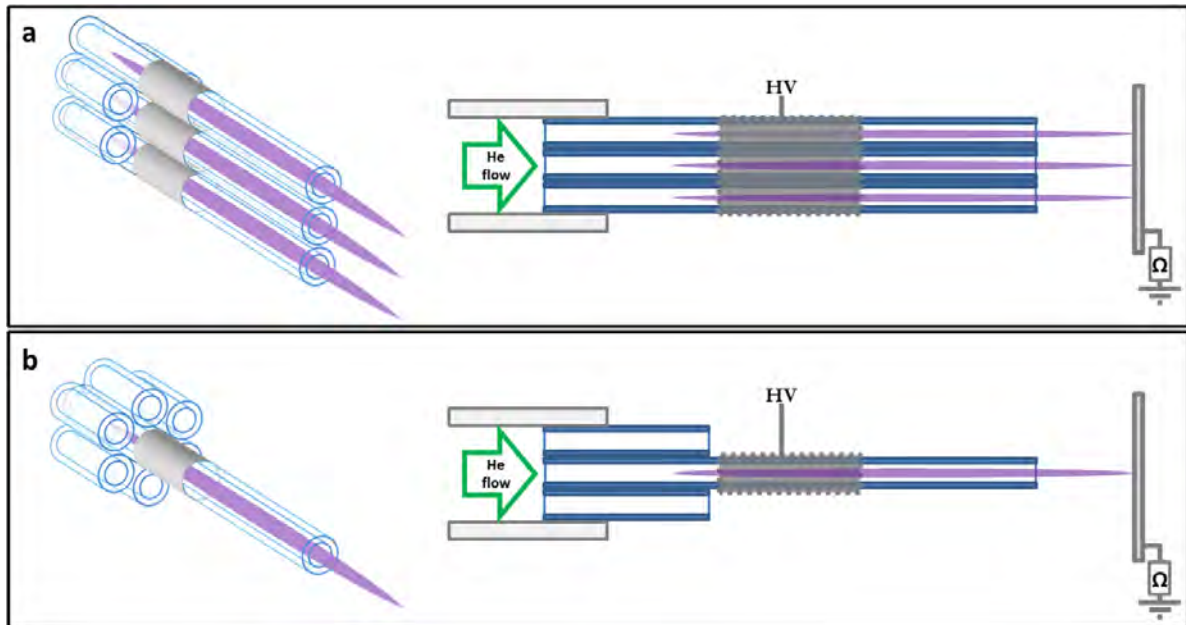


Figure 3. Schematic of the modified version of the multijet source: (a) 3-jets version with only three powered electrodes; (b) single jet version with only one powered electrode.

single element source; in this case the gap distance between the plasma source and the grounded plate was reduced to 5 mm and the total gas flow rate was fixed to 4.5 slm to achieve a more stable plasma plume.

2.2. Mass spectrometry setup

We have used a quadruple-based molecular beam mass spectrometer (MBMS) for determination of the ion composition of the plasma generated by the multijet source. A photo of the multijet plasma source during spectrometry measurements is shown in figure 1(e). HIDEN HPR-60 mass spectrometer with its three-stage differentially pumped inlet system is specially designed and well tested for operation at atmospheric pressures. We have used the secondary ion mass spectrometry mode (SIMS $+/-$) in order to investigate the mass spectra of positive and negative ions. SIMS $+/-$ mode operates without MBMS internal ionisation source turned on, so the positive and negative mass spectra obtained were originated only from within the plasma. For both negative and positive ion measurements the MBMS was tuned to get good intensity of the ion signals. In order to get the best possible signal from MBMS, the alignment of the plasma source with respect to the sampling orifice was necessary before each set of measurements. The multijet plasma source was placed in front of the MBMS sampling orifice at a distance of 5 mm (single jet version), 7 mm (3-jet version) or 9 mm (7-jet version) according to the adopted plasma source version. The plasma source was positioned in such a way that the axis of the central capillary was normal to the mass spectrometer plate and matched the axis of the orifice. The set of three stage pressure orifices that was used for all plasma source configurations was made of: front plate orifice $\text{\O} 0.3\text{ mm}$; cone 1 orifice $\text{\O} 0.3\text{ mm}$; cone 2 orifice $\text{\O} 1.0\text{ mm}$. This set was chosen in order to get reasonable levels of signal even for the single jet configuration so the

comparisons could be made between all the configurations. Before starting the experiments it was necessary to check the plasma interaction with the MBMS system. This was done by monitoring the energy distributions of oxygen and nitrogen ions. The pressure values in the three stages ($P_1 = 6.5 \times 10^0$ Torr; $P_2 = 2.4 \times 10^{-5}$ Torr; $P_3 = 4.1 \times 10^{-7}$ Torr;) were kept constant during the measurements. The presented ion mass spectra are the average values of three different scan cycles for the same operating conditions.

2.3. iCCD and electrical measurements setup

The time evolution of the discharge structure in the two modes of operations was investigated by means of an Andor iStar DH734I iCCD camera with mounted a NIKON AF-Nikkor 105 mm $f/2.8$ lens. We have set the lens aperture at its highest value ($f=2.8$) in order to collect maximum of the light emission from the plasma and to get clear and bright images. The lens of the iCCD camera was set perpendicular to the multijet plasma source so we were able to obtain the axial plasma profiles. For this analysis, the multijet plasma source was positioned at the distance of 9 mm in front of a copper plate that was grounded through a $100\text{ k}\Omega$ resistance. The voltage waveform was recorded by means of a high voltage probe (Agilent N2771B) while the current values were obtained indirectly, measuring the voltage drop at the $100\text{ k}\Omega$ resistance with a passive voltage probe (Agilent 10076A). The temporal evolution of the discharge was scanned over an entire voltage pulse period ($12.5\ \mu\text{s}$). Sequential iCCD acquisitions (25 ns gate width, to enhance the recorded signal multiple exposures were integrated on chip) were taken with a time step of $0.5\ \mu\text{s}$ to cover all the discharge evolution during one pulse period. A wider range of operating conditions that granted coupled mode of operation was also investigated by means of iCCD acquisitions at longer exposure time $12.5\ \mu\text{s}$ (one period).

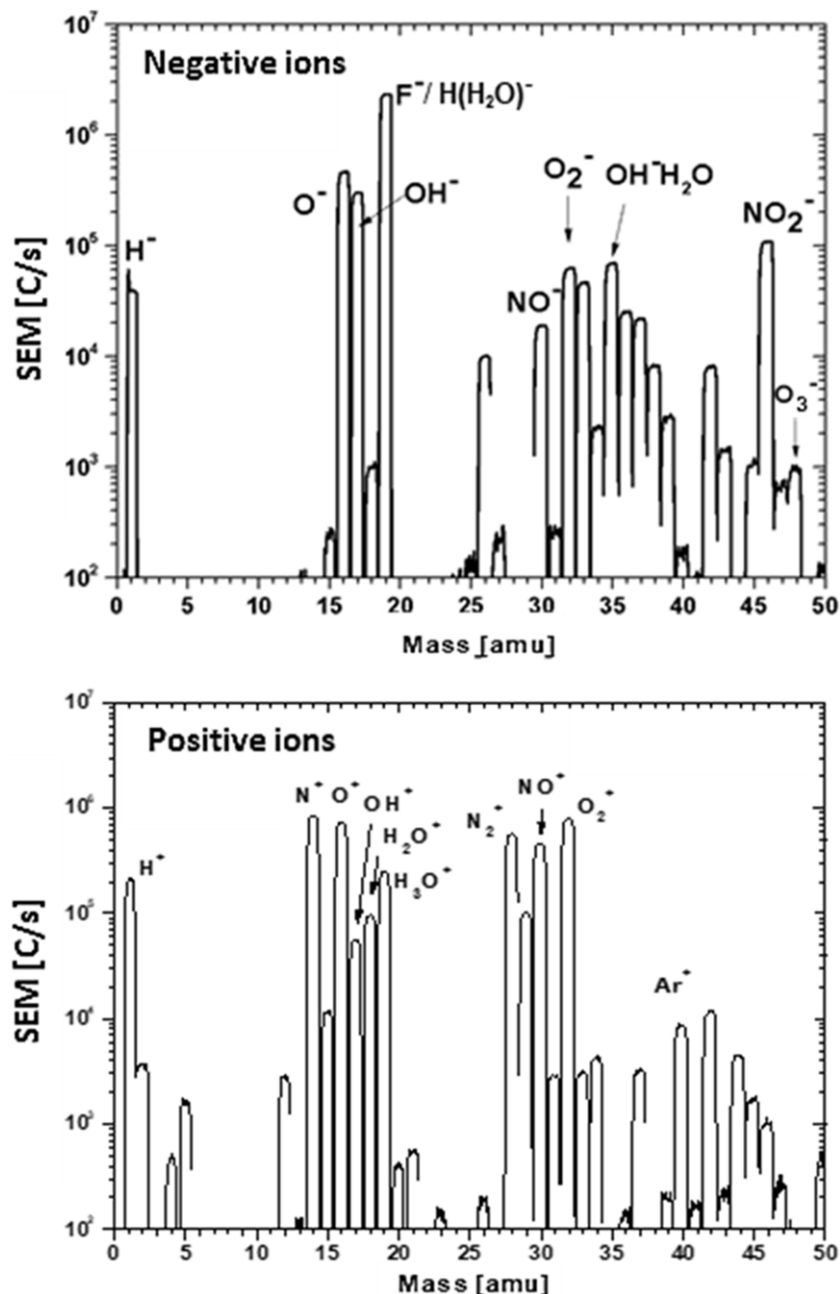


Figure 4. Negative (top) and positive (bottom) ions for coupled mode (5kV, imposed voltage 4.14 Vpp, 5 mm, 4.5 slm He).

3. Results and discussion

3.1. Mass spectroscopy

3.1.1. 7-jets configuration. In figure 4 we have shown negative and positive ion mass spectra of the multijet atmospheric discharge. The recorded positive and negative ions represent plasma products due to the mixing of the working gas (helium) with humid ambient air. The processes involved in the production of negative and positive ion species are mainly dissociative electron attachment and dissociative ionization.

The mass spectrum of negative ions characteristic of coupled mode is shown in figure 4 (left); we can see that several of the most abundant negative ions, such as O₂⁻, O⁻, O₃⁻, OH⁻, H⁻, NO⁻ and NO₂⁻, are of interest in biomedical treatments

[54, 55]. Nevertheless, the highest counts belong to the mass 19 amu. This mass can correspond to H(H₂O)⁻ but also to the F⁻ ion that originates from the PTFE capillaries used in the multijet source structure. The H(H₂O)_{*n*}⁻, as well as OH(H₂O)_{*n*}⁻ clusters, are formed through dissociative electron attachment followed by hydration. The reaction rate through dissociative electron attachment for forming H⁻ ($k_{\text{H}^-} = 7.8 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1}$) is higher than that for OH⁻ ($k_{\text{OH}^-} = 2.6 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$). On the other hand, the reaction $\text{H}^- + \text{H}_2\text{O} \rightarrow \text{OH}^- + \text{H}_2$ has a two orders of magnitude higher rate coefficient than the k_{H^-} , resulting in the overall dominance of OH⁻ ions and their clusters, as visible in the negative ions mass spectra in figure 4 (left).

The positive ion spectrum that is obtained under the same plasma conditions is shown in figure 4 (right). As expected,

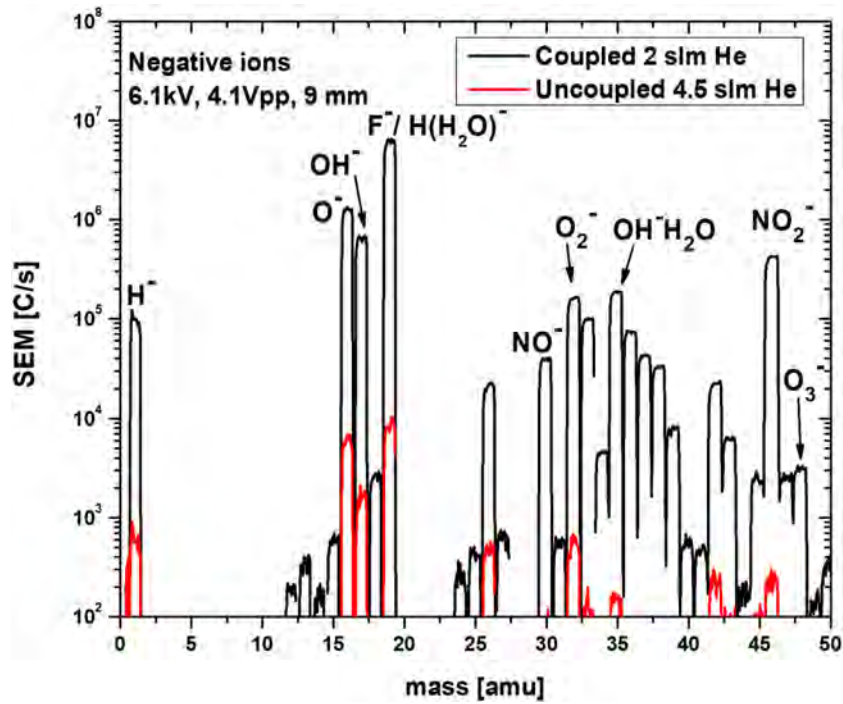


Figure 5. Negative ions: coupled mode (6.1 kV, imposed voltage 4.1 Vpp, 9 mm, 2 slm); uncoupled mode (6.1 kV, imposed voltage 4.1 Vpp, 9 mm, 4.5 slm).

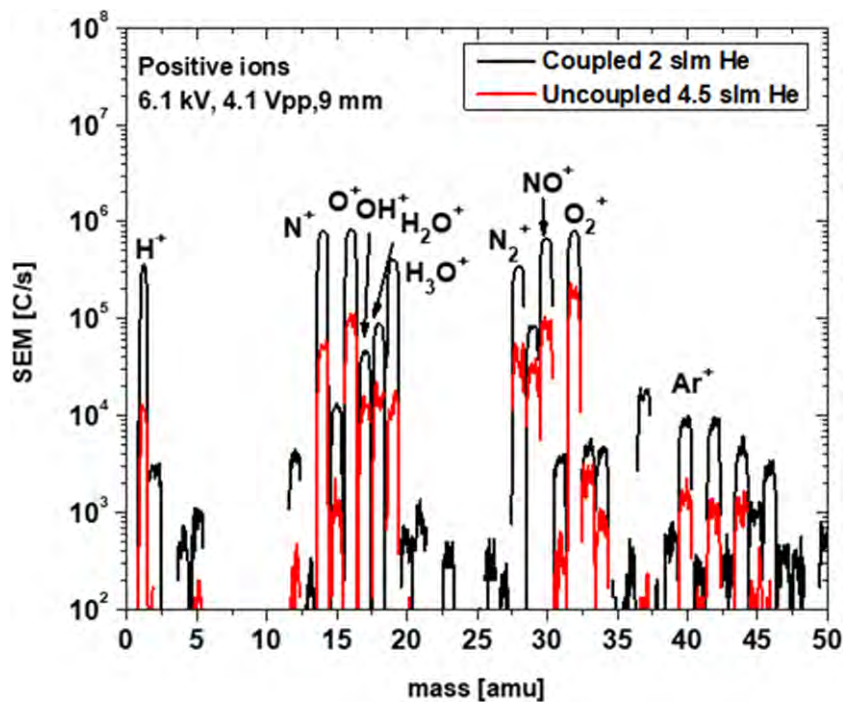


Figure 6. Positive ions: coupled mode (6.1 kV, imposed voltage 4.1 Vpp, 9 mm, 2 slm); uncoupled mode (6.1 kV, imposed voltage 4.1 Vpp, 9 mm, 4.5 slm).

the positive ion spectrum has higher abundance of nitrogen molecular ions. Besides the positive ions of oxygen species, we can see that the most abundant ions are N^+ , N_2^+ and NO^+ . Except for negative fluorine ion, that is characteristic of this plasma source structure, the recorded dominant ions agree with those recorded by McKay *et al* [56] in a cold atmospheric pressure helium plasma jet.

We have detected only $H(H_2O)^+$ and $H(H_2O)_2^+$ water ion clusters in the positive ion spectrum. The H_3O^+ ion is the dominant ion in discharges with very low humidity in the gas flow. It is formed through the following reaction $H_2O^+ + H_2O \rightarrow H_3O^+ + OH$ ($k = 1.85 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ [41, 57]). Water ion clusters with higher molecular mass are created predominately through the collisions of the smaller

ionized water clusters with water molecule ($\text{H}^+(\text{H}_2\text{O})_n + \text{H}_2\text{O} \rightarrow \text{H}^+(\text{H}_2\text{O})_{n+1}$) and through the breakdown of larger clusters ($\text{H}^+(\text{H}_2\text{O})_n \rightarrow \text{H}^+(\text{H}_2\text{O})_{n-1} + \text{H}_2\text{O}$) [41, 57].

With an increase of humidity in the feed gas the reactions producing higher order clusters are favored resulting in their higher intensity in the mass spectra [41]. This is valid for formation of both negative and positive ion clusters. In our case during the measurements the humidity in the surrounding air was between 30% and 50%, but water percentage in the helium gas flow is very low (order of few ppm). This can explain why we were only able to detect smaller water clusters and none of the higher ones.

In figures 5 and 6 negative and positive ion spectra for coupled and uncoupled modes are compared. We can see that in the case of coupled mode the intensity of the signal, for both positive and negative ions, is much higher than in case when the 7 jets are not merged into one single channel. Another difference is the abundance and variety of negative ion species found only in coupled mode. In case of the uncoupled mode the number of detected ion species, characteristic of the coupled mode, is smaller. Again, the mass 19 ion is present with high counts and out of oxygen species we could detect O^- , OH^- , O_2^- ions while O_3^- ion and nitrogen ions could be barely (or not at all) detected in the uncoupled mode. The ratio between the measured intensities of negative ions in coupled and uncoupled mode is more than two orders of magnitude (note the logarithmic scale). On the other hand, the ratio between the intensities of positive ions in coupled and uncoupled mode is not that high (figure 6) while still remaining in favour of the coupled mode, which always results in a higher count. The positive ions composition remains similar between the coupled and the uncoupled mode.

Since the shift from coupled to uncoupled mode is achieved by increasing the gas flow, we can assume that the axial gas speed and the radial diffusion of the species play a significant role in formation of these two modes. According to the literature [34], the higher the He flow and gas velocity the smaller the radial diffusion of the species, which, in turn, favors the maintaining of unique He channels with a higher He mass fraction. In coupled mode, the lower gas velocity ($\sim 6.1 \text{ m s}^{-1}$ in coupled mode, $\sim 13.6 \text{ m s}^{-1}$ in uncoupled mode) leads to the merging of jets helium flows and therefore to the interaction and merging of the ionization fronts. Moreover, a greater diffusion of the air species in the He channels is expected for this case. According to Darny *et al* [58], the negative ions are likely to be generated mainly by transfers at the boundary of the cylindrical helium channels, where the mixing with air is also the highest. For the multijet plasma source this condition is indeed favoured in coupled mode, when helium flow is lower. Furthermore, based on what has been reported for plasma actuators in air [59], we can assume that the difference in the negative and positive ions ratio between coupled and uncoupled mode is to be attributed to the longer lifetime and greater variety of the negative ions produced. The relatively high repetition frequency (80 kHz) of the power supply driving the multijet and the relatively long lifetime of some

of the recorded negative ions, such as NO_2^- ($\sim 1 \text{ s}$) and OH^- ($\sim 1 \text{ ms}$) [60], could lead to a build-up of negative ions in the gap between the source and the target.

3.1.2. 3-jets configuration. In order to emphasize the complexity of the interaction of the plasma jet with the target, we have used a particular version of a multijet plasma source presenting only 3 powered jets (see figure 3(a)) and therefore close to the plasma source configuration numerically investigated by Babaeva *et al* in [34]. Like in the 7-jets version, also in this case, the shifting between operating modes was achieved by changing the He flow (coupled 4 slm, uncoupled 7 slm). The measurements were done at a 7 mm distance between the multijet nozzle and the MBMS to have a similar level of signal as with the 7-jets version of the plasma source. Comparing the negative ions spectra for this 3-jets plasma source (figure 7) we can see that the difference of negative ion signals between coupled and uncoupled mode is reduced. In this case a ratio of about 2.5 (coupled/uncoupled) was obtained, differently from the 7-jets plasma source where this ratio was more than two orders of magnitude. A similar situation can be observed also for the positive ions (figure 8): the difference in signal levels between the coupled and uncoupled mode being very small and for some ions negligible (N_2^+ , O_2^+ , H_2O^+ , O^+ ...). From these results it can be concluded that the coupling phenomenon could be achieved even with a reduced number of jets, producing, in accordance with modeling results reported in [34], a stronger and amplified ionization wave that results in higher (especially negative) ion concentration.

3.1.3. Single jet configuration. In order to check if the level of the total ion signal in the coupled mode is just a simple addition of the separate single jet signals, mass spectrometry measurements were performed with a single jet plasma source (figure 3(b)) and compared it with the coupled mode of the 7-jets source. In order to get a good level of signal we had to use 5 mm distance between the plasma source nozzle and the MBMS. It must be noted that for this distance it was not possible to achieve an uncoupled mode with the 7-jets plasma source and therefore this case was not investigated.

The mass spectra for negative and positive ions for the described configurations are given in figures 9 and 10, respectively. Even at a first glance the presented results show that the total signal obtained in the coupled mode is orders of magnitude higher than the ion signal of a single jet. Even ideally multiplying the single jet signal 7 times (as if we were simply summing the contribution of 7 jets) we see that we can not achieve values as high as those of the coupled mode. For the negative ions the mass spectra for single jet is almost non-existent, having only significant counts for O^- and F^- . In case of positive ions, there is a significant ion count for several species in the single jet, however, the spectrum results significantly different from the one of the coupled case. Moreover, even in the case of positive ions, the coupled mode has a much higher ion signals compared to the single jet as the difference is of several orders of magnitude.

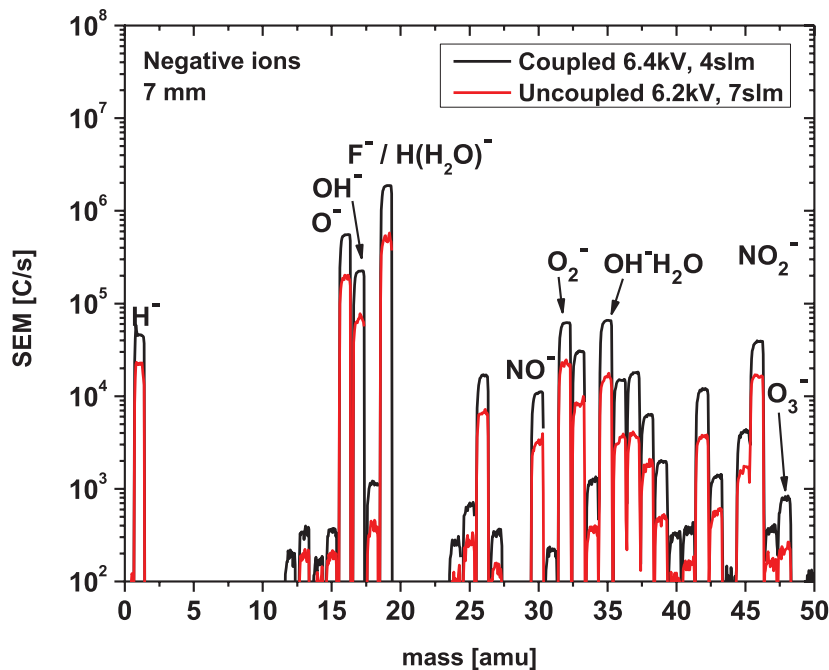


Figure 7. Negative ions—3 jet source: coupled mode (6.4kV, imposed voltage 6 Vpp, 4 slm, 7 mm); uncoupled mode (6.2kV, imposed voltage 6 Vpp, 7 slm, 7 mm).

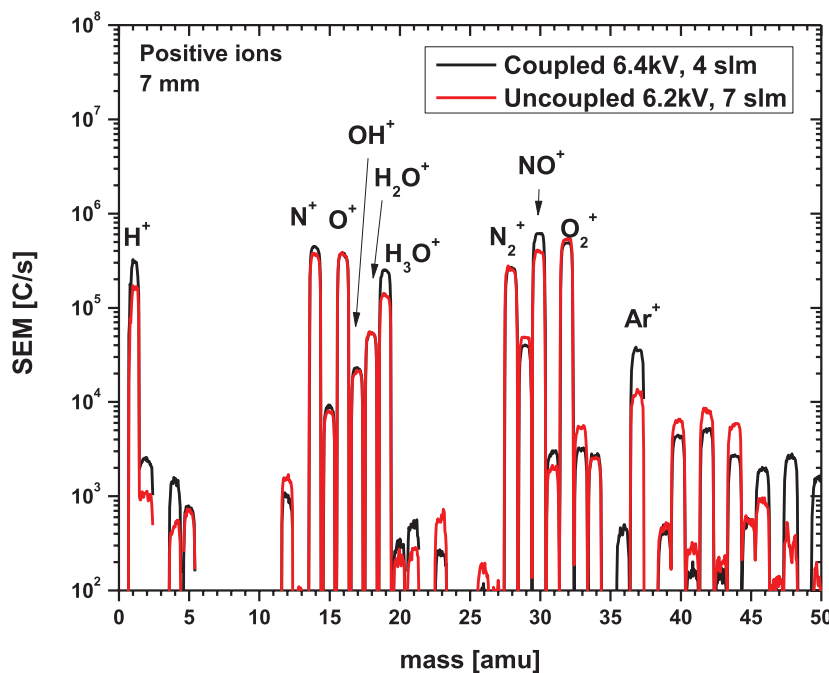


Figure 8. Positive ions—3 jet source: coupled mode (6.4kV, imposed voltage 6 Vpp, 4 slm, 7 mm); uncoupled mode (6.2kV, imposed voltage 6 Vpp, 7 slm, 7 mm).

3.2. *i*CCD and electrical analysis

3.2.1. Comparison of coupled and uncoupled modes by means of *i*CCD imaging. The temporal evolution of the plasma discharge generated by the multijet plasma source was investigated by means of an *i*CCD camera. In figure 11 time resolved images for the coupled and uncoupled modes of operation are shown. We have presented the time resolved imaging of the plasma development and the scales for coupled

and uncoupled mode were set to 10000 and 5000, respectively (figure 11). The scales were set to these values in order to avoid saturation of the emission intensities. If those scales are reduced to lower values we can see that throughout the whole period there is a feeble plasma channel connecting the multijet and the copper target. For both cases nine *i*CCD acquisitions related to different instants of the voltage pulse are presented ($t = 0 \mu\text{s}$ is positioned at the start of the positive half-period of the sinusoidal voltage pulse). The voltage and current

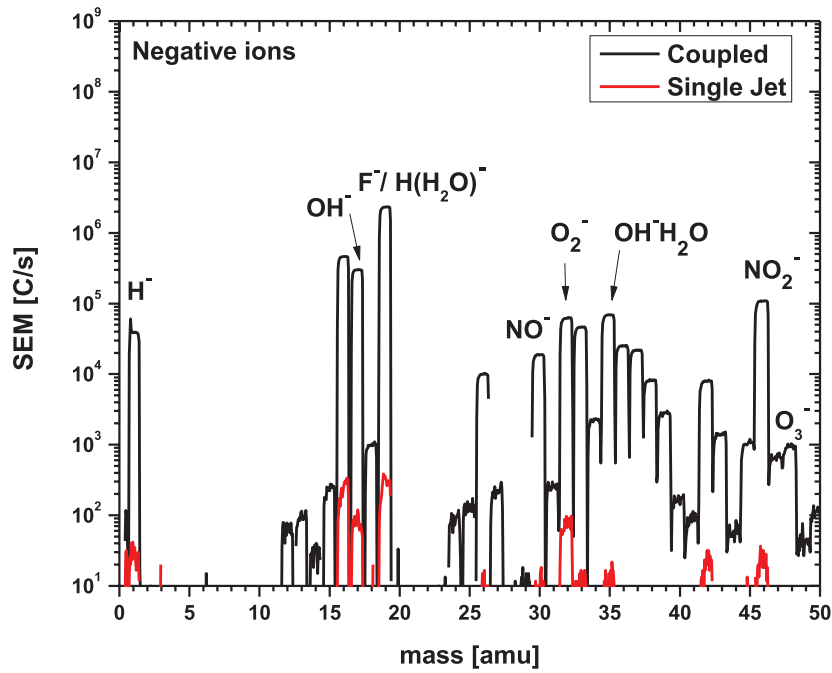


Figure 9. Negative ions: coupled mode of 7 jets source (5 kV, imposed voltage 4.14 Vpp, 5 mm, 4.5 slm); single jet (5 kV, imposed voltage 4.14 Vpp, 5 mm, 4.5 slm).

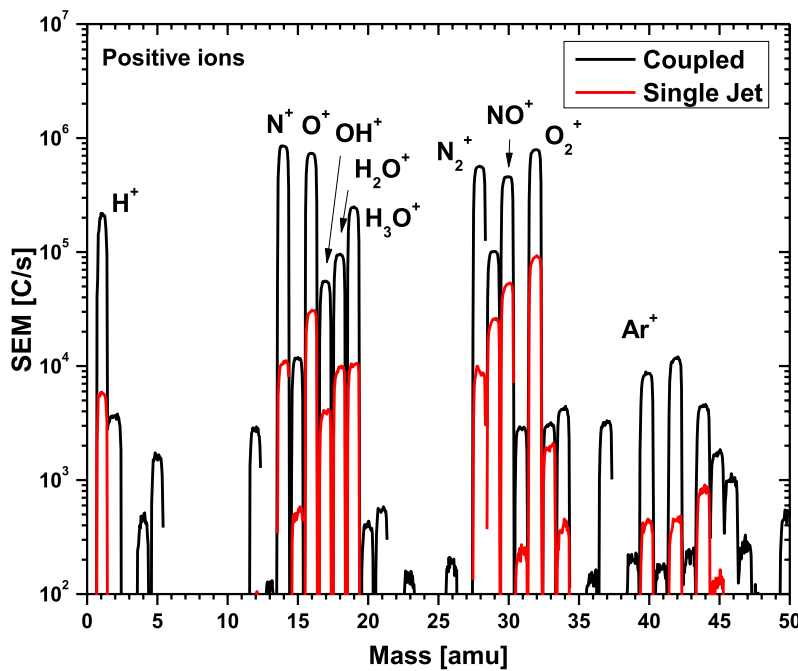


Figure 10. Positive ions: coupled mode of 7 jets source (5 kV, imposed voltage 4.14 Vpp, 5 mm, 4.5 slm); single jet (5 kV, imposed voltage 4.14 Vpp, 5 mm, 4.5 slm).

waveforms for both cases are also reported at the top of the figure. The acquisition reveals the in-air dynamic behaviour of the discharge impinging on the conductive surface. The time evolution of the discharge structure can be separated into four main steps.

First, at the start of the positive half period ($0 \mu\text{s}$ – $3 \mu\text{s}$, figures 11(a)–(c), plasma propagates from the source outlet towards the grounded target, expanding inside the flow of high He fraction. The distribution of the He concentration is

different in the two cases because of the different He flows and, as expected, it leads to multiple ionizing fronts in the uncoupled mode (even if not all the jets are visible at the same time). Only one ionization front was detected for the coupled mode. The current was measured through the ground connection of the target the only signal that was collected was the current signal due to the main plasma channel of the multijet. We can see that the system does not show the appearance of the additional streamers that can be seen as additional peak

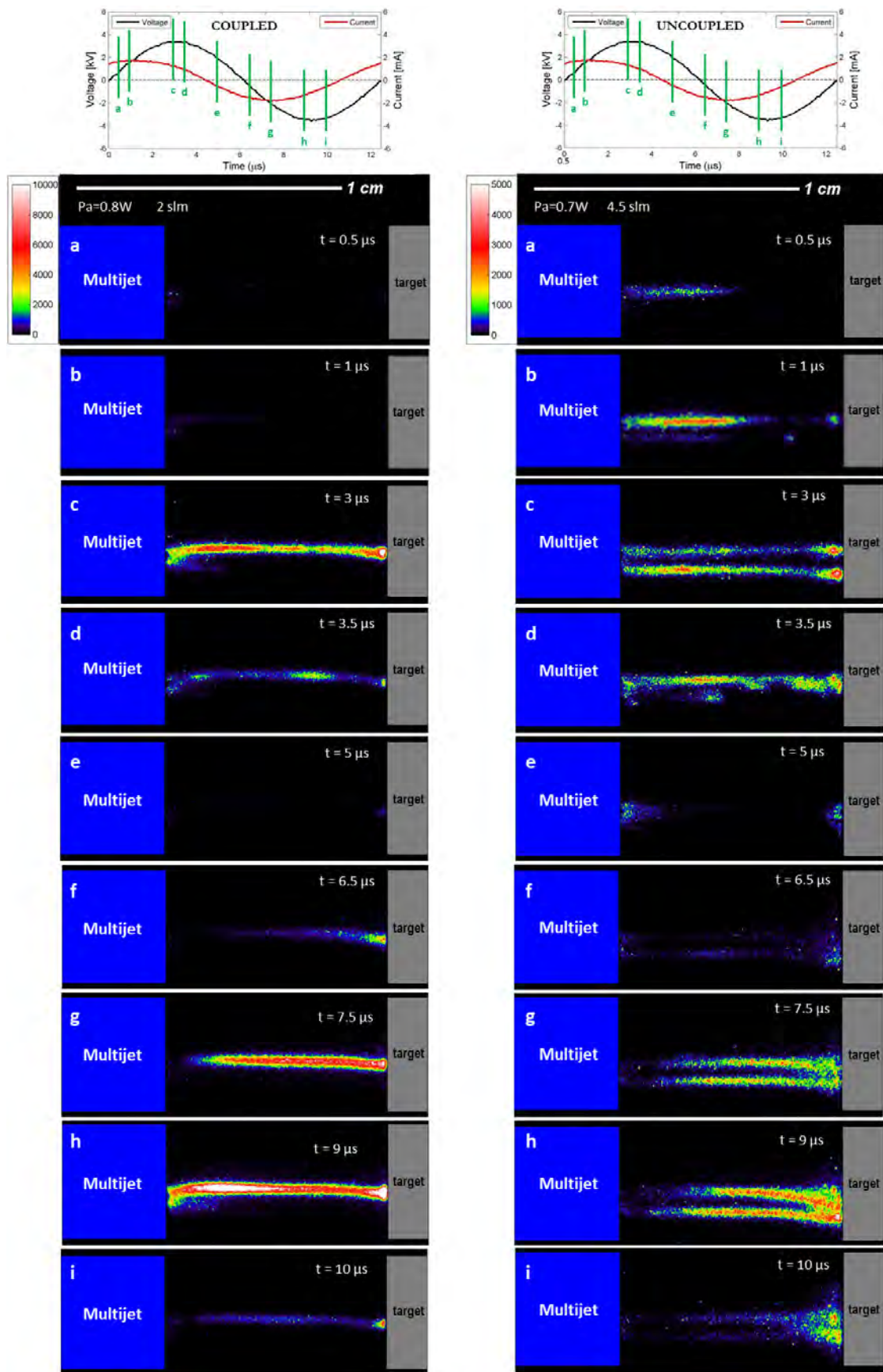


Figure 11. Coupled (left column, He 2 slm) and uncoupled (right column, He 4,5 slm) voltage and current waveforms (top) with iCCD acquisitions (bottom) at different time instants. The timing of iCCD acquisitions is labeled with green lines and letters (a)–(i) on the waveforms.

in the current waveform. Also, the time scale of the development of the higher emission intensity is of the order of half a period ($1/2 T = 6.25 \mu\text{s}$) and this is not fast enough in order to get sharp peak in the recorded current. Similar behavior can be observed in both positive and negative half period of the voltage signal.

Highlighting the differences, it is possible to observe that in the coupled mode plasma propagation starts later than in the uncoupled one and reaches the target about $2 \mu\text{s}$ later. This difference is probably due to the different percentage of air mole fraction, which is higher in the coupled case and may require a higher electric field to sustain the discharge propagation. Therefore, the condition for the discharge propagation could be reached earlier in the uncoupled mode and later, when the voltage of the sinusoid is higher, in the coupled one. The ionization front in coupled mode crosses the gap within the time-lapse between two acquisitions ($2.5 \mu\text{s}$ and $3 \mu\text{s}$) propagating faster than the fronts in the uncoupled mode ($0 \mu\text{s}$ – $1.5 \mu\text{s}$, acquisition not shown). This could be explained by the combined effect of two factors: in uncoupled mode the ionization front propagates later, when the voltage pulse is closer to the positive peaks, and the electric field in the gap is presumably higher; a higher degree of air impurities in coupled mode may also lead to an increase in the ionization front propagation velocity as reported in literature for small nitrogen admixture or higher air/helium fraction [37, 61]. As reported in the literature, gas flow rate can greatly influence the propagation of plasma generated species [62]. Anyway, in our case, the several orders of magnitude of difference between the gas velocity and the ionization front velocity (m s^{-1} versus km s^{-1}) lead us to exclude a direct relation (not mediated by flow regime variations and air impurities) between the different gas flows and the ionization front velocities recorded for the two modes. As the streamers have crossed the gap, strong and luminous conductive channels form between the target and the high-voltage electrode. These plasma channel emissions reach maximum intensity at the maximum of the positive voltage peak ($3.1 \mu\text{s}$) for both cases, but result considerably higher for the coupled case (note different scale used for the two cases in figure 11).

In the second phase ($3.5 \mu\text{s}$ – $6.5 \mu\text{s}$, figures 11(d)–(f), for both cases, there is a quasi-extinction of the plasma emission from the ionized channels. It should be emphasized that, at times $6.25 \mu\text{s}$ (the beginning of the negative half cycle), even if only a small light emission is detected, remnant conductive ionized channels are still present in all the regions crossed by the streamer. Hence, when the voltage powering the high voltage electrode reverses, the potential across the remnant columns instantaneously reverses and the electrons and ions are now accelerated in the opposite direction. As a consequence, in the third phase ($6.5 \mu\text{s}$ – $9 \mu\text{s}$, figures 11(f)–(h), the counter propagation of a secondary streamer from the target surface to the high voltage electrode through the remnant conductive ionized channel left by the primary streamer can be observed. For both the positive and the negative parts of the

voltage waveform we have propagation of cathode directed streamer (CDS). This observation is in agreement with what has been reported in the literature by other groups investigating the interaction of APPJs and conductive or semi-conductive targets [63–66]. Interestingly, in our case, multiple counter propagating streamers are observed for the uncoupled mode due to the still present separation between the remnant conductive channels. As reported by Ries *et al* [63] the position where the counter-propagation stops could depend on various parameters among which the gas flow rate, such that the higher the gas flow rate the shorter the counter-propagation is. Consistently, in figure 11 we can observe that while for the coupled case, with lower He flow, the counter propagating secondary streamer clearly crosses the gap, for the uncoupled case the streamer's head approaches the proximity of the multijet plasma source outlet but presents a significantly less intense emission in this region. The streamers reach in both cases the maximum intensity at $9 \mu\text{s}$ in correspondence of the negative peak voltage. In accordance with the mass spectrometry ion analysis, the emission intensity of the coupled mode appears to be always higher than the uncoupled one due to a higher ionization degree.

In the fourth phase ($9 \mu\text{s}$ – $12.5 \mu\text{s}$, figures 11(h) and (i), the slow disappearance of the plasma channel produced by the secondary streamer takes place. It must be noted that also in this case remnant conductive ionized channels are still present after the end of the negative half period. This will guide the new ionization fronts along the same path of the previous ones. As reported by Darny *et al* [58] a critical role in inducing strong modification of the gas flow propagation can be attributed to the large and long lifetime negative ions (eg. O_2^- , O_3^- , NO_2^-) during repetitive operations in the kHz regime as in our case. Since we know, from MS analysis, that negative ions are present in relatively large amount, especially in the coupled mode discharge, we are inclined to believe that these ions play a major role in the induction of the jet-to-jet coupling phenomenon here investigated.

3.2.2. Parametrical investigation in coupled mode. Electrical properties of the discharge generated in coupled mode were further investigated by means of iCCD camera and voltage and current probes. Measured voltage and current values are presented in figure 12 while a selection of iCCD camera acquisitions is reported in figure 13. Figure 12(left) represents the dependence of voltage and current amplitude peak-to-peak values as a function of the voltage given by the signal generator (imposed voltage peak-to-peak value). In figure 12 (right) we have voltage–current characteristics of the multijet system obtained for the coupled mode operation. Electrical measurements have shown that there is not always a direct correlation between the voltage imposed on the pulse generator driving the multijet plasma source and the voltage recorded at the high voltage electrode. In fact, after a certain value, the recorded voltage does not increase with the imposed voltage but, starting from 4.6 V of imposed voltage, reaches a plateau. The

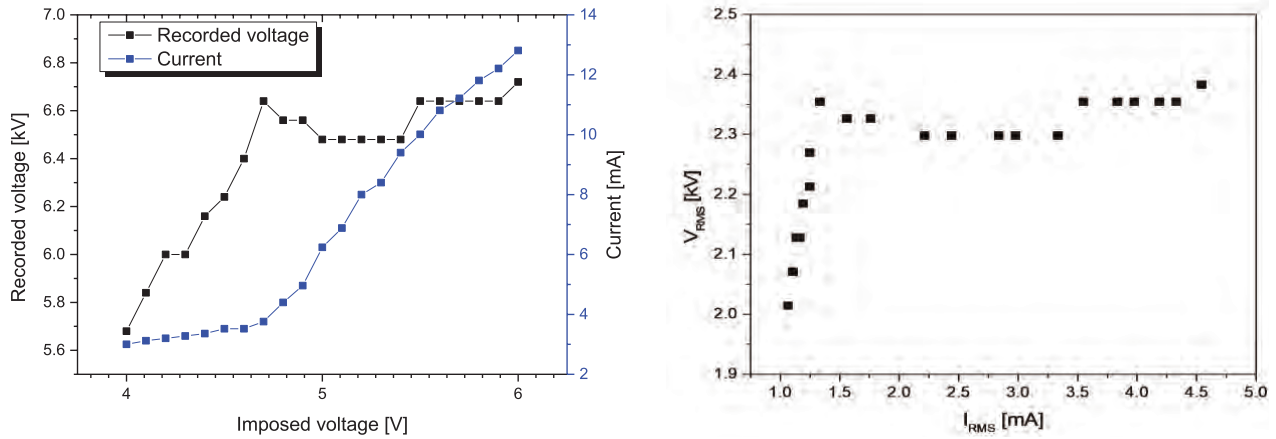


Figure 12. Recorded voltage and current values over pulse generator imposed voltage (left) and recorded rms voltage over current (right), during operation in coupled mode (7-jets version, He 2 slm, 9 mm gap).

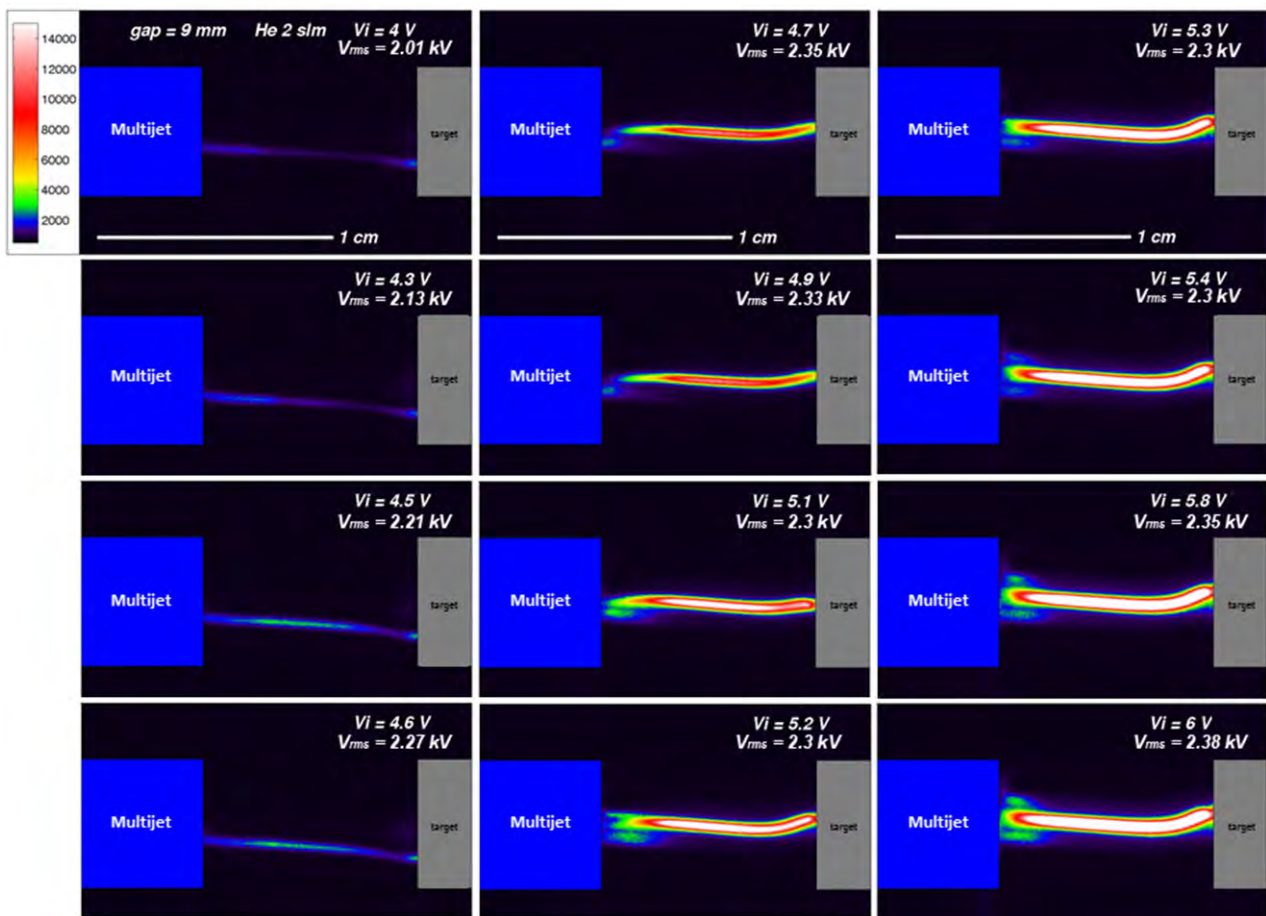


Figure 13. iCCD acquisitions (12,5 μ s gate) of the multijet plasma source in uncoupled mode (7-jets version, He 2 slm, 9 mm gap) for different imposed voltages.

current on the other side present two different trends, with different gradients, that meet in correspondence to the 4.6 V. After this value, the current starts to increase more steeply with the increasing imposed voltage. Similarly, iCCD acquisitions show a plasma discharge of gradually increasing intensity and width from 4 V to 4.6 V of the applied voltage. From 4.6 V to 4.7 V a drastic variation of the discharge, that becomes significantly brighter and wider, is observable. A further increase of the applied voltage does not induce any

increase in the recorded voltage but leads to a widening of the plasma discharge and an increase in the light emission that may be associated with the recorded increase in the current.

As reported by other groups for similar setups with microsecond voltage pulse excitations [65, 67], when the ionization channel left by the ionization front is conductive enough, a glow-like discharge may occur and lead to a strong conduction current. Since the conductivity of the ionized channel supposedly increases with the voltage,

it is plausible to assume that this transition to a glow like discharge in our case occurs between 4.6 and 4.7 V of the applied voltage. This behavior could be explained by the role of the conductive plasma column, which electrically connects the plasma source and the grounded target; the ionized channel can in fact be regarded as a transient connection of low resistivity between these two. In turn, the voltage drop due to the channel impedance and the current flow limits the voltage recorded on the high voltage electrode. The fact that this voltage reaches a plateau while the current increases suggest that the total impedance of the plasma column decreases with an increased applied voltage. This hypothesis is in agreement with ICCD recordings that show a plasma channel with increasing emission intensity, probably related to a higher ionization degree and therefore higher conductivity.

4. Conclusions

The results reported in the paper present new insights into the complex jet-to-jet coupling phenomenon. Using ICCD imaging, mass spectroscopy and electrical measurements, it was shown that a variation of the helium flow can completely change the nature of the discharge generated by an array of plasma jets, leading to different modes of operation (coupled and uncoupled) with substantial differences in the ionization fronts number, temporal evolution and ionic composition. Results suggest a major role of large and long lifetime negative ions, possibly due to variations in the air/helium mole fraction, which are orders of magnitude more abundant in the coupled mode as compared to the uncoupled one.

The counter propagation of secondary streamers from the conductive target towards the plasma source was also observed and associated to the presence of the remnant conductive ionized channel following the first streamer. Interestingly, multiple secondary streamers were observed in the case of uncoupled mode suggesting the repetitive ionization of these channels, relatively fixed in space, during the plasma source operation.

Moreover, a non-linear behaviour of the plasma discharge in the coupled mode has been observed for increasing values of the applied voltage. After a critical value a glow-like regime, characterized by high current and stable voltage at the electrodes, is recorded. Results suggest that this behaviour may be ruled by the formation of a plasma column of sufficiently high conductivity that acts like a transient connection between the grounded target and the plasma source.

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Long and short term effects of plasma treatment on meristematic plant cells

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In this paper, we will present results of plasma treatments of meristematic cells of *Daucus carota*. Plasma needle was used as an atmospheric pressure/gas composition source of non-equilibrium plasma in all treatments. Activity of antioxidant enzymes superoxide dismutase and catalase was measured immediately after plasma treatment and after two weeks following the treatment. Superoxide dismutase activity was increased in samples immediately after the plasma treatment. On the other hand, catalase activity was much higher in treated samples when measured two weeks after plasma treatment. These results show that there is a direct proof of the triggering of signal transduction in the cells by two reactive oxygen species H_2O_2 and O_2^- , causing enzyme activity and short and long term effects even during the growth of calli, where the information is passed to newborn cells over the period of two weeks. © 2014 AIP Publishing LLC.

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In the last decade, the expansion of the plasma medicine and its demand for *in-vivo* treatments resulted in fast development of various plasma devices that operate at atmospheric pressure. It is much more difficult to achieve non-equilibrium (non-thermal) mode of operation (as opposed to the thermal mode) at atmospheric pressures. Nevertheless, nonequilibrium operation is essential for treatment of biological samples for a number of reasons. For example, temperature of the tissue treated by plasma has to be kept below the threshold for the denaturation of the proteins. Another demand is that atmospheric plasma sources have to be able to do precise and localized treatments. This is so since some of the reactive species which have beneficial effects in small doses (as low as a single molecule serving as a trigger) may have very harmful effect in larger quantities.

In spite of difficulties to achieve nonequilibrium operation, numerous atmospheric pressure plasma sources fulfill all demands for application on biological samples. So far, atmospheric pressure discharges (APPD) were used for sterilization,¹ treatment of wounds,² improved blood coagulation,³ treatments of skin,⁴ treatment of cancer,⁵ etc.

One of the sources that meet all the requirements needed for treatment of biological material is plasma needle. This device was first described by Stoffels and coworkers,⁶ and it has been used to induce apoptosis and necrosis of cultured eukaryotic cells,⁷ bovine aortic endothelial cells,⁶ or human (epithelial cells—MR65 cells originating from non-small cell lung carcinoma (NSCLC)⁸) tissues.

We have developed a somewhat improved version of this device and used it for sterilization of planctonic samples of bacteria, MRSA biofilm, for improved differentiation of human periodontal stem cells into osteogenic line and for treatment of plant meristematic cells.^{9,10} The derivative probe measurements of power delivered to the plasma and mass spectrometry of the plasma generated by the needle have already been presented in our previous work.^{11,12} Plasma needle generates reactive oxygen species (ROS) and reactive

nitrogen species (RNS) radicals like N, O, O_3 , OH, and NO that strongly affect metabolism of living cells. One of the open issues is to correlate external plasma products (electrons, ions, RNS, ROS, photons, strong fields, etc.) with the immediate internal response which triggers or induces effects in the living cell. For that purpose, we study the kinetics of two enzymes which are typical indicators of the identity of reactive species from the plasma created environment that can trigger signal transduction in the cell and ensue cell activity.

In plants, ROS are always formed by the inevitable attachment of electrons to O_2 from the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments.^{13–15} All ROS are extremely harmful to organisms at high concentrations. When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of “oxidative stress.” In order to avoid the damage caused by ROS, plants have evolved molecular defense systems that both limit the formation of ROS and promote its removal.¹⁶ The plant enzymatic defenses include antioxidant enzymes, such as the phenol peroxidase (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT), which together with other enzymes of the ascorbate–glutathione cycle promote the scavenging of ROS.¹⁷

In this paper, we will show results for the SOD and CAT activity in the *Daucus carota* calli cells immediately after the plasma treatment and two weeks after the treatment. SOD is considered to be the first line of defense against ROS.¹⁸ It catalyzes the dismutation of O_2^- to H_2O_2 and molecular oxygen. CAT is present in the peroxisomes of nearly all aerobic cells, and, to a lesser extent, in mitochondria.¹⁹ It serves as protection of the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O .²⁰ Thus, both serve as good markers of internal chemical effects of the external plasma products and may also inspire similar procedures in animal and in human cells as well as for the bacteria.

For plasma treatment of calli, we have used plasma needle which is powered by sinusoidal signal at 13.56 MHz radio-frequency (RF). Construction and diagnostics of this device are described in more detail in Refs. 11 and 12. We have used helium as the working gas and the flow was 1 standard liter per minute (slm). As the working gas mixes with the air from the surrounding atmosphere, free radicals are generated within the plasma, such as reactive oxygen, nitrogen, nitrogen oxides, and ozone. The yields that were obtained for H_2O_2 and O_2 were 0.03% and 7.2%, respectively, in the helium flow of 1 slm while O_2^- was detected but no quantitative measurements could be made so far. Electrical circuit that supplies plasma needle consisted of signal generator which provided sine wave signal at RF frequency. This signal was amplified through a linear amplifier. We have used home-made derivative probes for measurement of the mean power transmitted to the plasma.

The embryonic calli cultures of carrot (*Daucus carota*) were established from storage root explants and maintained on solid MS medium²¹ supplemented with 2, 4-dichlorophenoxyacetic acid (2,4-D, 1.0 mg l^{-1}). Calli samples (10–30 mg) were placed in 96 wells micro-titer plate. The distance between the edge of the well and glass tube was 0.5 mm in all treatments. Well diameter was larger than diameter of the glass tube by 0.5 mm. Also, tip of the needle is located 1.5 mm outside the glass tube thus created plasma was in a mixture of helium and ambient air. During all experiments, plasma was covering whole surface of the samples and average distance between the needle and the culture surface was 4 mm. Culture was attached to the gel environment placed at the bottom of the micro titer plates. The treatment times were 10, 30, 60, and 120 s at two powers of 0.4 W and 1.6 W. Control calli samples were kept in micro-titer plate during the experimental procedure to ensure equal treatment conditions. Samples were also subjected to the treatment of only helium flow without the discharge with the exposure time of 120 s in order to determine whether helium flow affects the samples. After the treatment, calli were immediately frozen in liquid nitrogen and kept at -70°C until use, or continuously cultured on fresh basal MS medium without plant growth regulators for 2 weeks before use. Plant tissue was grinded to a fine powder, and proteins were extracted in 100 mM potassium-phosphate buffer (pH = 6.5) supplemented with protease inhibitor cocktail for plant tissue extracts and 5 mM ascorbate. Protein content was determined according to Bradford (1976) using bovine serum albumin as a standard²² and separated by native PAGE. The protein amounts applied to each well for the measurements of enzyme activity were $30 \mu\text{g}$. Gels were stained for CAT activity as in Ref. 23. SOD activity (in units of enzyme activity mg^{-1} soluble protein) was determined as in Ref. 24.

ROS that include hydroxyl radicals ($\text{OH}\cdot$), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) are produced during normal cellular functioning. Under standard conditions antioxidant systems of the cell minimize the perturbations caused by ROS. If ROS generation is increased either externally outside the cell or internally, the result of this imbalance is oxidative stress.^{25–27} Aerobic organisms evolved defenses that include both non-enzymatic and

enzymatic antioxidants in order to minimize the damaging effects of ROS.^{25,28} Because ROS are toxic, but also participate in signaling events, plant cell requires at least two different mechanisms to regulate their intracellular concentration by scavenging the ROS: one that will enable the fine modulation of low levels of ROS for signaling purposes and one that will enable the detoxification of excess ROS, especially during stress.²⁹ Major ROS scavenging mechanisms of plants include SOD, CAT, and different types of peroxidases. Their role is to directly scavenge superfluous amounts of the ROS created inside the cell or coming into the cell from the outside source. The SOD scavenges super oxide anion catalyzing its dismutation to hydrogen peroxide, while CAT reduces hydrogen peroxide to water.³⁰ Figure 1 shows the reaction paths inside the cell for the SOD and CAT.

We can see that the first enzyme that is usually employed in the cell defense is SOD followed then by the catalase that is responsible for the destruction of the products coming from the reactions involving SOD enzyme. We have measured activity of the SOD and CAT immediately after the plasma treatment and two weeks after the treatment. The analysis for the SOD activity performed immediately after the plasma treatment (Figure 2(a)) showed that enzyme activity of the carrot calli for the treatments with 0.4 W plasma did not alter significantly compared to the control samples. On the other hand, enzyme activity increased gradually with the treatment time for the higher power of 1.6 W. The highest SOD activity was observed for the treatment time of 120 s. Increase in the enzyme activity implies that more superoxide anions are present inside the cell and that calli cells are under oxidative stress. In order to cope with the elevated level of ROS antioxidants mechanisms in carrot calli had been triggered and enzyme activity was correlated with the stress level.

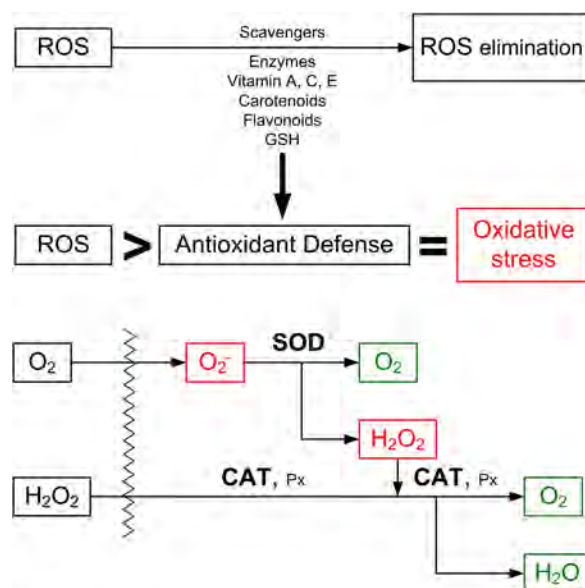


FIG. 1. The sources and cellular responses to ROS. Oxidants are generated as a result of normal intracellular metabolism in mitochondria and chloroplast, as well as from enzyme systems. A sophisticated enzymatic and non-enzymatic antioxidant defense system, including CAT, SOD, peroxidases (Px), and glutathione (GSH), counteracts and regulates overall ROS levels to maintain physiological homeostasis. When ROS production exceeds antioxidant defenses, oxidative stress occurs.

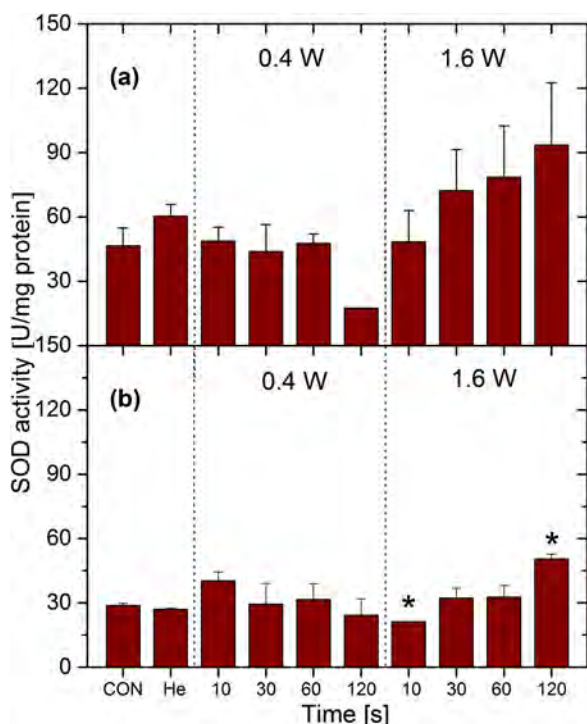


FIG. 2. SOD activity of carrot calli measured immediately (a) or two weeks after plasma treatment (b). Values are means \pm SE obtained from two independent experiments. The asterisk represents statistical significance in comparison with control value (* $p < 0.05$ and ** $p < 0.01$).

SOD activity in carrot calli after continuous two weeks growth showed much lower values (~ 2 fold), confirming that under non-stressful conditions the antioxidant defense system provides adequate protection against ROS (Figure 2(b)). Nevertheless, in two weeks grown calli SOD activity retained higher levels in comparison to control with a clear plasma power dependence. Comparison of SOD activity in calli cells immediately after the plasma treatment and two weeks after the treatment illustrates the importance of SOD for antioxidant defense of plants under excessive stress conditions.

The explanation for elevated levels of the SOD activity immediately after the plasma treatment can be found in the presence of the radicals and ions delivered by the plasma to the cell membrane. The oxygenation of the tissue was already observed in treatment of cancer cell.³¹ The triplet state of molecular oxygen is not a highly reactive species when it comes to chemical pathways inside the cells. On the other hand, singlet oxygen molecule ($^1\Delta_g\text{O}_2$) more easily reacts with other species forming superoxide anion. In case when plasma is present, the concentration of the singlet delta oxygen (SDO) molecules is especially increased. Atmospheric plasmas are known as a good source of the singlet delta oxygen species.³² When comparing atmospheric sources, it is shown that those operating at RF frequencies, like plasma needle, are much more efficient in production of SDO than the kHz sources.³³ The production of the singlet delta oxygen molecules is also more efficient with an increase of the power transmitted to the discharge.³³ This coincides with an increased activity of SOD for the higher power of 1.6 W. Apart from the singlet oxygen molecules in plasma, we have negative ions present including the superoxide ion. Its

contribution should not be neglected even though concentration of O_2^- reaching the surface of the sample is quite low. The reader has to have in mind that we are here neglecting whole cocktail of species created in plasma and concentrating only on these two types of species since SOD is involved only in the reactions including O_2^- .

The product of the SOD catalytic reaction inside the cells is hydrogen peroxide. This molecule is lately recognized as signaling molecule in the chemical pathways of the cell,²⁸ but mainly, in higher concentration, it is highly dangerous and leads to cell damage and consequently in cell death. The reactivity of H_2O_2 is the result of its reduction by metal ions to form the highly reactive hydroxyl radical.³⁴ Hydrogen peroxide is a participant in a number of reactions, and it is capable of diffusing across membranes.³⁵ This means that H_2O_2 created in the plasma also has significant influence on the hydrogen peroxide balance inside the calli cells. CAT is one of the most active enzymes which decompose hydrogen peroxide molecules at extremely rapid rate. Another important point is that, depending on the concentration of H_2O_2 , different reactions are activated. Hydrogen peroxide is decomposed by oxidizing some of the hydrogen donors inside the cell (e.g., ethanol, ascorbic acid) at lower concentrations. This is done through the reaction: $\text{RH}_2 + \text{H}_2\text{O}_2 \rightarrow \text{R} + 2\text{H}_2\text{O}$. In case of higher concentrations of H_2O_2 molecules, CAT decomposes it in a pure catalytic reaction: $2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$. Catalase is unique among H_2O_2 -degrading enzymes in that it can degrade H_2O_2 without consuming cellular reducing equivalents. Hence, catalase provides the cell with a very energy-efficient mechanism to remove H_2O_2 . Therefore, when cells are stressed for energy and are rapidly generating H_2O_2 through “emergency” catabolic processes, H_2O_2 is degraded by catalase in an energy-efficient manner.³⁶

In this study, CAT activity was measured immediately after the plasma treatment (see Figure 3(a)) and in the carrot calli that were grown within two weeks after the treatment (Figure 3(b)). The CAT activity increased when compared to the control samples, but we could not obtain a clear picture about its dependence on the applied power or treatment time. In our experiments, we have measured enzyme activity shortly after plasma treatment. 1–2 min after the treatment samples were frozen in liquid nitrogen and prepared for measurements. We assume that this time was not enough for the enzyme to react adequately. Especially in the case of the CAT which is the second in line and it is activated after the SOD enzyme. Evidently, it is necessary to evaluate the activity of these enzymes in a period of few hours after the plasma treatment.

However, CAT activity in calli samples that were grown for two weeks increased gradually with the increment of plasma power and time of treatment exhibiting 1.5 fold higher values in comparison with CAT activity measured in calli samples immediately after the plasma treatment. This means that in the treated cells, even two weeks later, the levels of H_2O_2 are elevated compared to the control samples. This long term effect of plasma could be explained by the increase in the morphogenic potential of the directly treated cells.^{37–39} The increase in the morphogenic potential may be through the activation of some section in the DNA

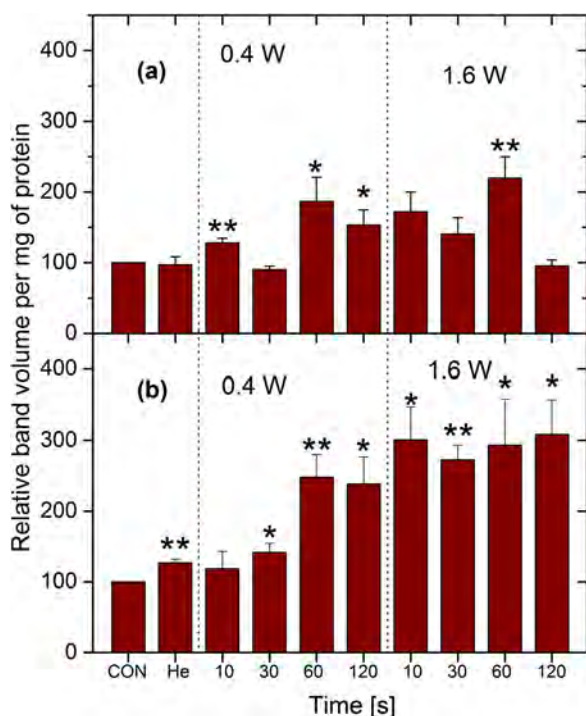


FIG. 3. Catalase activity of carrot calli measured immediately (a) or two weeks after plasma treatment (b). The band volume was recorded using densitometric analysis. Data were normalized using control signal. Values are means \pm SE obtained from three independent experiments. The asterisk represents statistical significance in comparison with control (* $p < 0.05$ and ** $p < 0.01$).

responsible for the proliferation and/or somatic embryogenesis (SE) of the cells. An increasing number of publications correlate ROS and SE. Kairong and coworkers suggested that plant SE is a special cell differentiation process and established a link or “partial overlapping” of ROS and cell differentiation.⁴⁰

Until now, we have shown that plasma has significant influence on several types of cells and bacteria. The effect of the treatment was investigated up to few days after the plasma treatment. This was the case for all studies: stem cell differentiation potential¹⁰ and sterilization.⁹ So far, the only long term effects were the increase of the fresh weight of the treated *Fritillaria imperialis* calli.¹¹ At the same time, the whole field in general is seeking means to connect the external agents produced by plasma to the intracellular events, agents, and triggering mechanisms. In this paper, we have presented results of enzyme activity that provides direct link between the external ROS and the activity of enzymes inside the cell.

The observable effects on enzymes were obtained immediately after the plasma treatment and also after a delay of two weeks. The SOD activity, while still being considerably greater than the activity in untreated cells, decreased after two weeks compared to the activity immediately after the treatment. The presence of increased densities of ROS may lead both to an increase or decrease of the enzymes. A decrease happens when the response of the living cell cannot keep up with an increased intake of ROS and in that case the survival of the cell is endangered. On the other hand, an increase may mean that, triggered by the ROS, production of enzymes is increased and final concentration is not under control of enzymes.

CAT activity was significantly increased in the samples in the period of two weeks following the treatment. This long term plasma effect due to exposure to the ROS and RNS generated by plasma on the treated meristematic cells is consistent with the previously observed increase of the morphogenic potential of cells.^{41,42} One should bear in mind that the observed increases over long term period are significant as the recorded values are always normalized to the mass of the growing calli (per mg of the protein isolated from the samples).

This paper provides a clear indication of the two facts: it is possible to associate increased activities inside the cell to the external plasma and one may claim a proof that at least H_2O_2 and O_2^- may pass through the cell membrane and induce a chain of events inside. In addition, some long term effects of plasma treatment on the cells in growing cultures have been observed where most likely triggering by ROS induces cell response and higher enzyme activity.

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Time-resolved optical emission imaging of an atmospheric plasma jet for different electrode positions with a constant electrode gap

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Abstract

The aim of this paper is to determine the influence of the position of the electrodes on the range of a plasma jet, for specific experimental conditions, by using time-resolved optical emission spectroscopy. The optimal position of the electrodes is determined for a fixed gas flow rate and applied excitation voltage. We characterize the helium plasma jet for different distances from the end of the glass tube, showing detailed results for four different electrode positions from the jet nozzle (7, 15, 30 and 50 mm). It was found that at the distance of 15 mm, the length of the plasma jet is at its maximum. The highest speeds of the plasma package travelling outside the glass tube of the atmospheric plasma jet are obtained for the same electrode configuration (15 mm from the jet nozzle). With the electrodes positioned at smaller distances from the nozzle, the plasma plume was much shorter, and at the larger distances the plasma did not even leave the glass tube.

Keywords: plasma jet, ICCD, time resolved, optical emission spectroscopy

(Some figures may appear in colour only in the online journal)

1. Introduction

Interest in plasma jets that operate at atmospheric pressure has been increasing in research literature in the last decade because of possible applications and also because of their unique characteristics and interesting physics. The most important characteristic of these atmospheric pressure plasma jets (APPJs) is low gas and ion temperature and abundant plasma chemistry. Atmospheric plasma jets produce relatively high concentrations of reactive chemical species, such as atomic oxygen and nitrogen, OH radicals, NO_x and ozone [1].

It is important to understand plasma jets because of their wide range of possible application in the new, fast developing field of plasma medicine [2] and treatment of organic materials [3]. In stomatology, plasma was used

in the removal of biofilms of bacteria responsible for the formation of dental plaque and caries [4–7] and also for the sterilization of bacteria responsible for periodontitis [8, 9]. Plasma sterilization of surgical instruments and heat sensitive implants [10, 11] is more efficient than using classical methods such as thermal and chemical sterilization. It is shown that the plasma can also accelerate blood coagulation, speeding up wound healing. It can also kill and remove cancer cells [2, 12–14]. Because of the fungicidal and antimicrobial properties of plasmas, low temperature plasmas may be used in dermatology for healing some chronic skin diseases that are non-sensitive to standard drugs [15].

Some of the pioneering work in the field has been done using a plasma jet needle, which, although it appears to be similar to plasma jets, has a different electrode configuration [3] and

regime of operation. Still, a large, perhaps even the largest, percentage of activities in plasma medicine are currently based on plasma jets. Many authors have reported results obtained by using various types of plasma jets. These jets can be divided into several groups using several criteria: operating gas, excitation frequency, type of excitation signal, electrode type and geometry. A noble gas, such as helium or argon, is usually used as the operating gas [16, 17] to reduce the breakdown voltage. Gas mixtures were used in order to control the concentration of desirable active species. Usually a small amount (~1%) of oxygen or nitrogen is added to pure argon or helium. Multiple bullets can appear in a single tube configuration too, by adding small amounts of nitrogen to helium feed gas. In this case, up to eight bullets are reported [18]. Air impurities such as water vapour can also affect plasma propagation and the production of reactive species [19, 20]. The operating frequency can be in the kilohertz, megahertz (13.56 or 27.12 MHz) or gigahertz domain, while the driving signal can be pulsed or sine wave [21, 22]. There are three main groups of electrodes that are typically used in plasma jet devices. The first type is with the electrodes separated from the buffer gas by an insulator, usually a glass tube [23] or capillary [24]; the second type is when they are in contact with the buffer gas [25]; and the third type is when the powered electrode is in contact with the buffer gas and the grounded electrode is isolated from the gas [26]. The electrodes can be cylindrical [27], flat [28], ring shaped [29], needle like [30], etc. Apart from results on systems with different electrode geometries, in the literature results obtained using different geometries of the glass tubes/gas flow may be found. While it has not been reported in most papers that a discharge may go beyond the grounded electrode (as observed by us), in [31] it is reported that in a T-shaped configuration, bullets appear at two ends of the tube asynchronously. This is, however, in both cases, motion of the bullet downstream. The electrode gap, geometry, frequency and type of gas used determine the behaviour of the plasma jet. These parameters have a large impact on the breakdown voltage, operating mode (chaotic, bullet or continuous [26]), voltage–current characteristics, dissipated power in plasma and the effluent length.

Several well-known techniques, such as optical emission spectroscopy, absorption spectroscopy, mass spectrometry, laser spectroscopy and electrical probes, can be used for the characterization and diagnostics of plasma jets. Fast time resolved ICCD imaging is the easiest way to investigate time/space development of plasma. From these high-speed images, it can be seen that plasmas in plasma jets are not always continuous. They sometimes appear to travel in a form of small plasmas that propagate from the glass tube filled with a flow of helium into the surrounding atmosphere [23]. These plasma packages, the so-called ‘plasma bullets’ or PAPS (pulsed atmospheric pressure streamers) [32], are not yet fully understood. Several theories explaining the formation of these fast travelling plasma packages have been proposed [23, 29, 33]. There have been some attempts to simulate the ‘plasma bullets’ as positive streamers [34], but there is some discrepancy between the simulation and experimental data

[35–38]. It is assumed that photons and Penning ionization [39, 40] play the main role in the propagation of ‘plasma bullets’.

In our previous paper, we have presented formation and time/space development of ‘plasma bullets’. The powered electrode was 5 mm from the edge of the glass tube and the electrode gap was 15 mm [40]. The point of that paper was to show the operation of a plasma jet with transparent electrodes, so that the development of the plasma could be followed at all times. The aim of this study is to take advantage of the technique presented earlier and provide systematic experimental data over a wider range of configurations and operating conditions. Apart from providing such data in order to provide theory with well-defined data that may be used for qualitative and even quantitative comparisons, we also try to determine the optimal distance of the electrodes from the edge of the glass tube at a constant electrode gap in order to obtain the largest plasma range for the conditions of our plasma source.

2. Experimental setup

A low temperature atmospheric plasma jet that can operate in the ‘bullet mode’ has been briefly described in [40]. The plasma jet consists of a Pyrex glass tube (inner diameter 4 mm and outer diameter 6 mm) and two transparent electrodes that allow the observation of plasma development inside the electrodes. The electrodes were made of polyethylene terephthalate (PET) covered with a thin conducting film of indium tin oxide. The electrodes were 15 mm wide with the inter-electrode gap of 15 mm. These dimensions were kept constant in the measurements. The electrode closer to the end of the glass tube was connected to the power source (powered electrode). The second electrode was connected through a resistor of 100 k Ω to the ground. Figure 1 shows an ignited plasma jet with the instruments used in the experiment. The parameter that was varied was the distance between the powered electrode and the external edge of the glass tube. We have followed the development of the plasma plume by varying the geometry for many geometries with 1 mm steps; we chose to only present the data for the four dimensions as selected in the text. Above 30 mm, the plasma did not leave the glass tube. Below 30 mm it did, and the maximum range occurred at 15 mm. Here we will show results for distances of 7, 15, 30 and 50 mm.

The operating frequency of the plasma jet was 80 kHz, adjusted by a signal generator. In order to power the plasma jet, the signal generator was connected to a custom built amplifier. The amplifier can produce voltages up to 1 kVpp, which is insufficient for plasma ignition. Therefore, an additional HV transformer was connected after the amplifier so that the voltage was increased up to more than 7 kVpp. Two commercial voltage probes were used for the current and voltage characterization of the system. The average power was calculated from the current and voltage signals obtained by these two probes. In all measurements, the amplifier power was kept at the constant value of 4 W. A very important note is that using a sinusoidal voltage simplifies the electrical power

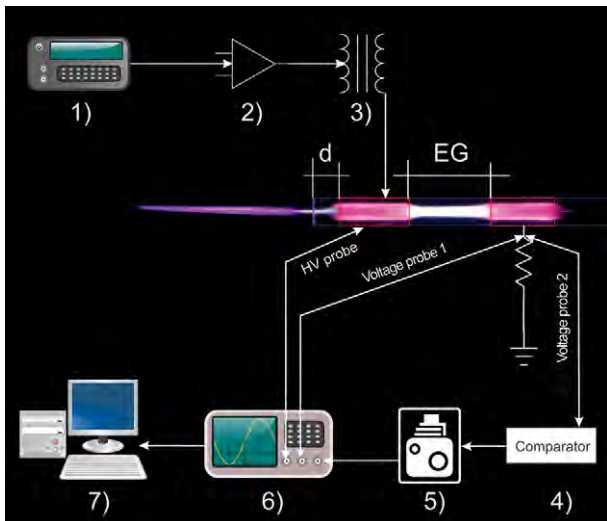


Figure 1. Experimental setup: (1) signal generator; (2) amplifier; (3) high-voltage transformer; (4) voltage comparator; (5) ICCD camera; (6) oscilloscope; (7) computer. EG = electrode gap (15 mm) and d = distance between the powered electrode and the edge of the glass tube ($d = 7, 15, 30$ and 50 mm).

supply and most measurements, but it also limits the range of plasma bullets (ionization fronts).

We had to introduce the third voltage probe in order to synchronize the ICCD camera (Andor iStar DH734I) with the driving signal of the plasma jet. This probe was connected at the same point in the electrical circuit as the probe for measuring the voltage on the $100\text{ k}\Omega$ resistor (voltage probe 2 in figure 1). The signal taken from this probe was introduced in a custom built voltage comparator. The voltage comparator enables triggering of the camera in each cycle ($12.5\ \mu\text{s}$) of the signal while keeping the relative time within the cycle. The stability of the voltage comparator is important since the ICCD camera has to record 160 images during the adjusted exposure time of 2 ms, while keeping the 25 ns gate width. All these single images were then integrated at the chip of the ICCD camera and transferred to the computer by Andor software. The position of the gate was scanned over the whole period of $12.5\ \mu\text{s}$ using the camera's internal delay generator. Thus, the emission over the duration of the entire period was recorded. We have checked our integration and timing jitter by comparing the single shot and the averaged recordings and looked for a change in shape or timing, which turned out to be negligible. In our experimental conditions presented in this paper we have always had only one PAPS formed during one cycle. The moment of formation corresponded to the voltage maximum. The results were experimentally reproducible and there was no evidence of the formation of multiple bullets. In all experiments, helium was flowing through the glass tube at the constant rate of 4 slm (standard litre per minute).

3. Results and discussion

3.1. Images for different distances between the electrodes and the edge of the tube

In this section, we show ICCD images for four different distances between the electrodes and the edge of the glass tube,

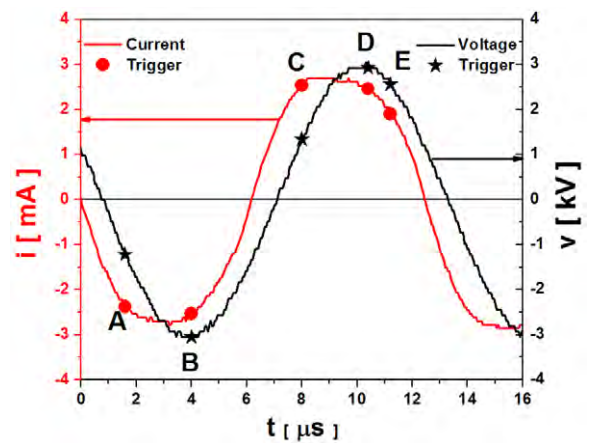


Figure 2. Current–voltage signals with trigger positions (for the distance of 15 mm). One period is $12.5\ \mu\text{s}$.

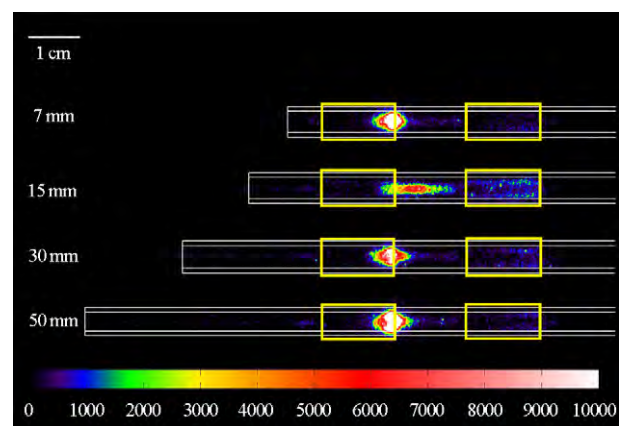


Figure 3. Time-resolved ICCD images at A ($1.6\ \mu\text{s}$).

along with the current and voltage signals with the triggering positions marked that correspond to the timing of those images. The current and voltage signals with the triggering positions A ($1.6\ \mu\text{s}$), B ($4.0\ \mu\text{s}$), C ($8.0\ \mu\text{s}$), D ($10.4\ \mu\text{s}$) and E ($11.2\ \mu\text{s}$) for the gap of 15 mm are shown in figure 2. The beginning of the period is chosen to be in the downward slope of the current and the voltage, when current is close to zero and the voltage is about 1 kV. The voltage signal is a pure sine wave, while the current signal is slightly deformed due to the superimposing of the plasma jet current on the displacement current. (The displacement current is observed directly when there is no plasma.) The peak-to-peak values of the voltage and current are 7 kV and 6 mA, respectively.

In figure 3 we show the emission intensities taken for the triggering time of $1.6\ \mu\text{s}$ after the beginning of the period (point A in figure 2). For this trigger time, the voltage signal is negative and at about one half of its amplitude, while the current signal reaches its maximum negative value. For all cases except for the distance of 15 mm, plasma is formed at the right edge of the powered electrode and fills the tubes almost uniformly. For the distance of 15 mm, however, plasma is somewhat shifted towards the grounded electrode and the main emission originates between the electrodes. It almost appears that the development at 15 mm is slightly ahead of those for other distances.

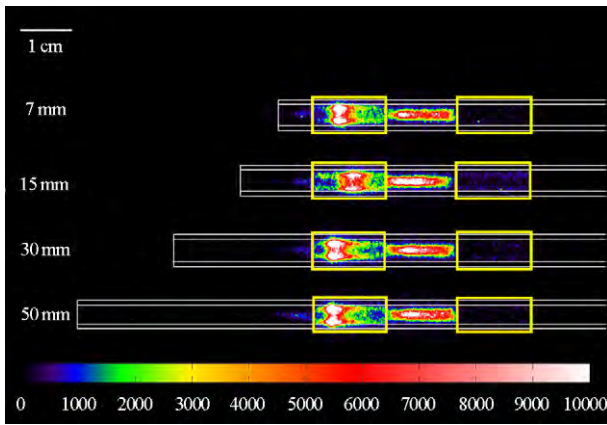


Figure 4. Time-resolved ICCD images at B ($4.0 \mu\text{s}$).

If we move further along the voltage/current cycle, the ionization wave indicated by the light emission starts propagating inside the powered electrode from its right edge to the left one (downstream of the helium flow and towards the exit of the tube). These images are shown in figure 4 and correspond to the triggering point B in figure 2. For this current/voltage combination, the plasma (as indicated by light emission) inside the powered electrode peaks close to the inner surface of the glass tube and has a ring-like shape. At the same time, the emission intensities originated from between the electrodes indicate that the plasma (emission) is confined at the axis of the glass tube. Comparing the images shown in figure 4, we can see that the plasma behaviour is again similar for all distances except for 15 mm. In the latter case, the position of the maximum emission inside the powered electrode is lagging compared to the results for the distances of 7, 30 and 50 mm. At the same time, the discharge for 15 mm appears to be ahead of other discharges in between the electrodes.

At $8.0 \mu\text{s}$ (C in figure 2), the voltage is at one half of its amplitude and the current is almost at the maximum in the positive half-cycle. Plasma in the inter-electrode gap reaches the left edge of the grounded electrode (see figure 5) and extends inside. At the same time, inside the powered electrode there is almost no light emission (for 7, 30 and 50 mm) except for the distance of 15 mm, where emission is detected inside the powered electrode. At this point, a PAPS precursor is being formed. We can see that there is a plasma channel connecting the tip of the plasma with the powered electrode. This channel is much stronger when the electrodes are further away from the edge of the glass tube and the ambient air. For the two smaller distances (7 and 15 mm), this channel produces weaker emission, but we can see that plasma is already in contact with ambient air, and PAPS are being formed. At the same time, for the distance of 15 mm, one can see a transition from a hollow emission distribution (peaking close to the wall-wall hugging) to the single peak on the axis in the transition when plasma leaves the electrode. In other words, the wall-hugging emission profile occurs when there is a conducting electrode on the other side of the glass.

When the voltage signal reaches its maximum, the PAPS is already formed and for the distances of 7 and 15 mm it is already travelling outside of the glass tube (see figure 6). For

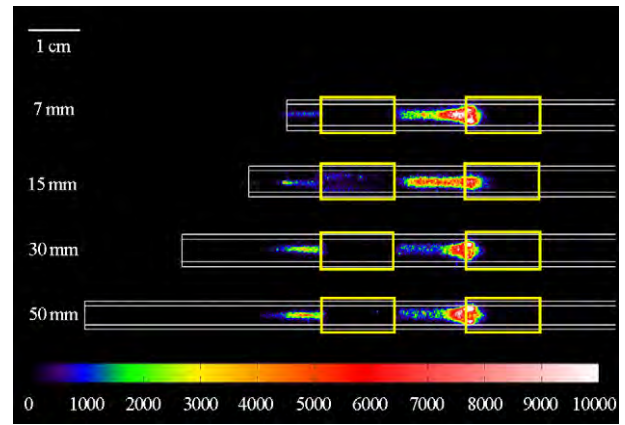


Figure 5. Time-resolved ICCD images at C ($8.0 \mu\text{s}$).

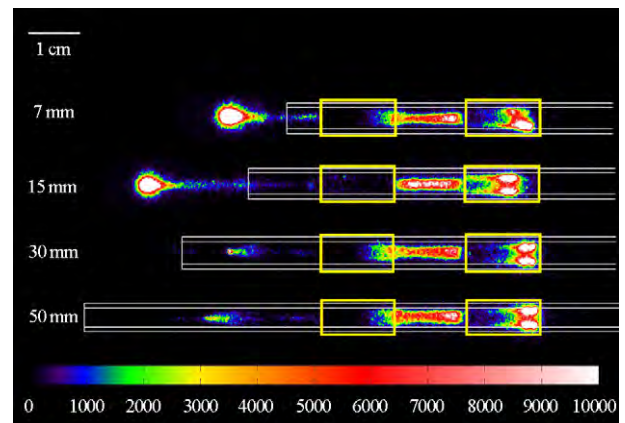


Figure 6. Time-resolved ICCD images at D ($10.4 \mu\text{s}$).

longer distances (30 and 50 mm), we only see the precursor inside the tube travelling downstream of the helium flow. It is also obvious that at the moment of departure of the plasma from the tube, it starts moving faster and becomes brighter. Whatever the explanation for the brighter glow of the plasma (Penning ionization mentioned in [40] and elaborated on in [39] or field distribution [41, 42]), the formation of the brighter region requires some time after the departure from the tube and it appears to be connected to the origin of plasma, thus supporting the streamer-like explanation. In the grounded electrode, the wall-hugging plasma propagates with varying velocities and a possible breakdown of symmetry occurs in one case.

Finally, the plasma reaches its maximum range when the voltage and current start to drop. This case is denoted by the point E ($11.2 \mu\text{s}$) in figure 2. ICCD images for this triggering time are presented in figure 7. The two bullets formed for shorter distances are progressing further away and dimming. We can see that for a distance of 30 mm, the plasma reached the edge of the glass tube with an indication of growth in brightness, whereas for 50 mm it is still inside the tube. For both these distances, the bright plasma ball (bullet) did not form as a separate plasma package outside the glass tube at any moment during the whole period of $12.5 \mu\text{s}$, although a continuous development inside the tube is observable. As for the grounded electrode, the discharge has reached its end, but it does not seem to penetrate any further.

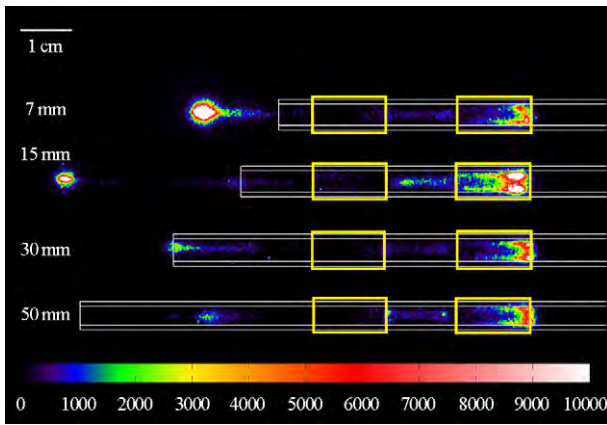


Figure 7. Time-resolved ICCD images at E (11.2 μs).

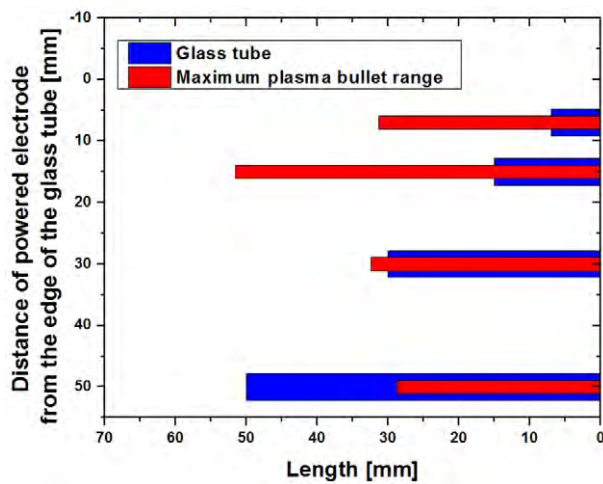


Figure 8. Maximum plasma bullet range.

The maximum ranges that plasma packages can reach are shown in figure 8. We repeat that the development of the bullet may be extended further by a pulsed dc power supply that would allow the existence of the potential gradient at the edge for a longer time. For clearer presentation, we present a histogram graph showing the maximum ranges of the plasma package and the positions of the glass tube. The maximum ranges are obtained from the corresponding ICCD images at 11.2 μs (point E in figure 2). The range is measured as the distance from the left edge of the powered electrode to the left edge of the plasma bullet. The left edge of the bullet was determined in such a way that the intensity of the measured points was not below 2000 a.u. The maximum range reached by the plasma bullet is almost the same when the distances are 7, 30 and 50 mm, and it is about 30 mm. For the distance of 15 mm, the plasma reaches a radically larger range of approximately 50 mm.

The main difference in results among all presented configurations is the maximum travelling range of PAPS. As can be seen from figure 8, the optimal distance of the powered electrode for the maximal plasma range is at 15 mm from the edge of the glass tube. Hence, this particular case will be presented in more detail.

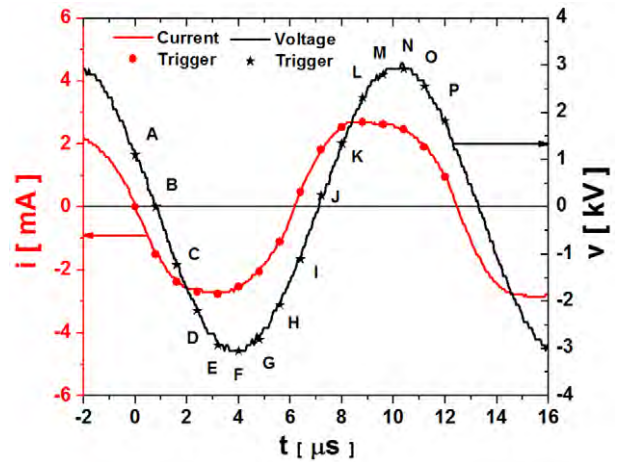


Figure 9. Current–voltage signals with trigger positions.

3.2. The optimal position of the electrodes

Current and voltage waveforms with marked triggering positions of the ICCD camera are shown in figure 9. The ICCD images (figure 10, left column) were taken for the delays from 0.0 to 12.0 μs , with a step of 0.8 μs , to provide detailed time development of the plasma jet. Each image and profile correspond to the letters (A–P) on the current–voltage signal. Axial profiles (figure 10, right column) of the plasma light emission along the glass tube axis were calculated from the obtained ICCD images. The presented profiles are calculated as a sum of the light emission coming from the plasma along the axis of the glass tube. The right edge of the grounded electrode was used as the zero distance. The position of the edge of the glass tube was at 6 cm.

In the downward slope of the current and voltage signal (A–E in figures 9 and 10), the plasma emission is mainly originating from within the electrode gap. As current reaches its maximum negative value, the discharge enters the powered electrode, taking the form of a ring shape. From the emission intensity profiles (figure 10, axial profiles A–E), we can see that the highest intensity inside the powered electrode is at the moment when the plasma is forming at the right edge of the electrode ($D = 2.4 \mu\text{s}$ in figure 10). After that moment, the plasma starts to move inside the powered electrode along the surface of the glass tube in the same direction as the helium gas flow. One should bear in mind that the transparency of the electrode film is less than 100%. With the movement of the plasma inside the powered electrode, the emission intensity is decreasing both inside the electrode and in the electrode gap (figure 10, $E = 3.2 \mu\text{s}$ to $I = 6.4 \mu\text{s}$). This coincides with the increase of the current and voltage in the negative part of the period (see figure 9).

The positive part of the period of the voltage starts with the point J in figure 9. Now the discharge is almost extinguished inside the powered electrode and the main emission originates from the electrode gap. With the increase in the voltage signal, the discharge is formed at the left edge of the grounded electrode (figure 10, $K = 8.0 \mu\text{s}$). The discharge inside the grounded electrode behaves similarly at some points as in the powered electrode. It is ring-shaped and travels through the

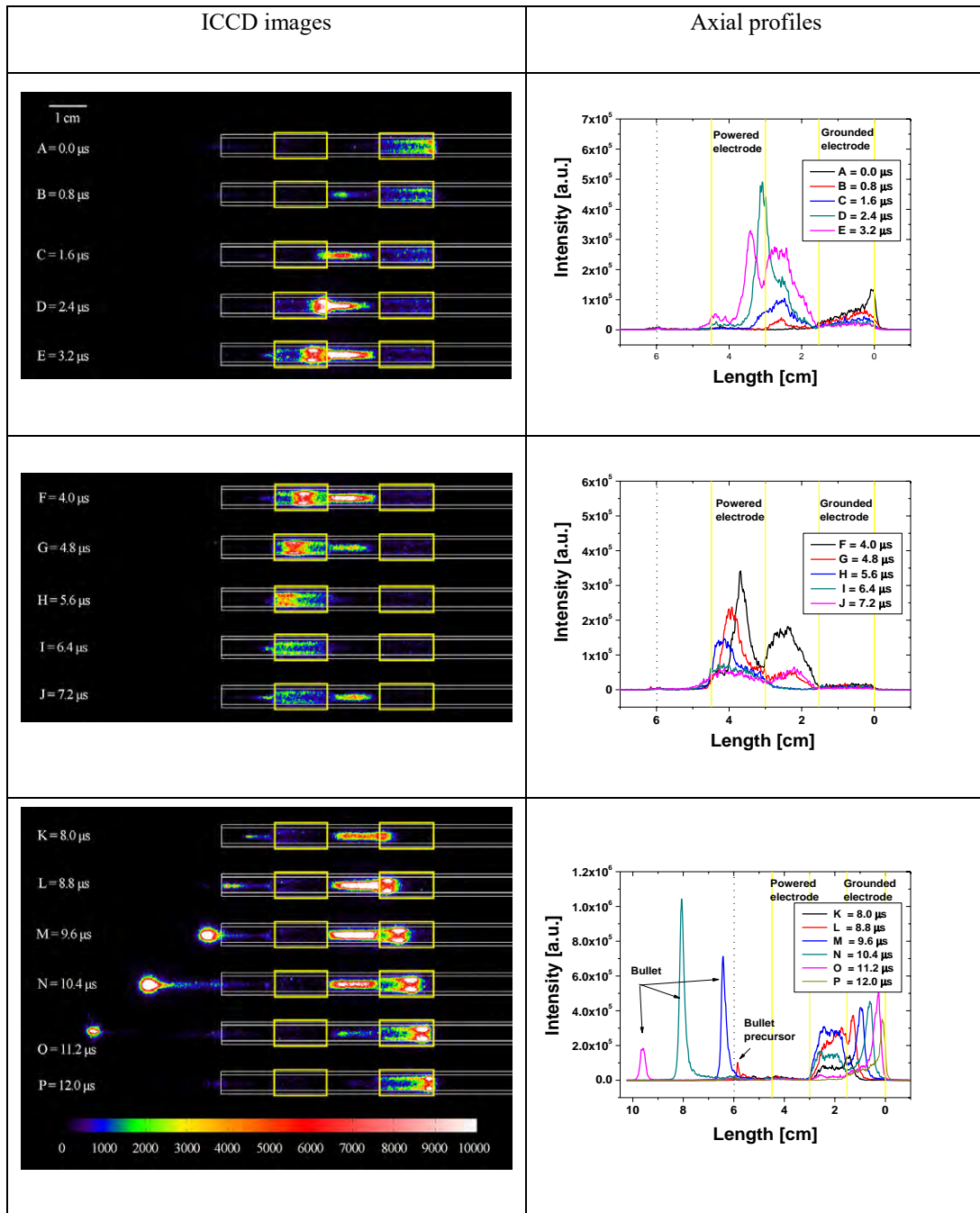


Figure 10. Time-resolved ICCD images for delays 0–12.0 μs (left) and axial light emission profiles for delays 0.0–12.0 μs (right), for 4 slm and power of 4 W.

electrode along the wall of the glass tube. The differences are that in the case of the grounded electrode, the discharge moves contrary to the gas flow and the maximal intensity of the discharge profile increases with the movement (figure 10, K–P axial profiles). In the case of both electrodes, the maximum emission intensity is always at the edge that is further away from the edge of the glass tube. At the same time, when the discharge enters the grounded electrode, a part of the discharge starts to travel away from the powered electrode and towards the exit of the tube (figure 10, $K = 8 \mu\text{s}$). While it travels through the glass tube, we can see that the highest emission is originating from the head of the formed plasma with a low-

intensity tail behind (figure 10, K, L images and profiles). We could say that a precursor is formed which will turn into the high-speed travelling package of plasma when it leaves the glass tube. Only upon exiting the glass tube, the plasma significantly expands in volume and the light intensity rises by several orders of magnitude. It appears that the main reason for a fast increase in the emission and volume of the plasma is the contact of excited helium atoms and metastables with molecules of nitrogen and oxygen from the ambient air [38, 39]. However, it appears that the field distribution outside the tube provides a greater potential drop at the front and thus increases ionization considerably.

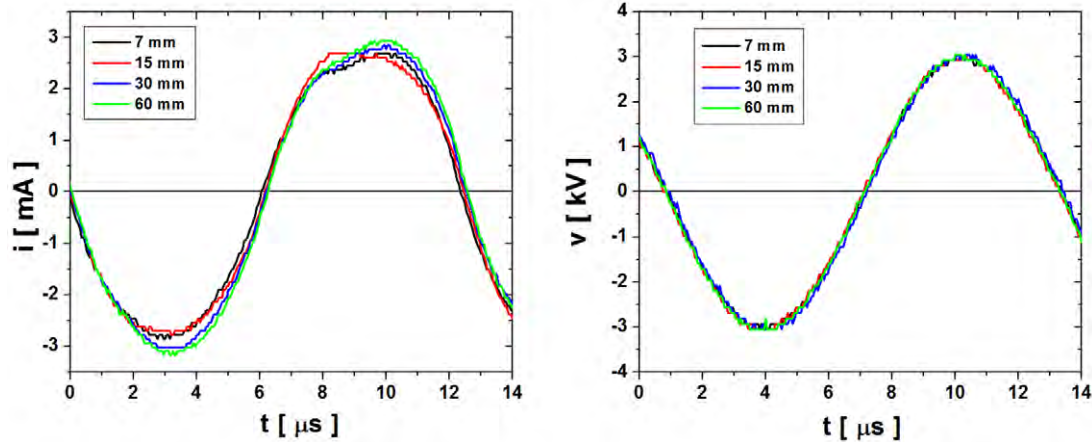


Figure 11. Comparison of current (left) and voltage (right) waveforms for different distances between the electrode and the edge for the glass tube. The flow of the helium was 4 slm.

The propagation of the plasma package in the open air can be explained as propagation of a positive streamer [19, 34]. The peak emission intensity of the propagating streamer is obtained for the delay of $N = 10.4 \mu\text{s}$. We can see that the highest intensity position corresponds to one half of the maximum distance travelled by the plasma. At this point, it is clearly seen that the ionization front is still connected to the main discharge inside the jet tube with a thin, hardly visible and apparently conductive, tail (figure 10, N). With further movement of the ionization front, this conductive tail is diminishing, accompanied by the diminishing intensity of the emission coming from the travelling plasma package. When the ionization front is at the maximal distance from the tube, the emission intensity is low and the plasma tail is thin. We have to point out that from the moment when the plasma package leaves the glass tube and enters surrounding air until the extinguishing of the package, the package appears to be constantly connected to the main discharge inside the tube with a long, streamer-like plasma tail.

The main difference that we have observed between the 15 mm configuration and other configurations is in the current waveform. It is smaller in amplitude during the negative part of the waveform compared to the signal for the other three configurations (see figure 11). Also, the positive peak of this waveform leads the other peaks (obtained for 7, 30 and 50 mm). This difference can be seen in more detail in the frequency domain. For the case of 15 mm, the 2nd and 4th harmonics are smaller in intensity compared to the same harmonics for other configurations. We can see that there is no significant difference between the voltage signals for different configurations. The main conclusion that can be drawn here is that the characteristics of the plasma jet and the plasma packages are influenced by the configuration of the electrodes, i.e., their dimensions and the distance from the mixing point of working noble gas with air. Also, the same system can be tuned by adjusting the electrode configuration with no need to change any component in the power supply system.

3.3. Velocities

The velocities of the emission peaks were determined (calculated from the ICCD spatial emission profiles at different

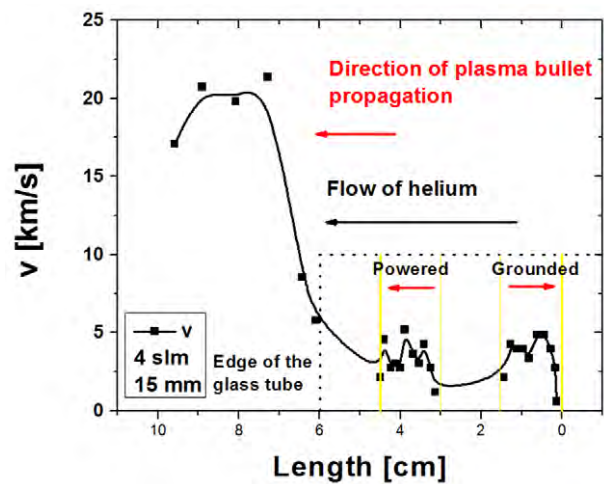


Figure 12. Plasma velocity inside and outside glass tube.

times) for the propagation of plasma through the powered, grounded electrode and outside the glass tube (figure 12). The velocity of the ionization front in the powered electrode starts to rise from the right edge of the electrode. At about 4 mm from the edge it reaches the speed of 4 km s^{-1} . The velocity is almost constant until the ionization front reaches the left edge of the electrode. The propagation direction of the ionization wave is downstream of the helium flow. When the electrode is grounded, the ionization wave starts to accelerate from the left edge of the electrode and reaches the speed of 4 km s^{-1} at 2 mm from the edge of the electrode. The velocity is almost constant until the plasma reaches the centre of the electrode. Thereafter, it starts to accelerate again and reaches the maximal speed of 5 km s^{-1} . Near the edge of the grounded electrode, the ionization front starts to rapidly slow down. The propagation of the ionization wave inside the grounded electrode is upstream of the helium flow. We have performed several experiments where we varied the helium flow while keeping the electrode geometry constant. These results are not in the scope of this paper. We have seen that helium flow does not influence the propagation velocity of PAPS outside the glass tube, but greatly influences its range.

Velocity outside the glass tube rises from the edge of the glass tube and reaches its maximum of approximately 20 km s^{-1} at the distance approximately 15 mm from the edge of the glass tube. The velocity is constant from 15 to 30 mm and thereafter it starts decreasing. The larger velocities outside the glass tube than inside may be due to the changes in the composition of the medium through which the plasma propagates. In the tube, we have pure helium, and outside the tube components of air diffuse into the stream of helium that leads to new and complex reactions in the plasma. Nevertheless, studies like this one, and quantitative comparison of models with the experimental data may be the best way to investigate these plasmas.

4. Conclusion

In this paper, we have shown results of optical emission spectroscopy for four different configurations of a plasma jet. In all configurations, the distance between the powered and grounded electrode was kept constant, while the distance of the electrodes from the edge of the glass tube was varied. Measurements were taken for distances of 7, 15, 30 and 50 mm from the edge of the glass tube.

Several conclusions can be drawn from the presented data that can be applied to all electrode configurations:

- while inside the electrodes, the plasma has a ring-like shape and it is mainly travelling along the walls of the glass tube;
- inside the glass tube (not in the electrode region) plasma is confined to the central axis of the tube and the main emission originates from that volume;
- only when the plasma comes into contact with the ambient air does the volume of discharge increase and the plasma form a sphere-like shape before it starts to travel in the open air;
- in all cases when PAPS is formed, we have seen that it has a trail of very small emission that ‘connects’ it with the main discharge inside the tube; the connection through the weakly lit plasma to the point of origin resembles streamer-like propagation and is consistent with proposed theoretical explanations.

The main difference between the configurations used in these experiments occurred in the maximum range that PAPS can reach before extinction. For the distances of 7 and 15 mm, PAPS is formed and it starts travelling outside the glass tube. In the case of 30 mm, the plasma reaches the edge of the tube, starts to increase in volume, but does not detach from the tube in order to start travelling in the open air. For 50 mm, the plasma stays inside the tube throughout the whole period of $12.5 \mu\text{s}$. Nevertheless, even if plasma does not leave the glass tube, the maximum distance that it can reach is around 30 mm, which corresponds to the ranges obtained for 7 and 30 mm. Only in the case when electrodes are 15 mm away from the edge of the glass tube, is the maximum travelling range of PAPS 50 mm. However, the travelling speed of PAPS for all configurations is almost the same and in open air it reaches about 20 km s^{-1} (for the 15 mm case, and 17 km s^{-1} for the 7 mm case). One should

bear in mind that we can measure the velocity only when the PAPS departs from the edge of the tube. It appears that the propagation of the plasma PAPS for the 7 mm case is slow when the plasma leaves the tube and forms the bullet, which could be related to the distribution of the electric field beyond the edge of the tube.

One could argue that the distribution of the field along the glass tube beyond the powered electrode is a key consideration in establishing the range of plasma bullets. In addition, one should consider that for 15 mm the transit time allows the plasma to leave the tube just when the voltage waveform goes through the maximum at a phase of 1.5π . All of these aspects, and other considerations, may be interpreted as the basis for an explanation within some plasma model that is sufficiently detailed and comprehensive. We hope that our results will promote and support such an analysis and its conclusions.

Acknowledgments

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Electrical and optical characterization of an atmospheric pressure, uniform, large-area processing, dielectric barrier discharge

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Abstract

A printed-circuit-board (PCB) based atmospheric pressure dielectric barrier discharge (DBD) capable of uniform processing over a large area was constructed consisting of two parallel plates. The first perforated plate is comprised of four layers: a RF powered metal layer, a polymeric dielectric layer, a floating metal grid and another dielectric layer. The second, grounded, plate was fluorine doped tin oxide (FTO) glass plate with surface of $100 \times 100 \text{ mm}^2$ and thickness of 2 mm. The PCB based atmospheric pressure DBD was characterized by (a) measuring electrical characteristics of the device using derivative $I-V$ probes, (b) ICCD imaging and (c) optical emission spectroscopy (OES). Optical and electrical characteristics, as well as plasma uniformity were measured by changing He flow rate and input power, while keeping the gap between the PCB and the FTO glass plate ground electrode constant at 2 mm. The plasma uniformity strongly depends on the applied power and on the flow rate of the buffer gas. When increasing the flow rate, the intensity of the nitrogen-dominated emission drops, while emission of helium and oxygen lines increases. The source allows low temperature, uniform plasma operation over a wide area of $100 \times 100 \text{ mm}^2$, which could be essential for numerous applications. Examples of etching rate and hydrophilization are demonstrated.

Keywords: optical emission spectroscopy, ICCD Imaging, plasma etching, hydrophilicity, dielectric barrier discharge, atmospheric pressure plasmas, RF plasmas

(Some figures may appear in colour only in the online journal)

1. Introduction

Atmospheric pressure plasmas have become a key emerging technology especially in the field of surface treatment of solids or liquids, both in industry and in research laboratories. One of their big advantages is their potential lower capital cost, when compared to vacuum plasma equipment, although operating (e.g. gas) costs have also to be considered. Several state of the art plasma sources have been designed for this purpose, such as atmospheric pressure plasma jets (APPJ)

and dielectric barrier discharges (DBDs) [1–16]. The most broadly used DBD designs consist of two parallel metal electrodes covered by a dielectric layer. The plasma is either a stable glow discharge or a filamentary discharge depending on working conditions [18–20].

Examples of the industrial use of DBDs include particle matter (PM) removal in catalysts for the car industry [21] and material surface modification/activation [8, 17]. The latter however requires uniform processing over large areas and, in this case, filamentary type of discharges should be avoided,

especially when soft materials are being processed. Typically, jet DBDs operating at kHz and MHz frequencies may process samples of the order of a few mm², which makes them useful for localized applications. Therefore, to address the need for large area processing of materials, a specific type of atmospheric plasma equipment has been designed and tested, such as roll to roll that makes use of cylindrical DBD sources, where one of the electrodes is a rotating metallic cylinder covered by a dielectric [22].

Characterization of the electrical and optical properties of generated plasma from these devices is a challenge. One technique for the electrical characterization is the use of derivative probes [23, 24], which provide crucial information regarding electrical properties such as Voltage, Current, Impedance and in general stability and discharge characteristics of the source. A further analysis can be achieved by making use of optical emission spectroscopy (OES), for detection of excited species and monitoring of plasma composition, especially regarding the role of each species in material processing [25–31].

We have recently presented a novel design of atmospheric pressure DBD that is based on printed circuit board (PCB) and is capable of large area operation [32, 33]. We have shown that the existence of a metallic grid in the PCB promotes plasma excitation uniformity, and stability and leads to higher processing rates. PCBs are used in electronics, and are a cheap and reliable source of materials for cost-effective manufacturing of the source.

In this work, we move a step further in the detailed characterization of the source in terms of electrical and optical properties of helium plasma. The aim of our work is to optimize plasma process focusing on the discharge uniformity and large-area operation and to confirm the scaling-up potential of our plasma source (from lab-scale to pilot-line). The characterization of the discharge is performed by using optical imaging via a new implementation of the PCB source with a bottom conductive glass electrode, OES and electrical measurements while varying helium flow rate and RF power. We perform measurements here with a variation of our design with respect to our first publication [32] by using a transparent ground electrode which allows us to quantify the optical characteristics of the discharge such as emission intensity, surface coverage and uniformity over areas of 80 × 80 mm². We use OES to quantitatively measure the emission of molecular nitrogen, helium and atomic oxygen line intensities versus helium flow rate.

2. Experimental setup

A schematic of the experimental setup is presented in figure 1. In figure 1(a) we show vertical cross section of the plasma source which is composed of two plane parallel electrodes with dielectric layers facing each other. The upper powered electrode (antenna) is a square-shaped (100 × 100 mm²) multilayer PCB with 2 mm diameter holes drilled equidistantly. The multilayer perforated PCB consists of 80 μm thick copper plate, a 1 mm thick dielectric material (FR4) and an 80 μm thick copper grid on the other side. From below, the

grid is uniformly coated with an additional 1 mm thick second dielectric material (poly-dimethyl siloxane—PDMS) [32, 33]. For all measurements presented in this paper, the source configuration includes a floating grid, while the lower grounded electrode, which serves as sample holder, is fluorine doped tin oxide (FTO) coated glass.

This is different from our recently published paper with a similar source [32] where, apart from the configuration with the floating grid, we also analyzed configurations of the source with a powered grid and without the grid. Moreover, in that paper a 50 mm × 50 mm PCB plate served as grounded second electrode. Since the surface and the type of the second electrode are crucial for the I - V characteristics of the atmospheric DBD plasma reactor, changing these characteristics with respect to [32] will partially alter electrical properties of the source.

In figure 1(b) we show the source with schematics of electrical and detection parts of the setup. The perforated PCB is fixed at the bottom of a Plexiglas box with a hole for gas inlet at the top side. This way, uniform gas flow through all PCB holes is achieved. The powered electrode is connected through an L-type custom-made matching network to a 13.56 MHz power supply. In all experiments we had reflected power set to the minimal value and kept below 3% of the forwarded power. This was set before the measurements and we did not need to change the settings of the matching network afterwards during the measurements. Therefore, the L - C values of the matching network did not change. All this implies that the DBD system is well balanced and its design gives close to optimal values when it comes to matching it to the power supply. The gap between electrodes was set at 2 mm. Pure helium was used as buffer gas at flow rates from 1 to 7 standard liters per minute (slm).

For the electrical characterization of the source, derivative probes were used to obtain voltage and current waveforms at the powered electrode. The voltage probe was a capacitive T-shaped probe and the current probe was an inductive Π-shaped probe, both placed inside a stainless-steel box which was connected in-line between the plasma source and the matching network [23, 24]. The V - I waveforms were recorded for different helium flow rates and power values using a 3000-X series digital oscilloscope from Agilent Technologies and then fast Fourier transform (FFT) was performed to obtain the voltage and current RMS values along with the total impedance of the source and the power dissipation (P_d). Power dissipation, voltage and current values V , I and phase difference φ were calculated using the waveform analysis software of the derivative probes. Due to the probe position, calculated dissipated power is not influenced by power losses from the cable and from the passive components of matching network and therefore represents only the power delivered to the DBD source.

For plasma imaging, we used an Andor ICCD camera iStar DH734I equipped with photographic lens NIKKOR 50 mm f/1.4 AIS [34]. Since electrodes were in horizontal position, a mirror was situated under the lower transparent FTO electrode reflecting the plasma emission to the camera. In addition to the surface plasma imaging described above, side on

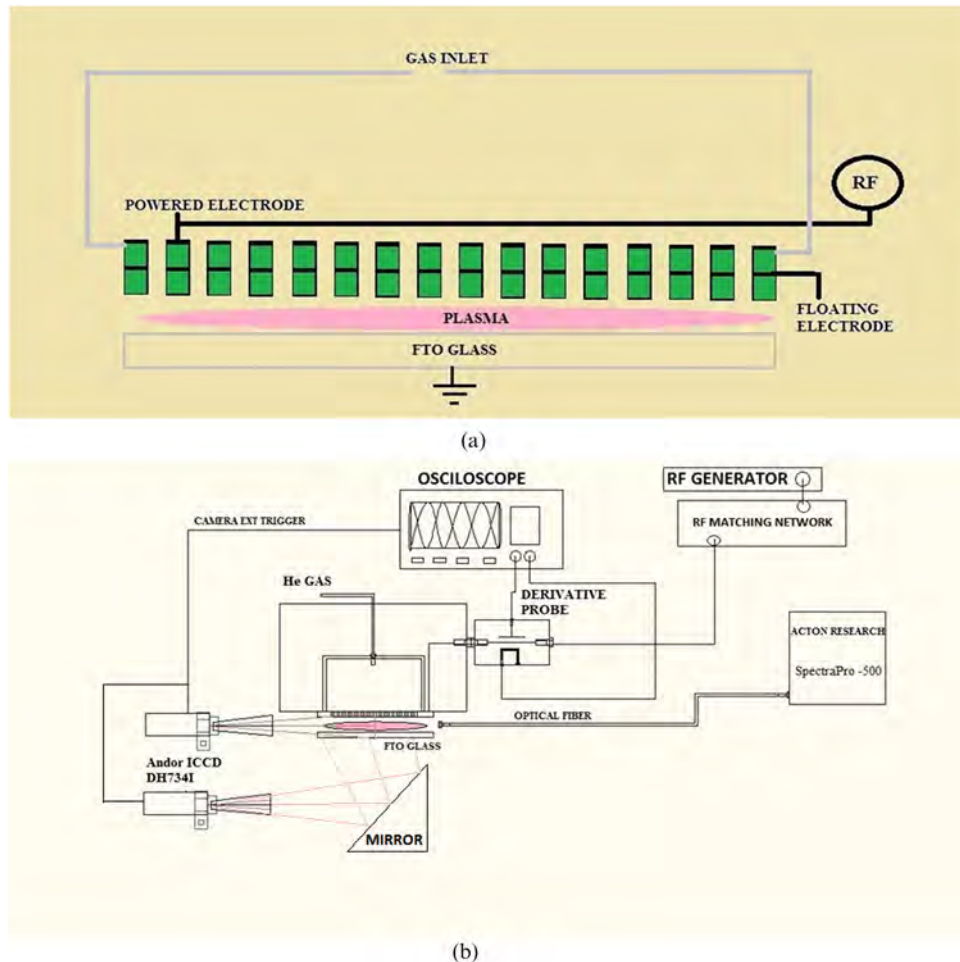


Figure 1. (a) Experimental setup of the perforated DBD source of plasma intensity and (b) vertical cross section of the perforated novel PCB source with power and ground connections.

capturing of the plasma intensity was performed. Thus, we obtained images spectrally integrated in the visible spectral range. As the exposure time was in the order of several hundred μs , obtained images were time integrated observation of discharge emission from many RF periods. The objective was transparent for the light above 375 nm.

For the OES, an Acton Research Corporation SpectraPro-500 spectrograph with a ICCD camera was used. Plasma emission was guided by a fiber, positioned in the middle of the electrode gap, to an entrance slit of the spectrograph. Thus, the fiber collected light coming from a wide angle making the observed emission integrated in space, over the whole acceptance angle. These measurements were also time-integrated since they were taken with 5 s exposure time while the spectrally resolved emission from the plasma was recorded in a wide interval of wavelengths from 300 nm up to 800 nm in steps of 10 nm. A VIS-NIR 1000 μm optical fiber with reduced emission below 360 nm and stable response above 380 nm was used.

Poly-methyl methacrylate (PMMA) films spin coated on silicon wafers, were used for plasma etching. Samples of size approximately $2\text{ cm} \times 2\text{ cm}$ were placed on the FTO electrode, and etching was performed for 120 s. A mixture of He/Air was used as etching gas, varying He flow rates at 2, 4 and 6 slm,

while keeping the power constant at 100 W using a 13.56 MHz RF generator. A Woolam M2000 Spectroscopic ellipsometer was used for the *ex situ* measurements of PMMA film thickness before and after the plasma etching in order to determine the etching rate. Our new results show the important role of He flow rate, and are consistent with our first observations [32] using a constant He flow rate, and a PCB based bottom electrode. The high etching rates of this source have been used to etch paper and subsequently render it superhydrophobic [35].

3. Results and discussion

3.1. Electrical measurements

Electrical properties of the DBD source were obtained for different power values and helium flow rates when plasma is present. For each condition, a set of I - V curves was recorded using derivative probes, followed by calculation of the dissipated power and the impedance in the circuit during the plasma-on period. A power range from plasma ignition up to 120 Watt was studied for He flows 1, 2, 4, 6 and 7 slm. Figures 2(a)–(d) show respectively the RMS voltage, current, impedance and impedance phase obtained versus the dissipated power. In figure 2(b), current–voltage characteristics

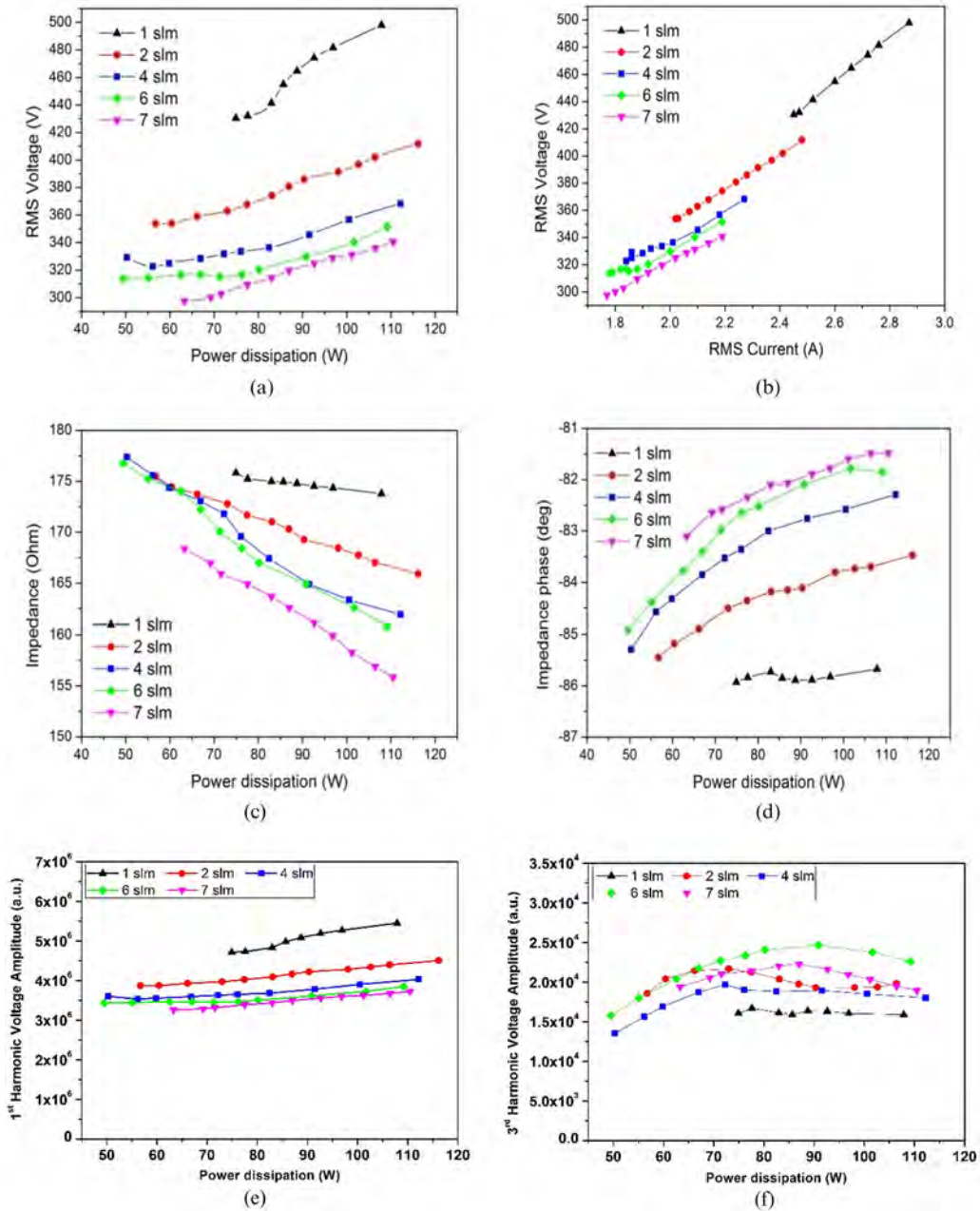


Figure 2. Electrical measurements of perforated DBD source: (a) voltage versus dissipated power and He flow, (b) current change versus dissipated power and He flow, (c) impedance behavior (source + plasma) with dissipated power and He flow change, (d) phase change with the variation of flow and power, (e) 1st harmonic (13.56 MHz) change with dissipated power and He flow and (f) 3rd harmonic (40.68 MHz) change with the variation of dissipated power and He flow.

obtained for different He flows are shown. In figures 2(e) and (f) we present changes in the voltage amplitude of the 1st and the 3rd harmonic as a function of dissipated power and different flows.

In figure 2(a) we can clearly see the effect of He flow on the ignition voltage for the same input power. Higher He flow results in lower ignition voltages. We also observe an increase of voltage with an increase of the dissipated power. Similar behavior is observed in the case of voltage variation with current, as shown in figure 2(b). From these two graphs, the amount of He has a significant effect on the discharge working point. Namely, higher He flow rate results in an increase of He percentage in-between electrodes, lowering

operating voltage of the plasma at a certain power. For the highest dissipated power of 110 W the difference in operating voltages between maximum and minimum flow rate is around 170 V. Additionally, current–voltage characteristic, shown in figure 2(b), indicate that current range also decreases with an increase of the flow rate. Therefore, at certain power forwarded to the source the discharge will operate at a lower current when the gas flow rate is higher.

As expected, from the voltage and current curves and assuming that the impedance of the source is constant, in figure 2(c) we see that the absolute values of the plasma impedance decreases both with increasing He flow rate and input power as a consequence of higher efficiency of the discharge

due to the presence of He [36], indicating enhanced electron density in the plasma. In figure 2(d) we present the phase difference between the voltage and current signals (related to the imaginary component of the impedance) as a function of both He flow rate and input power. The negative sign of the phase difference indicates a capacitive behavior of the source that decreases slightly with an increasing input power. On the other hand, the increasing He flow rate has a more important effect on the impedance phase, leading to lower phase difference values, i.e. less capacitive behavior of the DBD source.

Plasmas are in general highly asymmetric systems and as a result this influences the appearance of a number of higher harmonics. The amplitudes of signals at higher harmonic frequencies, other than the main operating frequency, are indicators of the asymmetry of the system. Design of the source i.e. its construction will govern the number of harmonics that will appear. The number and intensity of harmonics are sensitive to impedance of the outer electrical circuit (matching box, cables and connectors) [37, 38]. In our case, for all He flow rates and all dissipated powers, FFT analysis showed an appearance of only the 3rd harmonic (40.68 MHz), which is two orders of magnitude smaller than the 1st harmonic (13.56 MHz). In figures 2(e) and (f) amplitudes extracted from the 1st and the 3rd harmonic after FFT analysis of the voltage signal are presented. The amplitude of the 1st harmonic increases with power. However, a decrease of the amplitude with an increase of the He flow is observed for all power levels. On the other hand, there is almost no change of the 3rd harmonic for all He flow rates and powers. This implies that asymmetry of our plasma system is not highly pronounced and that system itself is well balanced.

3.2. ICCD imaging

Recording of discharge images by ICCD camera was used to study plasma coverage of the electrode surface and it allowed us to establish optimal conditions for achieving uniform plasma operation over the entire electrode area. Measurements were performed for different He flow rates (1–7 slm) and for different dissipated power values. The end-on images obtained by using ICCD camera are presented in figures 3 and 4.

In figures 3(a)–(f) we show images taken at 7 slm of helium flow, for powers starting from ignition of the plasma at 63.3 W up to the maximum dissipated power of 110.5 W. We can clearly see that the increase of power causes the plasma to spread over the whole electrode area. The emission intensity coming from the plasma also increases. In figures 3(f)–(l) we present images obtained when the dissipated power decreases having the same values as in figures 3(a)–(f). For example, the ICCD image in figure 3(g) was obtained for the same value of dissipated power as figure 3(e). The lowest dissipated power, where we could sustain plasma discharge was 51.8 W, and with further decrease of power plasma was turned off. This power value is lower than the one needed for the discharge ignition, demonstrating a hysteresis effect of the plasma and confirming that the ignition power is always higher than the power needed to sustain plasma. As the dissipated power decreases, at certain power values, the discharge coverage

shown in figures 3(g)–(l) was slightly different (compared with the images figure 3(a)–(e)) with the emission intensity decreasing more rapidly with power. The appearance of the dark patches distributed almost randomly across the electrode surface could be observed as lower power was fed to the plasma. It appears that plasma is switching off at some local channels before other even neighboring channels. This all may be due to different conditions at the surface or slightly different distance of not perfectly parallel electrodes.

In figures 4(a)–(e) we show the effect of He flow rate on uniformity and emission intensity of the plasma. The experiments were performed at 120 W input power varying He flow rate from 1 up to 7 slm. As the discharge, current varies with the flow change, dissipated power is changing for different flows. We observe that full coverage of the electrode is obtained for He flow values of 2 slm and above (figures 4(b)–(e)). Moreover, as He flow rate increases the intensity of plasma emission drops. The main reason for this drop is the reduction of the percentage of air in the air/He mixture, which is further analyzed in section 3.3.

In figures 5(a)–(c) we present side-on images of the PCB based atmospheric pressure DBD device for three different flow rates. The experiments were performed at 120 W input power by using He flow rates of 2, 4 and 7 slm. We observed that when He flow rate increases the plasma intensity across the inter-electrode gap decreases. It is also clearly seen that there is a small convergence between the plates leading to a higher distance in the right side of the board. This may be due to lack of perfect flatness of the PCB boards, or not perfectly equal spacers separating the two PCB boards. Moreover, this differentiation of distance leads to a gradual increase of the plasma intensity and an increase on the plasma variance, as one moves from the left side to the right side. The increase in the emission intensity could be explained by the higher amount of surrounding air in the admixture.

To evaluate optimum conditions when full plasma coverage of the electrodes is achieved, a more comprehensive analysis was performed. A software code was developed for automatic image processing to find the mean intensity, intensity variation (error bar of the mean intensity) and the size of plasma covered area from the discharge images. This way we performed quantification of the plasma uniformity versus RF power as well as the effect of power on the mean plasma emission intensity.

In figure 6(a) we present the percentage of the DBD surface area covered by plasma as a function of nominal power forwarded from the power supply unit for He flow rate of 2, 4, 6 and 7 slm. The electrode part is considered as covered by plasma if the intensity level of that part of the image is above 92% of the maximum intensity in the image. Then, all such areas are summed and compared to the total electrode area. Herein we consider as electrode area only the area covered by holes (showerhead) which is equal to $80 \times 80 \text{ mm}^2$ and not the entire electrode area ($100 \times 100 \text{ mm}^2$) as the edge effects may affect significantly the measurements. We see in the figure that for all flows, as power increases, plasma spreads over the surface of the DBD and eventually covers the whole surface. The area covered by plasma between 92%

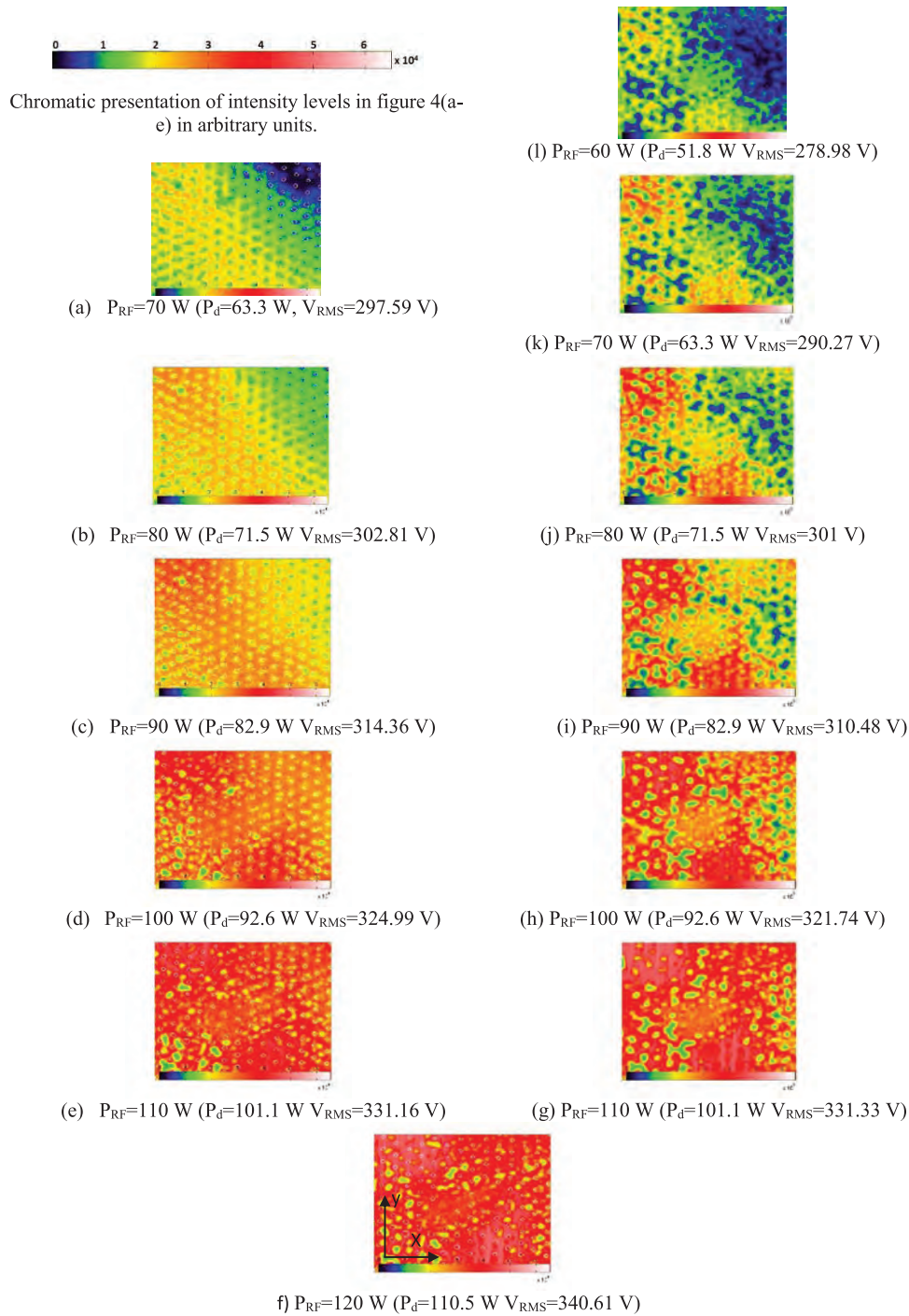


Figure 3. ICCD images of atmospheric pressure DBD operating with 7 slm of He for different RF input powers (P_{RF}). Calculated dissipation powers (P_d) and measured RMS voltages are shown also. Images (a)–(f) taken with power increase and (g)–(l) with power decrease. Chromatic presentation of intensity levels in images is in arbitrary units.

and 100% is defined as full coverage. At higher He flow rates full coverage is reached at lower power levels. Namely, at 7 slm full coverage is reached from 75 Watts, while at 2 slm it is achieved at a power level above 110 Watts. Nevertheless, we observed that even with the lowest flow (2 slm) conditions, full plasma coverage can be achieved.

In figure 6(b) we present the mean plasma emission intensity measured at 2, 4, 6 and 7 slm as a function of the nominal power for cases when the area covered by the plasma was larger than 92% i.e. with full coverage. The mean intensity values are

calculated by dividing the sum of all pixel intensities by the pixel number (240×240 pixels). As expected, the mean intensity increases with power for all He flow rates. The intensity for 2 slm is the highest while for higher He flow rates the intensity drops.

To quantify in more detail the uniformity of the plasma on the PCB, we calculated the mean pixel intensity and its standard deviation for each column along the X direction for figure 3(f), corresponding to typical operating conditions of 7 slm He and 120 W. In figure 6(c) we show the plasma variation obtained from such averaged emission intensity data

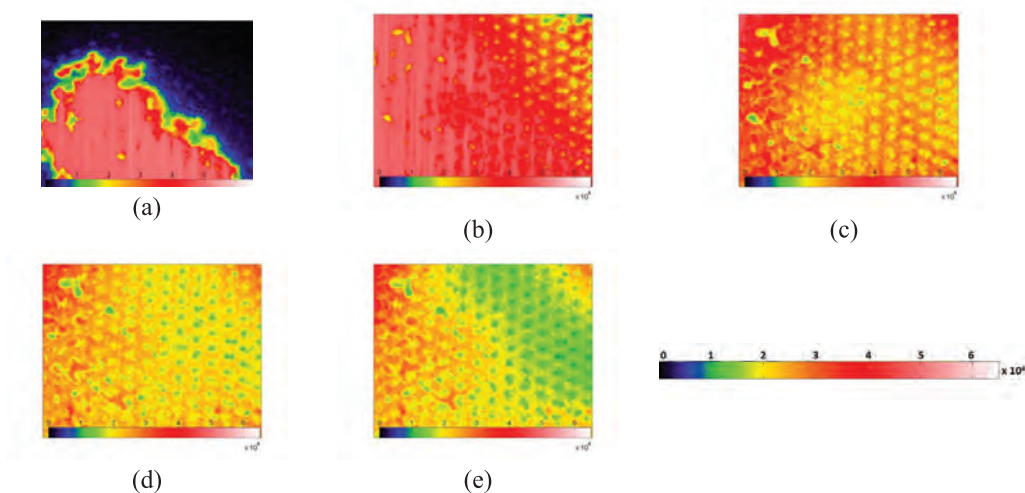


Figure 4. ICCD images of atmospheric pressure DBD for different helium flow rates at constant nominal power of the RF power 120 W. Dissipated power is indicated in parenthesis: (a) 1 slm He (109.8 W), (b) 2 slm He (108.3 W), (c) 4 slm He (110.1 W), (d) 6 slm He (111.9 W) and (e) 7 slm He (110.5 W). Chromatic presentation of intensity levels in (a)–(e) in arbitrary units.

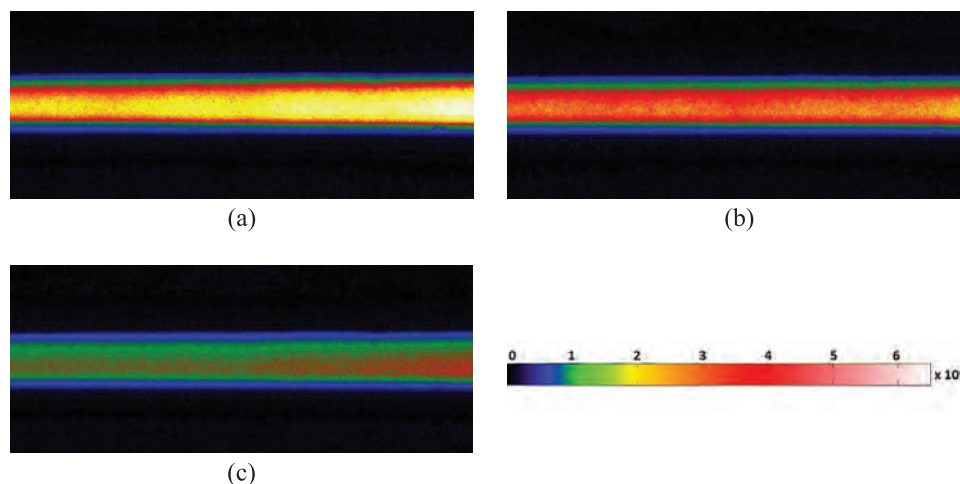


Figure 5. ICCD images of atmospheric pressure DBD for different He flow rates at constant nominal power of the RF power 120 W. Dissipated power is indicated in parenthesis: (a) 2 slm He (108.3 W), (b) 4 slm He (110.1 W) and (c) 7 slm He (110.5 W). Chromatic presentation of intensity levels in (a)–(c) in arbitrary units.

(from figure 3(f)) along the 240 columns in the *X* direction (i.e. from left to right). In figure 6(d) we plot again these data to show the uniformity of the plasma along the *X* direction. The uniformity (in %) is calculated as $(1 - ((\text{standard deviation}) / (\text{mean Intensity}))) * 100$. The uniformity variation shown is due to the showerhead-type of the powered electrode. The holes on the PCB cause small local emission intensity variations, i.e. slightly lower plasma emission intensity in the position of the holes compared to the position in between the holes. Nonetheless, the level of plasma uniformity stays almost constant in a wide range of operating power, especially for higher He flow. This feature of the DBD source is very important for homogeneous treatments of large area samples.

3.3. Optical emission spectroscopy

In figure 7 we present emission spectra obtained at 100 Watt nominal power obtained for a wide range of wavelengths between 300nm and 800nm. The atomic O, the first negative

system (FNS) of N₂ ion as well as the second positive system (SPS) of N₂ molecular transition lines are visible in the spectrum. For He there are no excitation thresholds below 19eV, and therefore most of the excitation and ionization is due to oxygen and nitrogen. Even at 19eV it is the metastable levels of He that would quickly transfer excitation to FNS lines of nitrogen and equivalent levels of oxygen. We also observe in figure 7 electron energy levels from He 707 line, i.e. from electrons with energy higher than 22eV.

The results of optical emission spectrometry (OES) obtained for different power values and flow rates are presented in figure 8: we show the total emission intensity of the plasma recorded by the spectrometer in the range between 380 to 800 nm, the intensities of atomic O at 777 nm, He at 707 nm and the intensity of (0–2) SPS N₂ molecular transition line (at 380 nm). We only present the 380 nm SPS N₂ line due to our optical fiber limitations, which reduces the emission below 360nm and has a stable response above 380 nm. The line intensities are corrected for the efficiency

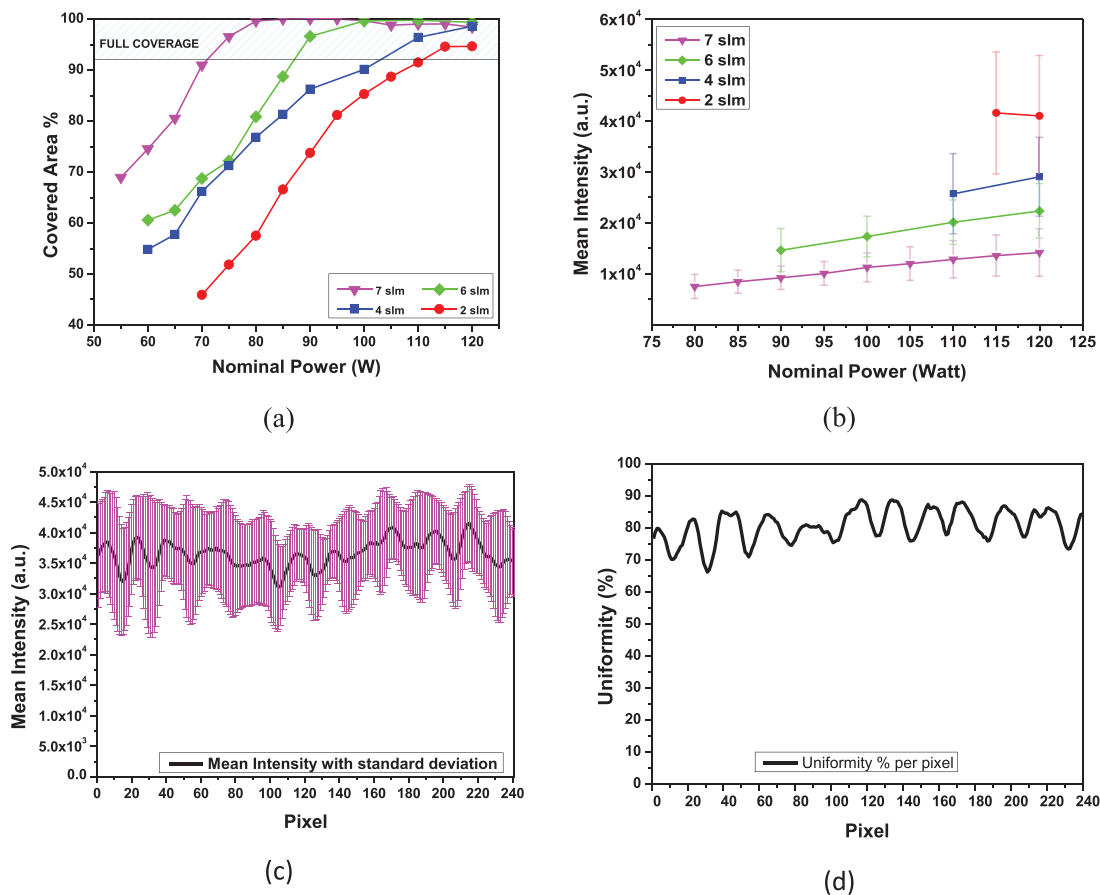


Figure 6. Results of quantitative analysis of ICCD images obtained for 2, 4, 6 and 7 slm of He. (a) Effect of power on plasma coverage of the DBD surface. (b) Effect of nominal power on the mean plasma intensity and its variation (c) column average emission intensity and its standard deviation along the X direction (i.e. from left to right), for figure 3(f). (d) Data of (c), plotted again as uniformity along the X direction.

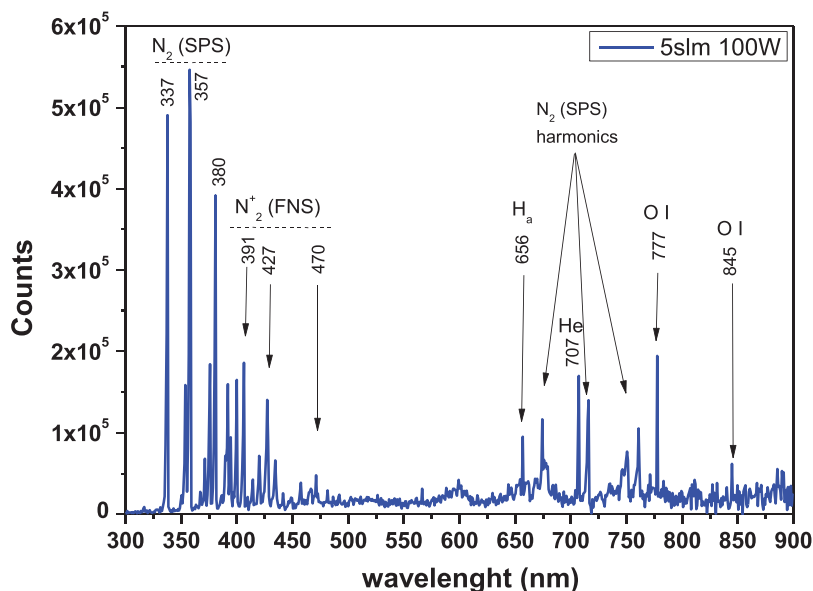


Figure 7. Optical emission spectroscopy of atmospheric pressure DBD at helium flow rate 5 slm and nominal power 100 W.

of the optical system using a curve obtained from the supplier, Princeton Instruments. However, correction due to the optical fiber limited response below 360 nm has not been applied.

In figure 8(a) left Y axis we can see the effect of He flow rate on the emission intensities when applying a constant 100 W input power. As expected, the He flow rate increase results in increase of the He 707 nm line intensity due to the higher

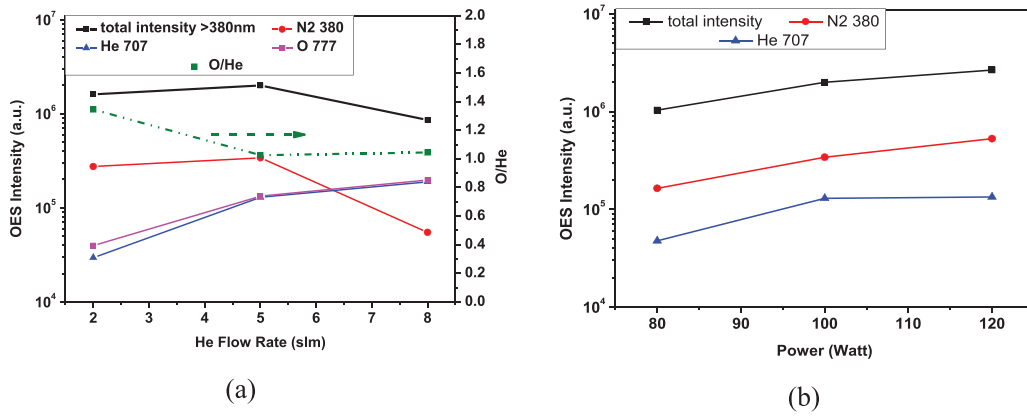


Figure 8. Effect of He flow rate and power to the total emission intensity (380–800 nm), intensity of O 777 nm, He 707 nm and SPS 380 nm N₂ band line. Atmospheric pressure DBD with (a) nominal power 100 W, (b) He flow rate 5 slm.

percentage of He in the He-surrounding air gas mixture. The emission intensity can be calculated as a function of the gas density, excitation coefficient and electron density. The emission of Oxygen also increases, although we expect a slight drop of its density at high flowrates. The increased Oxygen emission is probably due to higher electron density and/or higher excitation coefficient (due to higher electron temperature); such increases may offset any decrease of Oxygen density [39]. If we assume that the excitation coefficient ratio of He at 707 nm and O at 777.2 nm remains almost constant with flowrate, we can estimate the effect He flow rate on O density, by taking the emission intensity ratio of O/He (see figure 8(a) right Y axis). We observe that the ratio of intensities decreases, and this is an indication of reduced oxygen atom density at higher flowrates. Similar effects have been observed in He–O₂ mixtures [25, 40]. As we will see below this reduction of the Oxygen atom density is consistent with the reduction of the etching rate at high flowrates.

Contrary to He and Oxygen emissions, the N₂ 380 nm intensity drops almost one order of magnitude as He flow changes from 2 slm to 8 slm and smaller amount of air is present between electrodes. Such behavior of total emission and N₂ 380 line intensities suggests that dominant part of the spectrally integrated emission belongs to lines in N₂ SPS band.

Figure 8(b) shows the effect of input power on intensities of the same lines using 5 slm He flow rate. As expected, the power increase results in higher peak intensities of the N₂ 380 nm line and He lines. Finally, in both figures 7(a) and (b) we see that the total emission intensity of the plasma follows the N₂ 380 nm line intensity. Therefore, we conclude that the dominant emission is the N₂ SPS molecular transition lines, which define the total emission intensity of the plasma.

3.4. Etching and wetting properties of PMMA films

Treatment of polymeric substrates was also performed with the DBD plasma source. PMMA (Poly methyl methacrylate) thin polymeric films spin-coated on silicon were etched with the DBD source in He/air plasma. Film thickness measurements were performed using multiwavelength ellipsometry before and after plasma etching and the etch rates of polymer

Table 1. PMMA etching rates and corresponding contact angle values after six weeks of ageing as a function of He flow rate at constant nominal power 100 W.

Power (Watt)	He (slm)	Etch rate (nm min ⁻¹ ± 10)	Contact angle after 6 weeks (±3°)
100	6	52	45
100	4	69	41
100	2	93	35

films were calculated. For the treatment with He/air we used 100 Watt as nominal power and He flows of 2, 4 and 6 slm. All the samples were etched for 2 min. In table 1 we can see the effect of the change of He flow rate on the etch rates, i.e. as He flow rate increases etching rate drops. This is consistent with decreased density of atomic oxygen at high flowrates, as observed by the emission intensity ratios shown above in figure 8(a) for Oxygen and Helium. At high flowrates it is also possible that the etching rate is reduced due to convective cooling of the sample from the He flow.

It has been shown before that plasma treatments may affect surface hydrophilicity [32]. Namely, PMMA films before treatments had contact angles approximately 70°. After the treatment surfaces became more hydrophilic, with a lower contact angle approximately 30°. We re-measured the static contact angles of the treated PMMA samples six weeks later between 40° to 50°. This result showed that aging of treated surfaces with He/air plasmas has not been significant and the surfaces remained hydrophilic, especially those treated with low He flow rates.

4. Conclusion

In this work, we present a comprehensive analysis of characteristics of the plasma generated by a novel PCB based atmospheric pressure DBD source. We performed electrical characterization, by using derivative probes, simultaneously with optical diagnostics: plasma imaging and OES. By using the data obtained from electrical measurements, we determined voltage–current characteristics and calculated power dissipation at the plasma source. We showed that behavior of

the electrical properties of the DBD are strongly affected by the He-surrounding air mixture, i.e. related to the He flow. From the analysis of higher-order harmonics we demonstrated electrical stability of operation of the source. In addition, ICCD recordings of spatially-resolved images and optical spectra of the discharge provided information about plasma structure at different operating parameters. We systematically studied the effect of He flow rate and the power forwarded to the device on the uniformity and emission intensity of the plasma, attaining the parameter range of powers and flow rates with high plasma uniformity. The high plasma uniformity makes the discharge source suitable for material processing over potentially large areas.

We also performed polymer treatment with the DBD source in terms of PMMA etching and wetting measurements. We concluded that PMMA surface aging has been significantly retarded and the surfaces remained hydrophilic even after six weeks, especially those treated with low He flow.

The source that has been built allows non-equilibrium plasma formation over a wide area. It presents high uniformity, all the benefits of DBD operating in glow regime without filamentation, and it is well controlled by adjusting the gas flow, mixture composition and power. Other modes of operation are worth exploring such as using Ar as a buffer gas, allowing a more complex chemistry and allowing pulsing and two frequency operation.

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Mass spectrometry of diffuse coplanar surface barrier discharge: influence of discharge frequency and oxygen content in N₂/O₂ mixture^{*}

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Abstract. Diffuse Coplanar Surface Barrier Discharge (DCSBD) has been studied extensively for industrial applications in recent decade. So far, limited information was available on the production of ozone or nitrogen oxides important for industrial deployment of DCSBD. In this paper results of mass spectrometry of DCSBD performed at atmospheric pressure are presented. DCSBD mass spectra were studied for different oxygen contents in N₂/O₂ working gas mixture at low flow rate (estimated residence time in discharge chamber was approx. 3 s). Influence of the driving frequency (15, 30 and 50 kHz) at constant high voltage amplitude was studied as well. Ozone and NO production in DCSBD are given as typical representatives. Production of ozone decreases with the driving frequency, which could be attributed to the gas heating at higher frequencies.

1 Introduction

Dielectric barrier discharges (DBDs) have a promising potential to succeed in many industrial applications [1]. In order to better understand the DBD's properties and to control the discharge parameters, DBDs were subject of extensive research in past decades [2]. So-called Diffuse Coplanar Surface Barrier Discharge (DCSBD) is the DBD with electrodes fully embedded in the dielectrics in plane-parallel geometry [3]. The DCSBD is generated above the dielectric plate in a form of numerous micro-discharges [4]. This results in a sub-millimetre thin macroscopically diffusive layer of high power density plasma without any contact with metallic electrodes [3]. This property together with an ability to generate a macroscopically (visually) diffuse discharge in the air at atmospheric pressure provide DCSBD with a big potential for high-speed in-line industrial applications [5]. On the other hand applicability of the discharge places a high demand on thorough investigation [6] of the discharge.

In case of applications of any type of plasma, ranging from low pressure up to the atmospheric pressure, the knowledge of chemistry in the discharge and of the composition is of utmost importance. When it comes to the

low pressure plasmas mass spectrometry is a well known technique that has been used for a very long time. Lately mass spectrometers that can operate at atmospheric pressure were developed¹ [7–9]. These unique systems are enabling studies of active species (molecules, atoms and ions) and their abundances even at the atmospheric pressure. So far we have used HIDEN HPR60 mass-energy analyser with several plasma sources ranging from kHz to MHz region [10–13]. In all cases it was necessary to optimize the operation of the mass-energy analyser and in some of them to overcome serious problems that can occur due to the influence of the plasma source on the HIDEN HPR60 mass-energy analyser [13].

In this paper we present the results of mass spectrometry of the DCSBD plasma source, following up the preliminary measurements realized in 2008 [12]. The measurements were made in several mixtures of nitrogen and oxygen and special attention was aimed at investigating behaviour of the reactive oxygen and nitrogen species (RONS) that are of the utmost importance in biomedical applications.

2 Experimental

2.1 DCSBD plasma source

Mass spectrometry of the DCSBD was performed on specially designed small-form-factor plasma reactor

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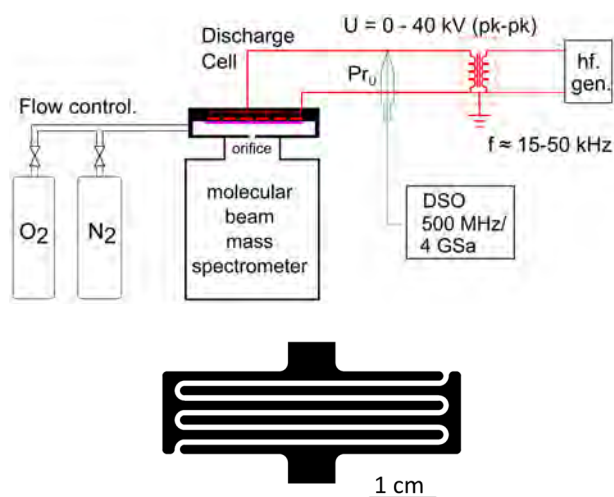


Fig. 1. Sketch of the experimental setup (top) and the details of DCSBD electrode system geometry (bottom).

of the total surface area of 7.5 cm^2 at atmospheric pressure. Firstly description of the plasma source is given followed by a more detailed description of the diagnostic technique and mass spectrometer.

A small-form-factor DCSBD plasma system studied in the presented paper consists of the discharge cell, oil cooling system and high-voltage power supply unit. The discharge cell consists of hollow polymeric cylinder cooled by the flow of oil. On top of the cylinder, DCSBD element was placed. The DCSBD consists of a ceramic plate (96% alumina) with an embedded electrode system. In Figure 1 the schematic drawing of the DCSBD discharge cell is given. The ceramic plate of the area of $80 \times 40 \text{ mm}$ and thickness of 0.2 mm was used. The electrode system consists of three plain-parallel (coplanar) electrode pairs of 45 mm length, 1.5 mm width and mutual distance (gap) of 1.0 mm . Plasma was generated on the top of the ceramic plate using an alternating sine-wave high-voltage of the amplitude of 11.2 kV and changeable frequency. Three frequencies were used in presented study – 15 , 30 and 50 kHz . The custom-made high-voltage power supply was used. The electrical parameters were controlled using Agilent DSO 6052A oscilloscope (in Fig. 1 labelled DSO) and high-voltage probe Tektronix P6015A (in Fig. 1 labelled Pr_U).

The discharge was operated in the cylindrical chamber of width of 10 cm and height of 3 mm that ensures operation in defined working gas. As the working gas the mixtures of nitrogen and oxygen (purity 5.0) were used, with a constant total mass flow rate of 500 sccm . The mass flow and mixing ratios were controlled using two mass flow controllers: OMEGA FMA5518 (nitrogen) and Bronckhorst MV-302 (oxygen). The discharge chamber was attached to the face of the mass spectrometer, protected by 0.3 mm thin PET foil. In case of surface discharges that operate at atmospheric pressure sparking can occur between the powered electrode and front plate of mass spectrometer without the additional PET foil (or some other type of

dielectric layer) [13]. Therefore, in order to have homogeneous discharge in the whole volume between the powered electrode of the DCSBD and the front plate of Hiden mass spectrometer we have used PET foil as dielectric layer. When measuring neutral mass spectra no observable changes were noticed with and without the PET foil.

2.2 Mass spectrometry originated radicals at atmospheric pressure conditions

In this experiment the quadrupole-based HIDDEN HPR-60 molecular beam mass spectrometer (MBMS) was used for detection of radicals derived from the DCSBD plasma source. MBMS has a three stage differentially pumped inlet system that enable mass spectrometer to sample at atmospheric pressure. The pressures in all three stages ($p_1 = 1.7 \times 10^0 \text{ Torr}$; $p_2 = 6.5 \times 10^{-5} \text{ Torr}$; $p_3 = 4.5 \times 10^{-7} \text{ Torr}$) were kept constant during the measurements. In all measurements DCSBD was attached to the front-side plate of MBMS with the specially designed housing and sealed with silicone rubber O-ring. This setup enabled us to operate the discharge at controlled working atmosphere and simultaneously analyse composition of the discharge. The following working gas composition/mixture ratios were used: N_2/O_2 : $100/0\%$, $80/20\%$, $60/40\%$, $50/50\%$, $40/60\%$ and $20/80\%$. The distance between the DCSBD's dielectric plate and inlet orifice of the MBMS was set to 2 mm to avoid arcing to the grounded front plate. The diameter of the first inlet orifice was 0.3 mm .

The mass spectrometry measurements were carried out in so-called residual gas analyser (RGA) mode. The measurements were performed in two distinct regimes: a) the mass survey spectrum recording and b) constant tracking of the selected species ('time sequence' or acquisition measurement – MID). The RGA mode uses an internal ionization source of MBMS for the detection of the neutrals derived from the analysed gas.

The mass analysis of plasma at atmospheric pressure condition is a challenging problem. In principle two approaches could be adopted to analyse the production of radicals in studied plasma system depending on the energy of electrons used for analysed gas ionization. The measurements can be carried out at a) certain fixed energy of ionizing electrons; or b) at variable energy of ionizing electrons – 'energy-resolved' measurements. Both approaches offers advantages, as well as serious drawbacks. It is worthy to note the major ones and therefore the short comments on the methods follows. If fixed energy of ionizing electrons is used, then the information on the origin of detected species is lost, unless a special attention to the measurement protocol is paid. This is because of the energy of ionizing electrons is usually as high as 70 eV , which is the level sufficient to ionize, but also dissociate the detected species, causing the detection of mass spectra signals of radicals, that are produced not only in studied plasma system, but also in the ionizer of the MBMS itself. Despite this drawback, this was the chosen regime for presented measurements, because the ionization yield can be substantially higher at this regime resulting in the sufficient

signal to noise ratio of detected species. In following text we will give the protocol of measurement and notes on the justification of the conclusions based on this approach.

The other approach is the adoption of ‘energy-resolved’ measurements, at which the energy of ionizing electrons is gradually increased and the signal of the particular radical is followed in the mass spectrum. The key benefit of this approach is the avoidance of parasitic mass spectrum signals, keeping the impinging electrons energy well below the dissociation threshold of species from which the particular radical could be created. Resulting energy-dependent signal course could be used to discriminate the radicals created in the instrument itself from those originating from studied plasma system. But the energy resolved measurements has also the serious drawbacks, which prevented us from the adoption of this method in our measurements and which we would like to briefly discuss.

The energy-resolved mass spectroscopy measurements were successfully used by co-authors for plasma-generated species monitoring (see e.g., [13–15]) but this technique has to be implemented unambiguously with careful consideration of the method limitations. Starting with the thresholds for dissociative ionization of N_2 and O_2 molecules which are 22 eV and 20 eV, respectively [7,16] and with the thresholds for dissociation by electron impact (that would produce neutral ground state atoms) that are 9.75 and 5.11 eV respectively [17] we see that the energy range is reasonable and yet it is rather narrow. In that energy range ionization of atoms may occur (thresholds for ionization are 14.5 and 13.6 eV respectively) but the cross section below 20 eV is still quite small thus providing a small likelihood of ionization of the fragments produced in the ionizer.

If we take into account the NO molecule that is an abundant product of the non-equilibrium atmospheric pressure plasma [18] with its thresholds for dissociative ionization (22 eV and 21 eV, respectively [7,19]) this range where we could find N and O atoms created ‘only’ in the discharge have to be between N and O ionization thresholds and <20 eV. By taking into account the cross sections for ionizations of N and O atoms we can see that by reducing the energy range of the ionizing electrons (for example 15–19 eV) we have low ionization efficiency. At 19 eV ionization cross section for both atoms is in the range of $0.2\text{--}0.3 \times 10^{-20} \text{ m}^2$ which is only around 10% of the cross section maximum. This indicates that in this range of energies the efficiency of detecting N and O atoms created in the plasma is quite low and prone to underestimation of the real number of neutrals in the plasma.

Here we may also mention N_2O molecule that is created in plasma ([18], see Fig. 4 of our paper). The dissociation thresholds for this molecule and creation of O and N atoms start already at around 8 eV [20,21]. This means that the signal collected for N and O atoms in energy resolved measurements in the range, for example, 15 eV to 19 eV also detects atoms created inside the instrument through dissociation of N_2O . The same issue is with CO_2 molecules with the threshold for dissociation of 11 eV [22]. These molecules are not very abundant in the atmospheric

pressure discharges, but still they may contribute to the detected signal and induce some uncertainty. In addition one has to take into account the thermal decomposition of the species. Even if we are measuring in the ‘safe’ range of electron energies the temperature of the electron emitting filament is about 2000 K that will lead to the thermal decomposition of the molecules and contribution to the signal of neutral atoms. This is a more of an issue for O atoms than in the case of N atoms [7].

To conclude, there are several limitations of the energy profile technique. Most important, in the present case it is its poor efficiency. The discharge studied in this paper is a diffuse planar discharge with low flux of particles at any point but with a large overall production of radicals and atoms. This rendered the energy-resolved measurement approach in case of studied DCSBD plasma source unusable, as the signal during the energy-resolved experiments of DCSBD was proven to be too low to be distinguished from the background noise. On the other hand the source, with its large area and other facilities provides numerous advantages in applications and it is certainly important to provide diagnostics of different species produced by the plasma.

One can easily dismiss approach where only measurements at 70 eV (or some such energy) are made, although even in the present day literature there is no shortage of papers employing such technique. In present paper a somewhat different approach was adopted, which was called a ‘time sequence measurement’. Three temporal stages were adopted in the measurement protocol, one when the plasma source is turned off, one when a valve in front of the ionization chamber is turned off and finally a stage when plasma is turned on and connected to the mass analyser. In other words the differences between the system with and without plasma were observed, allowing for the plasma products to be detected and tracked. The same type of time series measurements at 70 eV was also used by Stoffels et al. for detection of the changes in nitrogen, oxygen and nitric oxide molecules (among else) when the discharge is present and when it is extinguished [18]. It should be noted, that measurements based on both approaches (‘energy-resolved’ and ‘time sequence’) were performed on the same source and consistent results were obtained [7,18].

The measurements presented in this paper were carried out with the 70 eV electron energy used for ionization of neutrals. The background signal was measured in all experiments (molecular beam was shielded by closing the SwageLock valve) and subtracted from the total signal values in order to get the net signal of analysed gas. For whole set of experimental conditions (gas mixture and frequency of the supply voltage) the mass spectra of neutrals were acquired in the range of 1–100 amu.

In order to get better insight into the behaviour of plasma generated species high-sensitivity ‘time sequence’ measurements were performed using the acquisition mode (MID scan). The following procedure was applied for all measurements: firstly, the molecular beam inlet of the mass analyser was shielded (thus blocking the signal

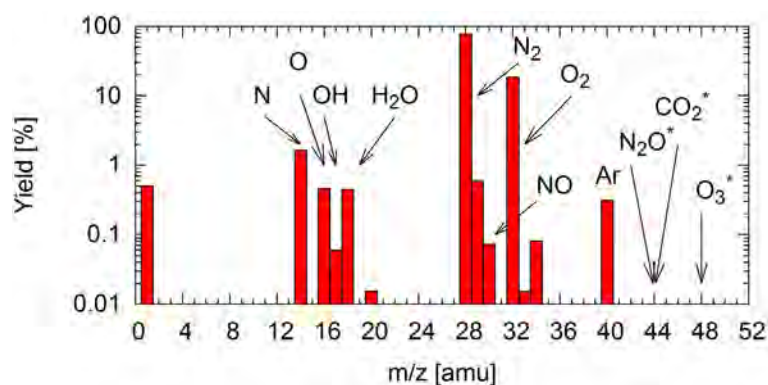


Fig. 2. The mass spectrum of working gas for the following conditions: N_2/O_2 ratio of 80/20 and DCSBD discharge operated at working frequency of DCSBD 15 kHz and voltage amplitude of 11.2 kV. The spectrum is truncated at 52 amu, due to lack of detectable signal at given experimental conditions. Asterisk-marked species are close to the limit of detection at given conditions.

from plasma-background signal acquisition), then the inlet of the MBMS was opened and the system was allowed to reach the equilibrium (≈ 2 min). After that, plasma was ignited and system was allowed again to reach the equilibrium (≈ 2 min). Then the discharge was switched off and after the final equilibrium was reached experiment was completed. The whole procedure of a MID scan took approx. 10–12 min. The behaviour of specific radicals could be derived from recorded changes of the signal when DCSBD plasma is on and off. Total variations were determined for O_3 and NO radicals for the pre-selected current at the ionization filament of $50 \mu A$.

3 Results and discussion

According to the described procedures of mass analysis this section is divided into two sub-sections. Firstly, the survey mass spectra are discussed and then results of the high-sensitivity measurements of selected radicals are given and discussed.

From the whole set of studied discharge conditions the limited sub-set was chosen for presentation. For survey spectra analysis, the typical discharge conditions used for plasma treatment of materials were chosen, i.e., the working gas composition of 80% of N_2 and 20% of O_2 , as the substitution of the ambient air. The high-voltage amplitude of 11.2 kV at the frequency of 15 kHz were used as the typical working conditions of DCSBD discharge. These results can be compared to the previous measurements made on the industrial-size DCSBD system at ambient air conditions in 2008 [12]. For the time-series measurements of selected radicals the two radicals (O_3 and NO) were selected as the representatives of volatile compounds that have to be controlled and monitored in industrial environment due to their biological effects.

Due to the used mass analysis method the interpretation of the data should be done carefully, concerning the other possible sources of the species, mainly from the reactions inside the MBMS. We based our analysis and conclusions on the published results and protocols on at-

mospheric pressure mass analysis of plasmas, mainly on the interpretation of the ‘time sequence’ measurements.

3.1 RGA analysis – mass spectra of the DCSBD discharge

In Figure 2 the mass spectrum of the working gas in the chamber of DCSBD discharge is given. The spectrum represents the discharge operated at frequency of 15 kHz at high-voltage amplitude of 11.2 kV in the gas mixture of 80% N_2 and 20% O_2 , i.e., artificial air conditions. The yields in Figure 2 represent relative percentage of distribution of the species. The following formula was used for the calculation of the yields: $Yield = Y_{mass}^i / \sum_i Y_{mass}^i$, where Y_{mass}^i is the count of specific species (like N_2 , O, etc.) at the detector (detected as appropriate positive ion there), see [8,11] for details.

The yields of the recorded mass spectra can give us the relative gas composition. From the spectra in Figure 2 it is evident that the dominant species in the discharge were molecular nitrogen and molecular oxygen. Atomic nitrogen and atomic oxygen are present as the result of plasma reactions as well as the NO radical. The OH radical is also present due to plasma reactions as well as the decomposition of water in the MBMS. Having in mind that the spectra were obtained for the electron energies of 70 eV the comparison of the mass spectra with and without ignited discharge is necessary.

In Figure 3 effect of the discharge on gas composition is shown. In the upper part of Figure 3 the comparison of working gas composition not-influenced (labelled ‘PL OFF’) and influenced (labelled ‘PL ON’) by the discharge is given.

Although the yields of species like N, O, or NO are relatively high, the relative gas composition of the rest of the species with/without plasma differs only very slightly, typically less than 1% of the relative composition (see lower part of Fig. 3). This could be mainly contributed to the fact that the most of detected species were produced in the ionizer. The system was also not able to sample

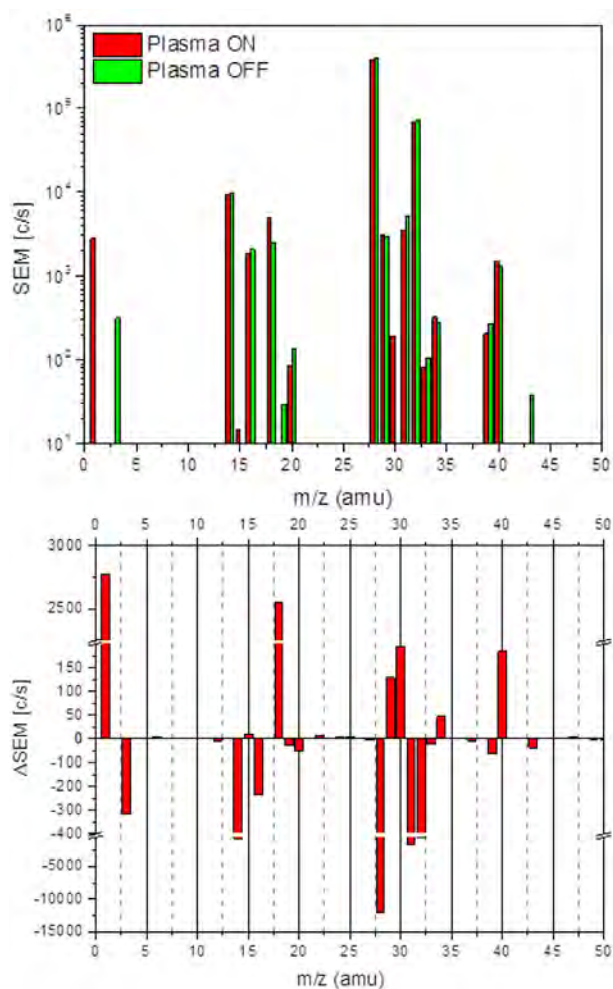


Fig. 3. The survey spectrum of working gas when the DCSBD discharge was turned on/off for the following conditions: N_2/O_2 ratio of 80/20 and working frequency of DCSBD 15 kHz (top); the corresponding difference of counts with/without plasma (bottom).

the gas directly from the plasma layer of DCSBD. The sampling of discharge atmosphere had to be realized keeping a minimum distance between the DCSBD discharge and the grounded MBMS front plate to avoid arcing to the grounded MBMS plate. Thus the post-plasma reactions of direct products of the DCSBD plasma-chemical reactions with the surrounding gas in the discharge chamber could occur also. This limits a better understanding of the reactions directly in the DCSBD plasma, but does not limit the goal of presented research, i.e., the research of the industrially important by-products of the DCSBD plasma deployment.

Although the background signal in the RGA analysis was relatively high, it can still give us an overview of the plasma influence on gas composition.

The absolute difference of MBMS signals with and without plasma is given in the lower part of Figure 3. Positive difference represents an increase of the signal of the radical after plasma ignition. We can see that the gas composition differs significantly for the N_2 and O_2 signals,

as these molecules are decomposed in the plasma. The increase of NO indicates production of these radicals in the plasma.

Stoffels et al. (see [7,18]) came to the (same) conclusion that NO is produced abundantly in the plasma and N_2 is lost due to the dissociation of the molecules in plasma itself. They have done extensive investigation for the various plasma parameters by using both methods presented in Section 2.2. In experiments conducted by Stoffels and Aranda Gonzalvo plasma needle was used as the plasma source and measurements were done, like in our case, by using HIDEN HPR60 mass spectrometer. The plasma needle is a source which is known not to produce significant amount of O_3 molecules and Stoffels and co-workers did not observe signal due to this molecule. In our case DCSBD produces significant amounts of ozone and therefore we have employed the same ‘time sequence’ method to record it. But the ozone molecule is not the most important and especially not the only relevant molecule for bio applications of plasma discharges. Actually its presence diminishes the use of plasmas if one needs to operate it in the vicinity of humans. The advantage of adopted MBMS technique (among other techniques able to give us the information on the ozone production) is in its ability to give us the information for all the desired molecules (RONS-reactive oxygen and nitrogen species) in real time and at the same time, which was our goal. When it comes to OH radicals we cannot certainly attribute their production to the plasma reactions, based on the conducted experiments. Its creation in the plasma was confirmed by mass spectrometry or other methods (see, e.g. [7,23–26], or EPR (Electron Paramagnetic Resonance) technique [27]), but in presented experiments we have used the pure oxygen and nitrogen gasses and we did not vary, nor measure the residual humidity of the processing gas. From the observation that the OH radical mass signal does not respond to the great extent on the presence of the discharge or not we can estimate that it is likely that the OH radicals could arise from the residual moisture inside the MBMS chamber.

3.2 MID scan – high-sensitivity measurement of selected species

While the results of mass spectra presented above gave us an overall view of the main species present in the discharge chamber, the nitrogen oxides and ozone affect the industrial environment even in much smaller concentrations (below the limit of detection used in obtaining mass spectra). Thus more sensitive measurements were performed only for the selected ‘important’ species, i.e., N_2 , O_2 , N, O, NO, NO_2 , N_2O and O_3 ². In the following, two representatives were selected for discussion, the ozone (O_3) and the nitric oxide (NO).

² The OH radical was measured also, but the production of OH was attributed to the MBMS residual gas water impurities, because of the time-series behaviour of OH radical did not exhibit the influence on the plasma presence in the discharge chamber.

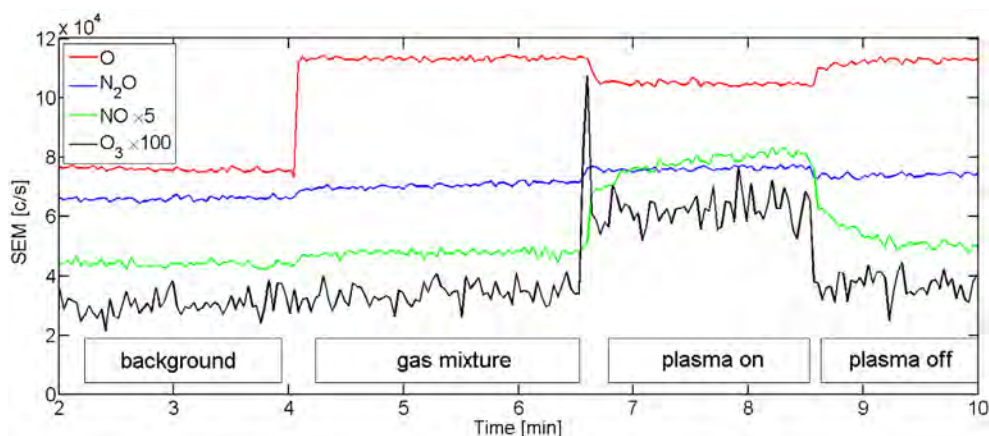


Fig. 4. The typical representative of the ‘MID scan’, i.e. the constant tracking of the selected species, four stages of experiment were marked using the labelled boxes. The conditions were as follows: gas mixture 80% N₂ and 20% O₂, DCSBD voltage frequency 15 kHz, ionization filament current 50 μ A.

In Figure 4 a typical representative of the ‘MID scan’, i.e., the ‘time sequence’ measurement of selected species is given. The four stages of the measurement can be identified (see the boxes in Fig. 4): the residual gas traces inside MBMS (labelled ‘background’) and the processing gas composition (labelled ‘gas mixture’) and the influence of the discharge presence (labelled ‘plasma on’, resp. ‘plasma off’). For the following calculations absolute differences of the averaged signals of the ‘plasma on’ and ‘plasma off’ stages were used. In Figure 4 at the beginning and at the end of the ‘plasma’ stage the inertia of the mass signal can be seen. The attribution of this behaviour to the parameters of the discharge or the whole measurement system is not clear at this moment and further analysis will be necessary.

In Figure 5 the results of the ‘MID scan’ data processing are given for the ozone (O₃) and nitric oxide (NO). The influence of experimental conditions is presented using absolute differences of the averaged signals of the ‘plasma on’ and ‘plasma off’ stages.

The first sub-figure (upper) shows the behaviour of ozone (O₃) production with the respect to the discharge conditions. The drop of the ozone production with the increase of discharge repetition rate (frequency) is clearly visible namely for oxygen-rich mixtures of operating gas. The same effect of the decrease of ozone production with increasing frequency was observed also in [30], but there the maximum frequency of 2 kHz was used and the decrease was not so pronounced as it was seen for studied DCSBD. The microdischarge of DBD can be treated as a small reactor, in which the plasma-chemical reactions occurs [1]. Assuming the average energy transferred per individual microdischarge of DBD partially heats-up the discharge channel [28] then the increase of the repetition rate (discharge frequency) could result in the increase of the average discharge channel temperature. Pietch and Gibalov stated in [29] that the increase of energy density is associated with the increase of processing gas temperature, to which the ozone producing chemical reactions are strongly dependent. The same temperature effect was observed

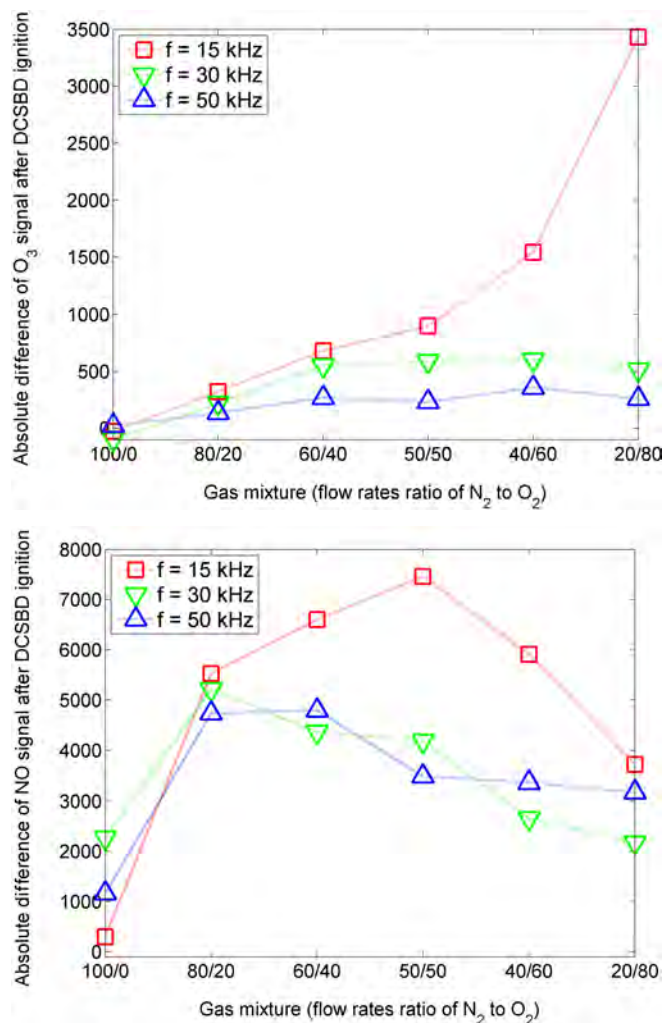


Fig. 5. The absolute difference of the MBMS signal after plasma ignition in the discharge chamber for ozone (top) and nitric oxide (bottom) as the results of ‘MID scan’ (‘time sequence’ measurement) signal analysis. For given species the dependence of their production is given with the respect to the discharge conditions shown in the figures.

also, e.g., in [31]. In [32] the decomposition of ozone was attributed rather to the repetitive exposure of the gas volume to the discharge in a microdischarge channel. The temperature and gas flow effect on ozone production studied in [33] indicated, that the ozone production must be treated as a complex interplay of the discharge condition hardly attributable to particular separated quantity. The possible mechanisms of the drop of the production of ozone in studied DCSBD discharge could therefore be attributed to the growth of energy density at higher frequencies, that leads to increase of the gas temperature through direct and/or indirect plasma-induced heating and/or to the higher plasma-exposure time at higher repetition frequency conditions, under the same gas residence time in the chamber (constant gas flow rate).

For low oxygen concentrations (labelled '100/0') only a few percent of remnant oxygen is present. For increasing amount of oxygen in the discharge chamber, the difference in ozone production due to variation in the frequency is evident. The DCSBD operated at lowest frequency, i.e., 15 kHz, produces considerably higher amount of ozone, than for 30 and 50 kHz. The behaviour of nitric oxide (NO) with the respect to the variation in frequency and gas mixture is given in the second sub-figure (lower). Production of NO has a peak depending on the abundance and with the peak position varying with the frequency. NO production is the greatest at the lowest frequency. The maximum of the production of nitric oxide for the lowest frequency is for the '50/50' gas mixture. In case of 30 kHz and 50 kHz the maximum of NO production is for the '80/20' gas mixture. For detailed analysis of RONS generation and equilibrium concentrations the full plasmakinetic model should be assembled, which is beyond the scope of this paper.

4 Conclusion

The mass spectrometry of DCSBD discharge generated at atmospheric pressure condition was performed. The effects of working gas composition (mixtures of oxygen and nitrogen) and the high voltage frequency were studied on the production of reactive oxygen and nitrogen species (RONS), e.g., N, NO_x or O₃, as they play important role in the plasma interactions with the living organisms and tissues. The results confirmed the substantial effects of the high voltage frequency and oxygen/nitrogen flow ratio on the production of RONS. The ozone (O₃) and nitric oxide (NO) molecules production was highest at the lowest frequency of 15 kHz from the studied range of 15–50 kHz. This demonstrated that the production of these species could be suppressed or enhanced with the selection of the frequency of the input high voltage and gas mixture.

Author contribution statement

All authors contributed equally to the paper.

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МИНИСТЕРСТВО НАУКИ И ВЫСШЕГО ОБРАЗОВАНИЯ РОССИЙСКОЙ ФЕДЕРАЦИИ
РОССИЙСКАЯ АКАДЕМИЯ НАУК
НАУЧНЫЙ СОВЕТ РАН ПО КОМПЛЕКСНОЙ ПРОБЛЕМЕ
«ФИЗИКА НИЗКОТЕМПЕРАТУРНОЙ ПЛАЗМЫ»
РОССИЙСКИЙ ФОНД ФУНДАМЕНТАЛЬНЫХ ИССЛЕДОВАНИЙ
ОБЪЕДИНЕННЫЙ ИНСТИТУТ ВЫСОКИХ ТЕМПЕРАТУР РАН
ИНСТИТУТ НЕФТЕХИМИЧЕСКОГО СИНТЕЗА ИМ. А.В. ТОПЧИЕВА РАН
КАЗАНСКИЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ

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Представляет интерес для специалистов, инженеров, молодых ученых и студентов, работающих и ведущих исследования в области физики низкотемпературной плазмы.

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**RECENT ADVANCES IN PHYSICS AND DIAGNOSTICS
OF ATMOSPHERIC PRESSURE NON-EQUILIBRIUM PLASMAS AND
THEIR APPLICATIONS IN BIOTECHNOLOGIES AND MEDICINE**

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In this paper we give a limited review of our studies in applications of Atmospheric Pressure Plasmas (APP) in some branches of Biology, Medicine, Biotechnologies and Materials Science. As those applications are critically dependent on the use of Non-equilibrium (Low Temperature) plasmas (NELTP) at the atmospheric pressure we start with some of our studies of the development of the required plasma sources and their diagnostics.

We have had experience with several NELTP sources including: plasma needle [1], Atmospheric Pressure Plasma Jet (APPJ) [2], dielectric barrier discharges, coronas and micro APPJ [3]. The most interesting aspect of the source diagnostics proved to be properties and spatial development of self-propagating streamer ionization fronts for APPJ with different electrode geometries. Inclusion of transparent electrodes [4] provided us with an uninterrupted view of the streamer development inside and outside the glass tube with the flowing buffer gas (helium in our case). Another novel observation was associated with the positioning of plasma inside the glass tube. Whenever electrode (ground or at some voltage) was beyond glass the plasma approached close to the walls and to the electrode (wall hugging). Whenever there were no electrodes, plasma was positioned in the center of the electrode [4].

For APPJ it is easy to apply standard probes to obtain current and voltage and thus deduce power transmitted to the plasma. For plasma needle that operates in MHz range it was necessary to develop specialized derivative probes, calibrated with high sensitivity so that we could ascertain observation of 0.1 W of power to plasma amidst large displacement currents three orders of magnitude greater than the plasma current [1]. In addition to optical emission spectroscopy, the main optical technique involved time resolved (fast) spatial profile recordings by the use of ICCD cameras [5]. Most importantly, application of mass spectrometry proved to provide the most relevant data for plasma-target interaction [3,6]. Detection of neutrals created in plasmas required a complex procedure to subtract the effects of dissociation due to the effect of ionizer beams in the mass analyzer. Ions originating from plasma could be detected much more easily and were used for comparisons with theoretical predictions.

Armed with all the available diagnostics our studies of biomedical applications of NELTP provided several new insights. Such plasmas may induce much more effi-

cient and faster sprouting of plants from the seeds and their faster growth [1]. Plasma provides signaling-triggering radicals that induce the growth of the plant and faster multiplication of the cells. On the other hand the same type of plasma may be used to remove excessive pesticides resolved in the water supply and used for plant or human consumption [7]. It proved that plasma treated water contains active species required both for disinfection and triggering of processes and may be beneficial for numerous applications.

The obvious medical application of sterilization proves to be more useful if studies are made for planktonic samples or even biofilms. In that case antibiotic resistant microbes are easily removed at the same rate as the non-resistant bacteria. More importantly it was possible to affect differentiation of stem cells by using plasma [8], to study short and long term enzyme activity induced by plasma and to test the plasma induced DNA damage and the resulting cell death [9]. Finally, a number of biocompatible materials may be treated by plasmas to improve their properties, adhesion and compatibility and often the same sources may be applied [10].

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iPlasmaNano-X
September 15-20, 2019
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Keynote: 40 min including questions

IT: 30 min including questions (25+5 min)

Short talks: 15 min including questions (12+3 min)

Poster Session:

**The posters will be presented throughout the whole conference in the conference hall.
The format of the posters is A0 Portrait**

Sunday 15-Sept		
14:00	Reception-registration	
17:00 – 17:15	CONFERENCE OPENING CEREMONY & OPENING SESSIONS Welcome: E. Kovacevic, J. Berndt, U. Cvelbar Chair: Eva Kovacevic	
17:15 – 17:55	S-1	K. Koga <i>Faculty of Information Science and Electrical Engineering, Kyushu University, Japan</i> <i>Center for Novel Science Initiatives, National Institutes of Natural Sciences, Japan</i> Time of Flight Size Control of Nanoparticles in Reactive Plasmas
17:55 – 18:25	S-2	J. G. Eden <i>Department of Electrical and Computer Engineering, University of Illinois, Urbana, IL</i> <i>61801, USA</i> Electromagnetic (MM-wave) and nanofabrication applications of microcavity plasmas
18:25 – 18:55	S-3	Z. Lj. Petrović <i>Institute of Physics, University of Belgrade POB 68 11080 Zemun Serbia, Serbian</i> <i>Academy of Sciences and Arts, Knez Mihailova 35, 11001 Belgrade, Serbia</i> Global Model and Diagnostics of an Atmospheric Pressure Plasma Jet in Mixtures of Helium and Water Vapour
19:15	WELCOME DRINK & DINNER	

Financial support: MOST grants MOST 106-2221-E-011-117 and MOST 107-2911-I-011-504, ANR grants ANR-15-CE06-0007-01 and ANR-11-LABX-0017-01), PHC “Orchid” grant 40938YL.

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Yang, J. S., Pai, D. Z., & Chiang, W. H. (2019). *Carbon*, 153, 315-319.

Global Model and Diagnostics of an Atmospheric Pressure Plasma Jet in Mixtures of Helium and Water Vapour

Z.Lj. Petrović^{1,2}, Ž. Mladenović³, S. Gocić³, N. Selaković¹, G. Malović¹ and N. Puač¹

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Detailed spatially resolved modelling of plasmas usually requires kinetic or may be performed by hybrid kinetic/fluid models. Using such models it is beyond current capabilities to include hundreds of different species that contribute to the rich, plasma-induced chemistry so for that purpose 0D global models are being used. The most complex systems that are being studied presently are atmospheric pressure plasma jets where initial helium flow is often mixed with some water vapour and/or oxygen and then upon generation of plasma it is mixed with the external air that may have its own humidity. We have found excellent agreement between our simulations [1] for the flow of 3 slm of He with 7600 ppm of water mixed with 1% of air and the mass spectrometry measurements at 23 mm from the end of the APPJ glass tube [2]. Our results are also consistent with those for measured OH densities [3] and for electron densities and dependence of rates on humidity from [4].

Acknowledgments

Authors are grateful to MESTD of Serbia projects ON171037 and III41011.

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Abstracts



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P2.14-50 LOW TEMPERATURE PLASMA NEEDLE INDUCES CELL CYCLE ARREST OF EPITHELIAL LUNG CANCER CELLS IN VITRO VIA A P21-DEPENDENT PATHWAY

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Background: Low temperature plasma sources which operate at atmospheric pressure produce reactive oxygen species (ROS) and reactive nitrogen species which can cause cancer cell death. In this study we investigated the effect of our low temperature plasma needle system on a non-small cell lung cancer cell line A549 and studied its mechanism of action. **Method:** The housing of the pen lookalike atmospheric plasma source (plasma needle) was made of Teflon. A Pyrex glass tube (o.d. 6 mm and i.d. 4 mm) through which 1 slm of helium was released as the feeding gas was positioned within the housing. The central electrode, a tungsten wire, was powered with a 13.56 MHz sine wave and placed within a ceramic tube inside the glass tube. The central electrode was sticking 1 mm outside of the ceramic and glass tubes, so the discharge occurred on its tip as a weak glow. The cytotoxic activity was determined using MTT assay. The potential of inducing cell cycle perturbations and apoptosis, and changes in the level of ROS was investigated by flow cytometry. The influence of plasma treatment on growth inhibition of multicellular tumor spheroids (MCTS) was also investigated. Evaluation of gene expression was performed by qPCR. All experiments were performed in triplicate, with statistical significance set at $p < 0.05$. **Result:** Our plasma needle exerted a cytotoxic effect with lower sensitivity towards BEAS-2B normal cells than towards A549 cells. A decrease in the number of cells in the G1 phase (up to 45%), increase in the G2 phase (up to 27%), and an increase in the sub-G1 phase (up to 12%) with fragmented DNA was detected upon treatment. The plasma treatment exerted a mild apoptotic effect (around 15% of apoptotic cells), and an increase in the level of ROS in a power dependent manner. There was no significant reduction in growth of MCTS after plasma treatment under investigated experimental conditions. A statistically non-significant proapoptotic effect (increase in Bax, decrease in Bcl2 and decrease in SKP2) was observed at the genetic level. A significant overexpression of the cyclin-dependent kinase inhibitor 1 (p21) was also observed at the genetic level in a power dependent manner. **Conclusion:** Our low temperature plasma needle induced cell cycle arrest of epithelial lung cancer cells through overexpression of p21. The effect of combined plasma treatment with existing treatment modalities (cisplatin, PARP inhibitors) is currently under *in vitro* investigation.

Keywords: cell cycle arrest, plasma needle, p21

P2.14-51 DUAL ALK FUSION PARTNERS AS POOR PREDICTIVE MARKER IN FIRST LINE CRIZOTINIB TREATED ALK REARRANGED NON-SMALL CELL LUNG CANCER

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Background: First line crizotinib response duration time differs with different fusion patterns in ALK-rearranged advanced non-small cell lung cancer (NSCLC) patients. Some former researches have elucidate the impact of EML4-ALK variants on crizotinib efficacy, however, there was little data about the efficacy of crizotinib considering different fusion partners including one patient with two or more fusion partners or non-EML4 partners. **Method:** 150 patients with NGS-identified ALK-rearranged NSCLC from March 2014 to July 2018 in Hunan Cancer Hospital were enrolled in this study. Among them, 112 patients received crizotinib as first-line treatment. Efficacy of crizotinib was evaluated and categorized according to different fusion partners. **Result:** Among 150 advanced NSCLC patients with NGS-identified ALK-rearranged, 181 fusion partners were detected including 43 novel fusion partners. 122 patients (81.3%) were identified with single ALK fusion partners, 28 patients (18.7%) were identified with dual or triple ALK fusion partner patients. Among 112 patients received first line crizotinib treatment, 89 patients were

identified with single fusion partner (79 for EML4, 10 for non-EML4). 23 patients were identified with dual fusion partner (20 patients with dual fusion partners, 3 patients with triple fusion partners). The overall response rate (ORR) was 85.2% and the median progression-free survival time (mPFS) was 11.7 months. The frequency of brain metastasis was high in dual fusion partner patients. Patients with dual ALK fusion partners have a significantly shorter mPFS compared with patients carrying single ALK fusion partner (6.1m vs. 12.0m, $p = 0.001$). Patients with EML4 partners have a significantly longer mPFS compared with patients carrying non-EML4 fusion partners (12.6m vs. 6.1m, $p = 0.004$).

Fusion partners	EML4-ALK fusion variants	Number of patient	Percentage of patient(%)
C9orf3-ALK	(2	1.3%
CLIP1-ALK	(1	0.7%
CYBRD1-ALK		1	0.7%
DEFA5-ALK	(1	0.7%
EML4-ALK	V1	31	20.7%
EML4-ALK	V2	10	6.7%
EML4-ALK	V3	47	31.3%
EML4-ALK	V5	11	7.3%
EML4-ALK	V7	3	2.0%
EML4-ALK	E3:A20	1	0.7%
EML4-ALK	E7:A20	1	0.7%
EML4-ALK,APOB-ALKE	V5	1	0.7%
EML4-ALK,ATXN1-ALK	V7	1	0.7%
EML4-ALK,BIRC6-AS2-ALK	V3	1	0.7%
EML4-ALK,C1QC-ALK	V3	2	1.3%
EML4-ALK,COL22A1-ALK	V3	1	0.7%
EML4-ALK,COL22A1-ALK	V1	1	0.7%
EML4-ALK,DIRC3-AS1-ALK,CDC42EP3-ALK	V3	1	0.7%
EML4-ALK,EHBPI-ALK	V3	1	0.7%
EML4-ALK,-FLJ14082-ALK	V3	1	0.7%
EML4-ALK,LBH-ALK	V1	1	0.7%
EML4-ALK,LINC00486-ALK	V3	1	0.7%
EML4-ALK,LINC01121-ALK	V1	1	0.7%
EML4-ALK,LOC102467222-ALK	V3	1	0.7%
EML4-ALK,LOC388942-ALK	V1	1	0.7%
EML4-ALK,LOC388942-ALK,	V3	1	0.7%
EML4-ALK,LR-RTM4-ALK	V3	1	0.7%
EML4-ALK,MBOAT2-ALK	V2	1	0.7%
EML4-ALK,MTA3-ALK,SP3-ALK	V1	1	0.7%
EML4-ALK,MYH7-ALK	V2	1	0.7%
EML4-ALK,PD-E6D-ALK	V1	1	0.7%
EML4-ALK,QPCT-ALK	V1	1	0.7%

Bulletin of the American Physical Society**50th Annual Meeting of the APS Division of Atomic, Molecular and Optical Physics APS Meeting
Volume 64, Number 4**

Monday–Friday, May 27–31, 2019; Milwaukee, Wisconsin

Session S01: Poster Session III (4:00pm-6:00pm)

4:00 PM, Thursday, May 30, 2019

Wisconsin Center Room: Hall A

Abstract: S01.00027 : Tracing Plasma Produced Atomic and Molecular species from Plasma into the Liquid and Living tissue for various applications[Preview Abstract](#)[← Abstract →](#)**Authors:**Zoran Petrovic
(Institute of Physics, University of Belgrade Serbia)Nikola Skoro
(Institute of Physics, University of Belgrade Serbia)Suzana Zivkovic
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Significant effects observed in applications of atmospheric pressure non-equilibrium plasmas have been shown to be due to the effect of plasma produced atomic and molecular reactive species. Some of those species are the ones acting as signaling agents initiating response of the living cells. At the same time, albeit in larger numbers, they may be chemical agents that can damage or dissociate unwanted living organisms, human cells or chemical components. We try to follow the trail of atomic and molecular physics starting from their formation, their passage into liquids and then passage into living cells or the reaction of cells that they invoke. Two examples will be the long term changes in enzymes regulating hydrogen peroxide in plant cells and destruction of malathion, a pesticide used in agriculture that may be a model of more lethal weapons of mass destruction. Finally we shall illustrate how presence of those active species in plasma treated water affects the germination of seeds. -abstract- Authors Zoran Lj Petrovic, Nikola Skoro, Suzana Zivkovic(t), Milica Milutinovic, Olivera Jovanovic, Nenad Selakovic,

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Tracing Plasma Produced Atomic and Molecular species from Plasma into the Liquid and Living tissue for various applications ZORAN PETROVIC¹, NIKOLA SKORO, Institute of Physics, University of Belgrade Serbia, SUZANA ZIVKOVIC, MILICA MILUTINOVIC, Institute Sinisa Stankovic, University of Belgrade Serbia, OLIVERA JOVANOVIC, NENAD SELAKOVIC, NEVENA PUAC, Institute of Physics, University of Belgrade Serbia — Significant effects observed in applications of atmospheric pressure non-equilibrium plasmas have been shown to be due to the effect of plasma produced atomic and molecular reactive species. Some of those species are the ones acting as signaling agents initiating response of the living cells. At the same time, albeit in larger numbers, they may be chemical agents that can damage or dissociate unwanted living organisms, human cells or chemical components. We try to follow the trail of atomic and molecular physics starting from their formation, their passage into liquids and then passage into living cells or the reaction of cells that they invoke. Two examples will be the long term changes in enzymes regulating hydrogen peroxide in plant cells and destruction of malathion, a pesticide used in agriculture that may be a model of more lethal weapons of mass destruction. Finally we shall illustrate how presence of those active species in plasma treated water affects the germination of seeds. -/abstract- Authors Zoran Lj Petrovic, Nikola Skoro, Suzana Zivković, Milica Milutinovic, Olivera Jovanovic, Nenad Selakovic,

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and
11th EU-Japan Joint Symposium on Plasma
Processing

Book of Contributed Papers

Štrbské Pleso, Slovakia
18-24 January, 2019

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DIAGNOSTICS OF ATMOSPHERIC PRESSURE PLASMAS AND THEIR APPLICATION IN AGRICULTURE

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Atmospheric pressure plasmas (APPs) have been intensively studied in the last decade due to their high potential in the applications in medicine, biology and, lately, in agriculture [1, 2]. They are generated in plasma sources of various geometries and configurations and with different electrode material in order to make them efficient for large variety of applications. The types of sources used are mainly atmospheric pressure plasma jets (APPJs), but since some applications require large areas to be treated plan parallel dielectric barrier discharges [3-6] are also utilized. Typical configurations of APPJs consist of a tube for conducting the flow of the buffer gas and set of electrodes where powered one can be in the contact with the plasma or covered by dielectric. The power supplies that are most commonly used can be divided by the type of signal as continuous or pulsed and they can operate in the large range of frequencies (from several kHz to GHz). The choice of the geometry as well as the type of the power supply used is governed by the application of the APP. Regardless of the system choice and configuration, one needs to perform detailed diagnostics of the plasma system to obtain the data that could be linked to the processes significant for the particular treatment. Then, the efficiency of desired application can be assessed and, which is also important in many cases, there is an opportunity up to some extent to compare the achieved effects with the other systems used for the same application. At the same time, the detailed diagnostics allows the studies of fundamental questions of APP behaviour. For instance, in the case of APPJs an interesting feature of formation of pulsed atmospheric-pressure streamers (PAPS) was observed and investigated. The fast ICCD imaging of this phenomena revealed that PAPS have a speed of several kilometres per second and their formation and propagation still needs to be explained in more detail.

However, from the point of applications the most important feature of the APP is that they create chemically highly active media (both in gas and liquid) with the properties that can be generally tuned according to the application's demands. The active chemistry of the plasma's gas phase can directly modify the treated surface or activate specific mechanisms inside the treated target, e.g. plasma treatment of cells leading to the improved differentiation or cancer cell death [7, 8]. Also, since APP can operate in direct contact with a liquid, the chemically active environment produced in the gas phase above the liquid can modify the physical and chemical properties of the treated liquid [9-11]. Extensive research in this type of experimental configurations has led to the development of the applications of APP in the field of agriculture. Two examples of such applications are direct treatment of seeds and treatment of water in order to create plasma activated water. It was shown that in both cases plasma treatment induces higher germination percentage, less contamination of the seedlings, higher water uptake and faster plant development. These and other observed effects are the result of the interaction of plasma formed reactive oxygen and nitrogen species (RONS) with the seed and plant cells. RONS can trigger various biochemical mechanisms that can be observed also at molecular level through the activity of enzymes or hormones in the seeds and plants.

Here we will try to give the overview of the detailed characterization of the APP systems that were used for both medical applications and applications in agriculture. Results of time-resolved plasma imaging using fast ICCD camera will show the development of plasma structure within one period of the power signal and provide an insight in kinetic effects such as PAPS. Moreover, by using optical emission spectroscopy spectra of excited species in the gas phase will be obtained allowing the qualitative assessment of excited species above the treated liquid. The results of electrical measurements of APP

sources, as another important diagnostics tool, will be presented. Comparison of the two different APP (in electrode geometry and applied voltage frequency) will be done by comparing both the results of plasma diagnostics and the response of the biological system treated by these plasma sources. Also, investigations of the applications of APP in the agriculture will be shown featuring the idea of plasma decontamination of water polluted by pesticides and its influence on germination of commercial plants.

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MASS SPECTROSCOPY OF AN ATMOSPHERIC PRESSURE PLASMA BULLET

N. Selaković, D. Maletić¹, N. Puač, S. Lazović, G. Malović, Z. Lj. Petrović*Institute of Physics, University of Belgrade, Belgrade, Serbia*

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Wide range of potential applications of plasma sources operating at atmospheric pressure, particularly in medicine and biology, has led to a large expansion in their development. Before using a new plasma source for treatment of bio-samples it is necessary to make detailed diagnostics of the plasma and, most importantly, examine its chemical composition. We have used Hidden HPR 60 mass-energy analyzer for the time-resolved measurements of the ionic species originated from atmospheric-pressure plasma jet.

Plasma jet was made of Pyrex glass tube with two transparent electrodes (15 mm wide) made of polyester (PET) foil. The gap between the electrodes was 15 mm. This source operated at excitation frequency of 80 kHz and applied voltage was in the range of 6-10 kV_{peak-to-peak}. The feeding gas was helium with flow rate of 4 slm. In all experiments distance between the plasma source and HPR60 orifice was 15 mm. We also used plastic side-covers around plasma source to prevent plasma flickering due to ambient air disturbance. The applied current and voltage signal and HPR 60 internal gate signal have been synchronized. The internal gate width of HPR60 analyzer was 0.5 μ s.

Mass spectrometer was operated in ion mass spectroscopy mode and we have measured positive and negative ion species coming from the plasma. The detected positive species from the plasma plume are N₂⁺ (36%), N⁺ (20%), O₂⁺ (18.5%), O⁺ (16.8%), H₂O⁺ (6.1%) and a few percentage of OH⁺, NO⁺, N₂H⁺ and Ar⁺ (see Fig.1). In case of negative ions we have detected O⁻ (34.3%), OH⁻ (24.2%), O₂⁻ (10.5%) and a few percentage of CO₂⁻, NO₃⁻ and NO₂⁻. The signal of detected ions was also tracked in time for the duration of 5 periods (5x12.5 μ s) of current and voltage signal.

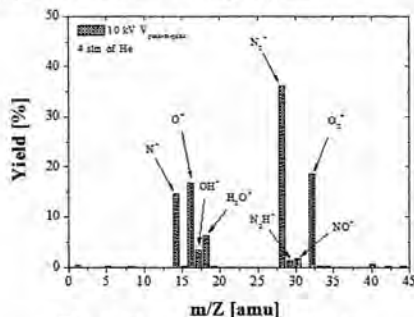


Fig. 1: Mass spectra plot of positive ions

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Time-resolved images of plasma bullet for different electrode geometries

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In this paper we will present time-resolved images of atmospheric pressure plasma jet obtained by using fast ICCD camera for several electrode settings. It will be shown that formation and position of the plasma bullet strongly depends on the electrode geometry. The main purpose of our investigation was the possibility of applying plasma bullet for the treatment of thermo-sensitive samples.

A possibility to obtain the discharges of various geometries at low gas/ion temperatures and at atmospheric pressure would be a good basis for numerous applications in the industry, biology and medicine [1-5]. Here we study properties of an atmospheric pressure plasma jet (APPJ) operating with sinusoidal voltage excitation at a frequency of 80 kHz. Construction of plasma jet [6, 7] allowed easy ICCD camera capturing of the time-resolved images of the discharge between and inside the electrodes, as well as, of the plasma bullet that is formed outside the tube/electrode system. Experimental setup is given in Fig. 1. We will use the common term "plasma bullet" for visible manifestations of plasma because these ionization fronts create appearance of a motion of a bullet even though plasma itself may have a very different motion.

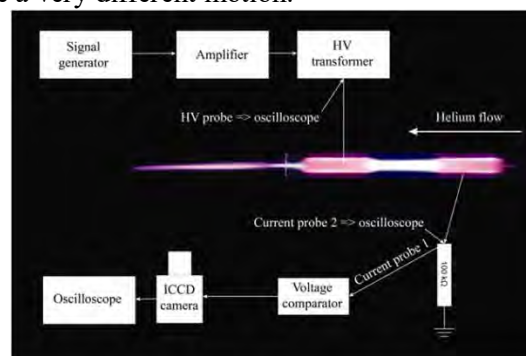


Fig. 1. Experimental setup

The body of a plasma jet was made of Pyrex glass tube 6 mm outer diameter and 4 mm inner diameter. The length of the coated PET electrodes was 15 mm and the distance between them was 15 mm. The distance between the electrodes was kept constant during all measurements. The right electrode was grounded and the other electrode, closer to the end of the glass tube, was the powered one (see Fig. 1.). The calculated mean power transmitted to the plasma was 4 W and the flow rate of the feeding gas (He) was 4 slm. The distance between the powered electrode and the end of the glass tube was varied and ICCD images were taken for distances of 7, 10, 30 and 50 mm. Voltage – current signals are shown in Fig. 2. with trigger position of 11.2 μ s.

In order to obtain the time-resolved images we have used integration on the chip because the light emission in a single shot is not always sufficient to obtain clear images with gate widths of less than 25 ns. In Fig. 3. (A, B, C and D) we can see time-resolved image of plasma jet obtained by ICCD camera for 4 different electrode distances to the end of the tube while the rest of the geometry is unchanged. All images were obtained for the same parameters of electrical circuit and ICCD camera settings. It is shown that, when the distances of electrodes to the edge of the tube are shorter, the

plasma bullet is formed (see Fig. 3. – A, B and C). At the same time there is no visible discharge in the powered electrode.

When plasma is moving through the tube, including both electrodes, it is at a much lower speed than the speed of the bullet. With an increase of the distance of the electrodes from the edge of the tube plasma bullet is formed but with lower emission intensity (Fig. 3. – C). For the longest distance bullet is not emerging from the plasma jet body throughout the whole cycle period of 12.5 μs . (Fig. 3. – D) and it simply dissipates.

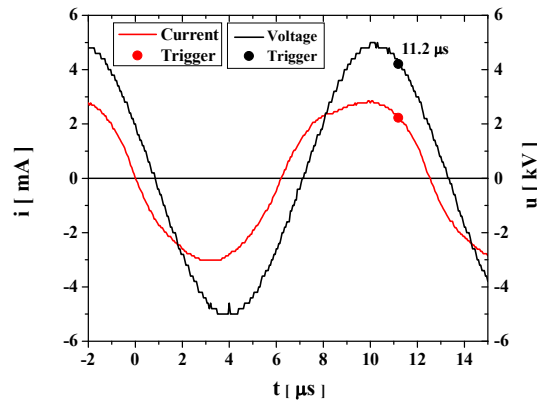


Fig. 2. Current – Voltage signals with 11.2 μs trigger position

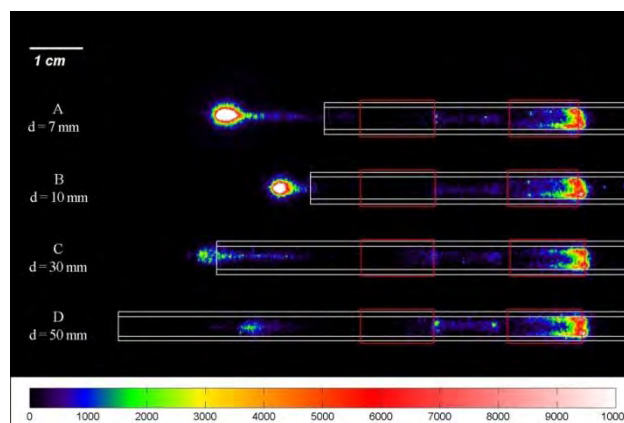


Fig. 3. Time resolved ICCD images for different geometry, delay of 11.2 μs , helium flow rate of 4 slm and average applied power of 4 W. Color bar represents intensities of emission.

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various properties of negative planar fronts. We discuss the practical and theoretical aspects of applicability of each fluid model.

¹Dujko *et al.*, *J. Phys. D* **46**, 5202 (2013).

11:45

DT2 8 Investigating the guiding of streamers in nitrogen/oxygen mixtures with 3D simulations* JANNIS TEUNISSEN, *Centrum Wiskunde & Informatica, The Netherlands* SANDER NIJDAM, *Eindhoven University of Technology, The Netherlands* EIICHI TAKAHASHI, *National Institute of Advanced Industrial Science and Technology, Japan* UTE EBERT, *Centrum Wiskunde & Informatica and Eindhoven University of Technology, The Netherlands* Recent experiments by S. Nijdam and E. Takahashi have demonstrated that streamers can be guided by weak pre-ionization in

nitrogen/oxygen mixtures, as long as there is not too much oxygen (less than 1%). The pre-ionization was created by a laser beam, and was orders of magnitude lower than the density in a streamer channel. Here, we will study the guiding of streamers with 3D numerical simulations. First, we present simulations that can be compared with the experiments and confirm that the laser pre-ionization does not introduce space charge effects by itself. Then we investigate topics as: the conditions under which guiding can occur; how photoionization reduces the guiding at higher oxygen concentrations and whether guided streamers keep their propagation direction outside the pre-ionization.

*JT was supported by STW Project 10755, SN by the FY2012 Researcher Exchange Program between JSPS and NWO, and ET by JSPS KAKENHI Grant Number 24560249.

SESSION DT3: EFFECTS OF PLASMAS ON BIOLOGICAL CELLS

Tuesday Morning, 4 November 2014; Room: State D at 10:00; Mounir Laroussi, Old Dominion University, presiding

Invited Papers

10:00

DT3 1 Application of atmospheric plasma sources in growth and differentiation of plant and mammalian stem cells* NEVENA PUAC, *Institute of Physics, University of Belgrade*

The expansion of the plasma medicine and its demand for in-vivo treatments resulted in fast development of various plasma devices that operate at atmospheric pressure. These sources have to fulfill all demands for application on biological samples. One of the sources that meet all the requirements needed for treatment of biological material is plasma needle. Previously, we have used this device for sterilization of planctonic samples of bacteria, MRSA biofilm, for improved differentiation of human periodontal stem cells into osteogenic line and for treatment of plant meristematic cells. It is well known that plasma generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) that strongly affect metabolism of living cells. One of the open issues is to correlate external plasma products (electrons, ions, RNS, ROS, photons, strong fields etc.) with the immediate internal response which triggers or induces effects in the living cell. For that purpose we have studied the kinetics of enzymes which are typical indicators of the identity of reactive species from the plasma created environment that can trigger signal transduction in the cell and ensue cell activity. In collaboration with Suzana Zivkovic, Institute for Biological Research "Sinisa Stankovic," University of Belgrade; Nenad Selakovic, Institute of Physics, University of Belgrade; Milica Milutinovic, Jelena Boljevic, Institute for Biological Research "Sinisa Stankovic," University of Belgrade; and Gordana Malovic, Zoran Lj. Petrovic, Institute of Physics, University of Belgrade.

*Grants III41011, ON171037 and ON173024, MESTD, Serbia.

10:30

DT3 2 Minimally-Invasive Gene Transfection by Chemical and Physical Interaction of Atmospheric Pressure Plasma Flow*

TOSHIRO KANEKO, *Department of Electronic Engineering, Tohoku University*

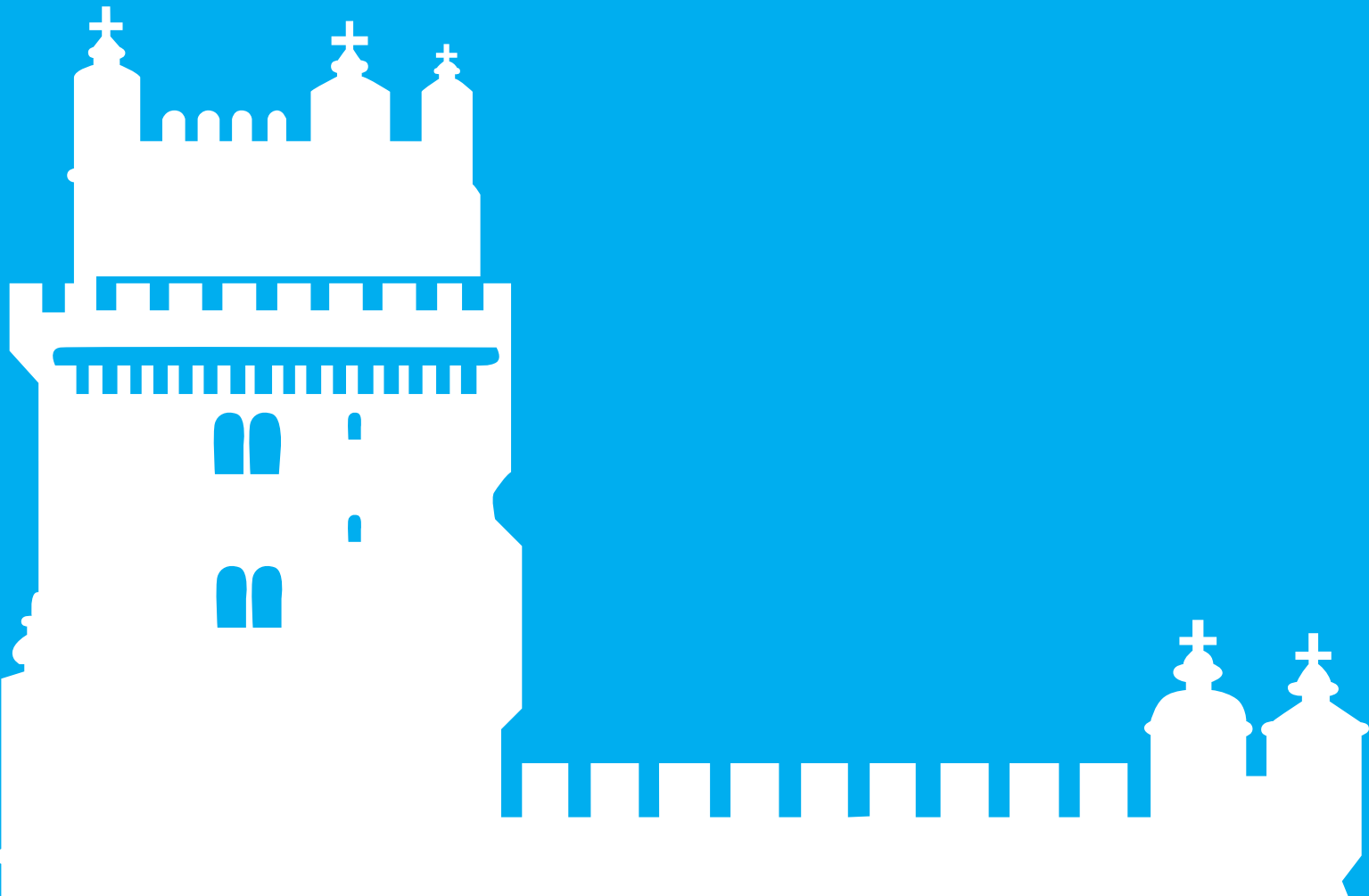
Non-equilibrium atmospheric pressure plasma irradiated to the living-cell is investigated for medical applications such as gene transfection, which is expected to play an important role in molecular biology, gene therapy, and creation of induced pluripotent stem (iPS) cells. However, the conventional gene transfection using the plasma has some problems that the cell viability is low and the genes cannot be transferred into some specific lipid cells, which is attributed to the unknown mechanism of the gene transfection using the plasma. Therefore, the time-controlled atmospheric pressure plasma flow is generated and irradiated to the living-cell suspended solution for clarifying the transfection mechanism toward developing highly-efficient and minimally-invasive gene transfection system. In this experiment, fluorescent dye YOYO-1 is used as the simulated gene and LIVE/DEAD Stain is simultaneously used for cell viability assay. By the fluorescence image, the transfection efficiency is calculated as the ratio of the number of transferred and surviving cells to total cell count. It is clarified that the transfection efficiency is significantly increased by the short-time (<4 sec) and short-distance (<40 mm) plasma irradiation, and the high transfection efficiency of 53% is realized together with the high cell viability (>90%). This result indicates that the physical effects such as the electric field caused by the charged particles arriving at the surface of the cell membrane, and chemical effects associated with plasma-activated products in solution act synergistically to enhance the cell-membrane transport with low-damage.

*This work was supported by JSPS KAKENHI Grant Number 24108004.

ICPIG 2017

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ON PHENOMENA IN IONIZED GASES

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Influence of humidity on formation of pulsed atmospheric pressure plasma streamers

N. Selaković¹, J. Voráč², N. Puač¹, G. Malović¹, P. Dvořák² and Z. Lj. Petrović^{1,3}

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Atmospheric pressure plasma jet (APPJ) falls into one of the most promising non-equilibrium low temperature plasma sources which are convenient for multiple applications. In order to achieve the best possible results in applications and explain the mechanisms that lead to the modification of the samples it is necessary to perform a detailed diagnostics of plasma source. Many studies showed that the low-frequency plasma jet's plume is made of fast pulsed atmospheric pressure plasma streamers (PAPS). In this study we show that the change in the concentration of water vapour within the tube, where the feeding gas flows, significantly affect the formation of PAPS.

1. Introduction

The expansion of low temperature atmospheric pressure plasma sources used in the treatment of heat-sensitive samples carries step forward in future bio technologies, methods of healing, etc. These kind of plasmas are particularly suitable for treatment of the samples that do not tolerate vacuum and, more importantly, they produce a huge number of reactive chemical species in its composition.

We have designed and performed detailed diagnostics of atmospheric pressure plasma jet sources with several types of electrode geometries [1]. It is shown that the formation and propagation of PAPS is influenced by electrode geometry, but also by the presence of the water vapour in the helium flow. The propagation of PAPS as a function of humidity of working gas was observed by using an ICCD camera.

2. Experimental set-up

In this experiment we used APPJ [2] that operates at 80 kHz and at 6.5 kV of applied voltage. We have used transparent PET foils coated with indium tin oxide as the powered and the grounded electrode (15 mm wide). The electrodes were wrapped around the Pyrex glass tube (O.D. 6 mm and I.D. 4 mm). As a feeding gas we have used 4 slm of helium and mixture of helium and water vapour. To perform humidity measurements within the flow tube we set up Vaisala DMT143 dewpoint transmitter in front of the glass tube. For PAPS evolution we set up the ICCD camera that recorded the discharge axially along the glass tube and the plume.

3. Results

During the active discharge (water vapour not added in mixture) we noticed that the humidity measured in the helium flow is decreasing and the concentration of H₂O molecules changes from 400 to 25 ppm. Around 100 ppm of H₂O we observe shorter range of PAPS. At the concentration of 30 ppm PAPS starts to lose its original shape and it becomes increasingly blurred. At concentration of about 20 ppm, the PAPS appear blurred. On the contrary, a high concentration of water molecules (above 1000 ppm obtained with mixture of helium and water vapour) creates a saturated environment in which discharge starts to be quenched.

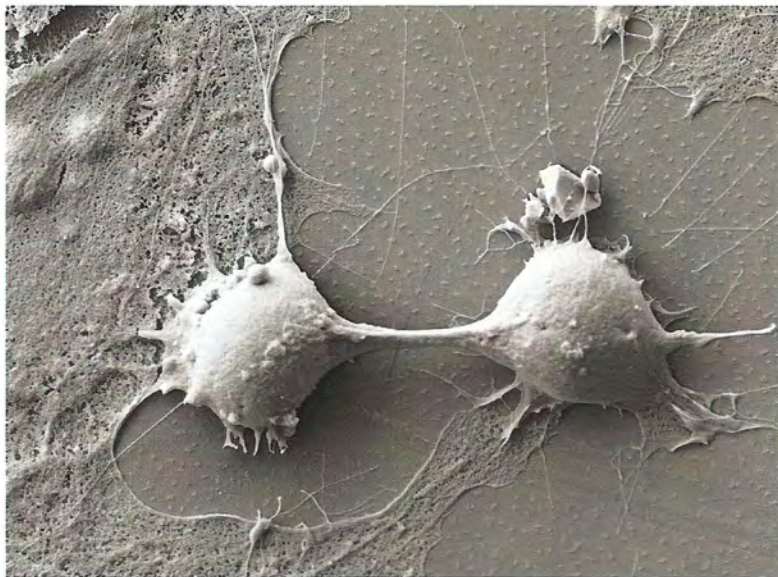
This research has been supported by the MESTD Serbia, project III41011 and ON171037 and project LO1411 (NPU I) funded by the Ministry of Education Youth and Sports of the Czech Republic

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69TH IUvSTA WORKSHOP ON
OXIDATION OF ORGANIC MATERIALS BY
EXCITED RADICALS CREATED IN NON-
EQUILIBRIUM GASEOUS PLASMA



Book of abstracts



69TH IUUVSTA WORKSHOP ON OXIDATION OF ORGANIC MATERIALS BY EXCITED RADICALS CREATED IN NON- EQUILIBRIUM GASEOUS PLASMA

ABSTRACTS

December 9th — December 13th 2011, Crklje na Gorenjskem, Slovenia

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Plasmait



Properties and bio-medical applications of non-thermal plasma

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Understanding of the complex mechanisms of interaction between the plasma reactive species and cells is among the major tasks in plasma medicine [1, 2]. Results show that treatment with atmospheric plasma can either improve the growth and development of cells and in some cases induces cells death [3, 4]. In order to investigate this phenomenon, we have used plant callus cells as a model of eukaryotic cells, due to their distinctive features and simplicity in handling. After the plasma treatment with different combination of discharge parameters which yield different plasma parameters (densities of charged species and neutrals, electron energies, UV radiation intensity), we have performed surface analyses (XPS) in order to determine plasma effects on the surface. Consequently we have monitored growth and viability of the callus cells (fresh weight increase, MTT test, fluorescent vital staining techniques).

Plasma treatment of plant tissue is demonstrated on fresh plant calli of *Iris germanica* var. "HP" (fam. Iridaceae), about 3 mm diameter in size. Calli were grown on Murashige and Skoog (MS) solid medium [5], containing 30 g-l⁻¹ sucrose, 7 g-l⁻¹ agar, 0.1 g-l⁻¹ myo-inositol, 0.1 mg-l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D), 0.1 mg-l⁻¹ 1-naphthaleneacetic acid NAA, 1 mg-l⁻¹ kinetin, 0.25 g-l⁻¹ proline and 0.25 g-l⁻¹ casein. Callus is a distinctively organized mass of proliferating cells, with specific morphology and anatomy, and may be obtained from almost any type of plant. According to the explants origin (and type of medium) compact or friable calli may be formed [6].

Plasma needle setup used in our previous research [7] was used to treat calli cells. Temperature was monitored not to exceed 40°C and it was found that there is no influence of the helium gas flow and plasma generated UV light (through the quartz window).

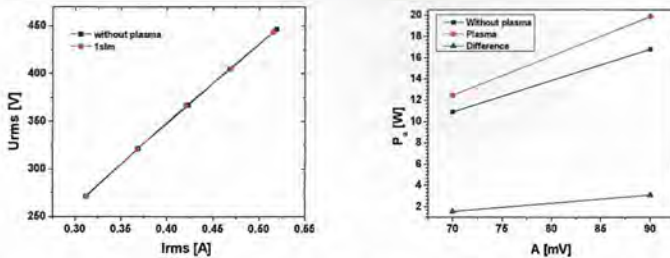


Fig.2. a) V_{rms} as the function of I_{rms} ; b) Average power delivered to the plasma (blue line).

Voltage-current characteristics show that the discharge is operating in alpha regime. Derivative probes were used to determine the power delivered to the plasma (see Fig.2. b) blue line). Low powers were used in order to avoid the sample overheating to more than 40°C. It was also found that there is no influence of the helium gas flow and plasma generated UV light.

After the plasma treatment, calli were stained or transferred to fresh half strength MS solid medium ($\frac{1}{2}$ of MS salts and vitamins) medium without growth regulators, in order to determine the plasma influence on the fresh weight of the calli. Fresh weight increase of the samples was measured every 7 days during six weeks. Evans blue stain was used for determination of cell death. Calli were transferred to a 2 ml plastic Eppendorf tube and submerged in 0.5 ml of 0.25% Evans blue for 20 min. This led to nonpermeating or exclusion dye leak through ruptured membranes and stained the content of the death cells. Calli were drained and rinsed by distilled water until no further dye eluted from the cells. Untreated plants and calli treated by absolute ethyl alcohol for 6 h represented control and negative control, respectively. Stained calli were observed using light microscopy. Staining of the plant material were repeated six weeks after the plasma treatment using the same protocol. Calli were grown under 16 h day/8 h night photoperiod, light intensity 50 ($\text{mol m}^{-2} \text{s}^{-1}$), and

temperature 25 ± 1 °C. Each treatment was performed in 3 replicates and each experiment was replicated twice.

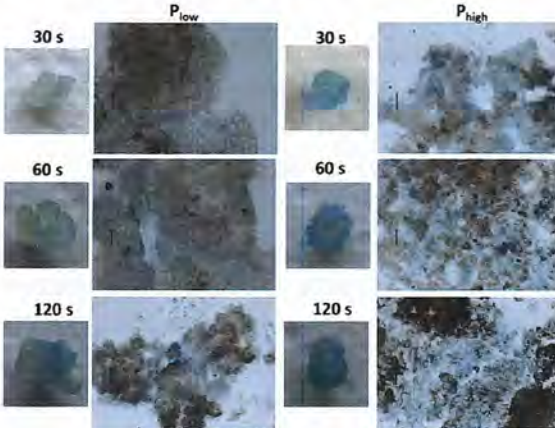


Figure 2. Plasma treatment of iris calli. Samples were stained with 0.25% Evans blue solution for 20 minutes, washed and observed using light microscopy. (Bar = 100 μ m).

Parameters such as the power delivered to the plasma, temperature, distance, gas flow rate were measured and optimized so that the treatment of calli of *Iris germanica* var. “HP” induced minimal injury of the surface plant cells layer, and calli continued their growth. Plasma needle treatment causes enhancement of the fresh weight of the iris calli. Moreover, values of the measured parameter significantly increased with the longer exposure times compared with the untreated samples. Increase of the fresh weight is an implication of calli growth accomplished by a combination of cell division and enlargement. Plasma treatment triggered the enhanced growth of the calli, probably influencing the cell division processes. The cells that divide repeatedly remains essentially meristematic (undifferentiated). These cells are small and oval, forming specific meristematic zones or centers. These zones were not observed in control samples. Furthermore, the XPS results show the increase of O/C ratio which is a sign of surface oxidation of calluses.

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23rd Europhysics Conference on Atomic and Molecular Physics of Ionized Gases



Proceedings

July 12-16, 2016 Bratislava, Slovakia

Proceedings of the 23rd Europhysics Conference on the Atomic and Molecular Physics of Ionized Gases, Bratislava, Slovakia, 12-16 July 2016.

Symposium organised by Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava; Society for Plasma Research and Applications in cooperation with European Physical Society and with support of the Ministry of Education, Science, Research and Sport of the Slovak Republic in hotel Saffron, Bratislava Slovakia, 12-16 July 2016.

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Modification of the dentin surface of human teeth by atmospheric pressure plasma needle

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In this study we have performed surface modification of dentin in extracted human teeth by a non-thermal atmospheric pressure plasma treatment. The plasma device that was used in the experiments was plasma needle. The treatments were performed for different powers transmitted to the plasma and at different plasma-to-sample distances. The results show that plasma treatments lead to significant reduction of the contact angle and increase in the surface energy. The focus of this modification was the improvement of surface properties relevant for tooth tissue interaction with dental adhesives.

Plasma needle is one of the most promising low temperature atmospheric pressure plasma sources with a broad spectrum of possible applications in material science, medicine and biology [1-3]. In the experiments presented here we have used plasma needle device to treat the exposed dentin surface of human teeth. The body of the plasma needle is made of Teflon. Inside the body there is a Pyrex glass tube which encompasses a ceramic tube. The central electrode passes through the ceramic tube and exits 1 mm outside of the tube. Helium flows between the glass and ceramic tube with the flow of 1 slm, which was held constant during the entire experiment. The plasma discharge was obtained with sine wave excitation signal at a frequency of 13.56 MHz. The discharge appears as a weak glow with a diameter of 1-3 mm at the tip of the central electrode depending on the distance from the target.

Dentin samples used in the experiment were 1 mm thick discs, cut midcoronally from the crowns of non-cariou third molars. Prior to the treatment each sample was stored in distilled water. Immediately before exposing to the discharge they were blot-dried to prevent the contact of plasma with any remaining water on the sample surface.

During the treatments the distance between the plasma needle tip and the surface of the sample was maintained constant at 2 mm, 5 mm or 8 mm, respectively. The power of plasma delivered to the dentin surface was 1 W and 3 W for all distances. The exposure time in case of each treated sample was 30 s. As a control we used untreated samples prepared in the same manner as the treated.

We used DSLR Nikon D7100 with Nikkor Micro 105mm f/2.8D lenses and Nikon Auto extension ring PN-11 in order to obtain images of liquid drops profiles on the dentin surface. These images were used in order to determine contact angles. We used distilled water, ethylene glycol and diiodomethane as the reference liquids.



Fig. 1: Images for the contact angle for the three reference liquids: (a) distilled water, (b) ethylene glycol and (c) diiodomethane

In the Fig. 1, we can see the profile of liquid drops that were placed on the untreated and treated (8 mm, 3 W) dentin surface. One can see that after the plasma needle treatment of dentin the contact angle decreased (compare upper and lower part of Fig 1.) and at the same time surface energy drastically increased. Obviously, plasma needle treatment causes physico-chemical changes in the micromechanical structure of dentin. These changes lead to an increase of the surface energy with a potential to allow better interaction between dentin and adhesive systems used in restorative dentistry.

This research has been supported by the Ministry of Education, Science and Technological Development Serbia, project III41011 and ON171037.

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MASS SPECTROSCOPY AND ICCD ANALYSIS OF COUPLED AND UNCOUPLED MODE IN A GATLING-GUN LIKE PLASMA SOURCE

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A novel type of plasma source, composed of an array of seven plasma jets arranged adjacent to one another similar in shape to a Gatling machine gun, has been recently developed to take advantage of the jet-to-jet coupling phenomenon and generate atmospheric pressure cold plasmas with higher intensity and energy with respect to singular plasma jets¹. This source can be operated either in “uncoupled” mode, where seven plasma jets are independently produced, or in the “coupled” mode, where plasma jets merge in a single combined very intense jet. Previous experiments demonstrated a higher antibacterial potential and surface activation efficacy of this source when operated in the coupled mode than in uncoupled one².

In the present study, the coupling phenomenon occurring in a *Gatling* plasma source driven by high voltage sinusoidal waveforms (80 KHz, up to 6.3 kV_{peak-peak}) was investigated using mass spectrometry, iCCD imaging and electric measurements. Different modes of operation were achieved by varying the feeding gas (He) flow between 2 slm (coupled mode) and 7 slm (uncoupled mode). A molecular beam mass spectrometer (HIDEN HPR60) was used to detect mass spectra of plasmas. Residual gas analyses (RGA) mode and secondary ion mass spectrometry mode (SIMS+/-) were used to investigate the mass spectra of neutral species and of positive and negative ions respectively, for different sets of operating conditions in both coupled and uncoupled mode. ICCD analysis and electrical characterization were also performed to investigate the temporal evolution of the plasma structure in the two modes.

Results shows that in the coupled mode ion concentrations are at least one order of magnitude higher as compared to the uncoupled mode for similar operating conditions. Consistently, ICCD acquisitions shows higher emission intensity in coupled than uncoupled mode and differences in plasma front propagation.

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27th Summer School and International Symposium on the Physics of Ionized Gases

August 26-29, 2014, Belgrade, Serbia

CONTRIBUTED PAPERS

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**ABSTRACTS OF INVITED LECTURES,
TOPICAL INVITED LECTURES, PROGRESS
REPORTS AND WORKSHOP LECTURES**

Editors:

Dragana Marić

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Belgrade, 2014

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METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* INHIBITION ZONE AREAS OBTAINED BY A PLASMA NEEDLE TREATMENT

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Abstract. Plasma needle, nonequilibrium plasma source that operates at atmospheric pressure, was used for treatments of methicilin resistant *Staphylococcus aureus* (MRSA). Free radicals and other ion species formed inside the gas mixture of helium and air in the plasma needle discharge are cause for the inactivation of bacteria. In this paper we will show how the inhibition zone areas depend on the applied plasma power, distance of the plasma source relative to the sample and the exposure time.

1. INTRODUCTION

The expansion of the relatively new field of medicine, plasma medicine, is reflected in the development of new plasma devices operating at atmospheric pressure that can be used in the treatment of biological samples. Some of these atmospheric plasma sources, such as plasma jet, plasma needle, DBD and plasma torch [1-4] have been already used and investigated for possible application in sterilization of wounds and medical equipment, treatment of dental caries, faster coagulation of blood, inactivation of cancer cells [5-8], etc.

Plasma needle, originally introduced by Stoffels [9], is atmospheric pressure plasma source suitable for the treatment of biological samples. We have developed slightly advanced version of the plasma needle and, so far, used it for sterilization of planctonic samples of bacteria, differentiation of human periodontal stem cells into osteogenic line and for plant stem cells – calli. [10-12]. Plasma needle is mild source of plasma, but with abundant chemistry that is essential for successful treatments. One of the most important limits that has to be determined is the area of the treatment i.e. the range of the radical and ion species from the plasma. Therefore, we have used MRSA samples in order to determine

zone of inhibition for different treatment times, powers and distances of the source from the sample.

2. EXPERIMENT

Plasma needle is made of glass tube with o.d. of 6 mm and i.d. of 4 mm. Ceramic tube (o.d. ~1 mm) with tungsten wire is placed inside the glass tube. Tungsten wire serves as powered electrode. Helium flows between the ceramic and glass tube. During all experiments helium flow was kept constant at 1 slm. The plasma appears on the tip as a faint glow with a diameter around 1 mm. We power this plasma device with sine wave signal at 13.56 MHz. Electrical circuit of plasma needle contains signal generator, linear amplifier and matching box. The MRSA isolate used in this study was recovered from a surgical wound and identified as MRSA by BD Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems, Sparks, MD). Bacterial suspension were prepared by suspending overnight grown culture in sterile physiological saline. The turbidity of bacterial inoculum was adjusted to 0.5 McFarland standard ($\approx 10^8$ CFU/mL) by using a Densimat photometer (BioMeriex, France). After that suspension was spread evenly on the surface of a solid growth media in Petri dishes and exposed to the plasma needle.

We divided each Petri dish into the 9 areas and each area was exposed to the treatment. The experimental parameters that we varied were distance between tip of the needle and sample surface (2 mm and 4 mm), exposure time (60 s, 180 s and 300 s) and power delivered to the plasma (1.5 W-3 W). All treatments were done in triplicate. As control we have used untreated samples and helium treated samples when there was no ignition of the discharge.

3. RESULTS AND DISCUSSION

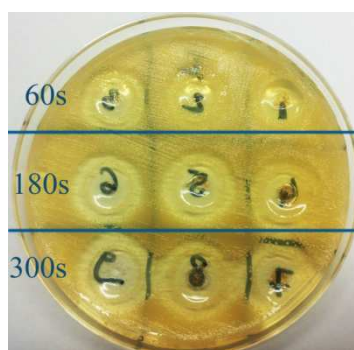


Figure 1. Treated samples of MRSA placed in Petri dish

In the Fig. 1 we can see inhibition zone areas obtained for the power of 1.5 W. Three upper fields were treated for exposure time of 60 s, the middle three fields 180 s and three fields below for 300 s. The needle in this plasma

treatment was set at a distance of 2 mm and it can be seen that the inhibition zone areas have larger diameter compared to the plasma needle.

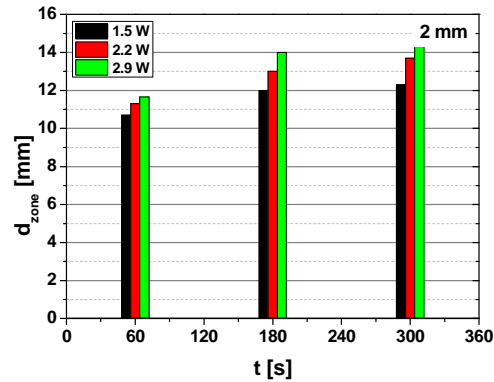


Figure 2. Comparison of MRSA inhibition area diameter for three different applied power of plasma needle and three different exposure times. The distance from plasma to sample was 2 mm.

Fig. 2 shows that the inhibition zone areas expand with the increase of the plasma power and the exposure time. Diameters of the obtained areas are in the range of 10-15 mm. We can see that there is no linear dependence of the measured diameters versus treatment time. With the increase of the treatment time diameters of the inhibition zones saturate at certain values. The higher the power saturation will occur for shorter treatment times. The largest inhibition area is obtained for maximum power 2.9 W, exposure time 300 s and the minimum distance of 2 mm.

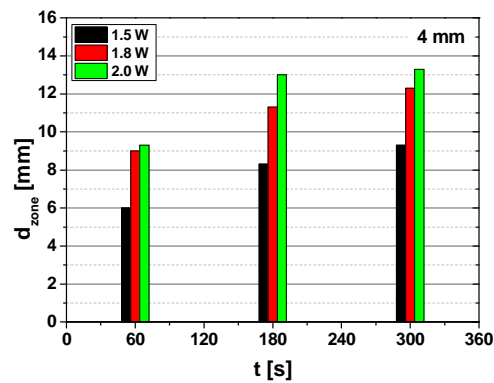


Figure 3. Comparison of MRSA inhibition area diameter for three different applied power of plasma needle and three different exposure times. The distance from plasma to sample was 3 mm.

For treatments with a distance of 4 mm we have observed smaller inhibition zones (see Fig. 3). This can be explain by following. Increase in the distance between the tip of the plasma needle and sample leads to a reduction in power and consequently we have reduced production of chemically reactive species. As in case for 2 mm distance increase in treatment time leads to saturation values of inhibition zones diameters.

4. CONCLUSION

We used the plasma needle in order to determine the surface area of the sample where the plasma makes inhibition effects. It was shown that the obtained inhibition zone areas are much larger than the size of the plasma needle.

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LOW TEMPERATURE PLASMA NEEDLE REDUCES THE SURVIVAL OF CANCER CELLS

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Abstract. The aim of this study was to investigate whether plasma needle induces cancer cell death. This atmospheric pressure plasma source generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are presumed to be major causes of cancer cell death. Here we report the influence of the power transmitted to the plasma and the exposure time on cell survival using two cancer cell lines, A549 and HeLa.

1. INTRODUCTION

The rapid development of plasma medicine, a new promising scientific field, occurred during the last decade. Using plasma physics in a wide range of sophisticated fundamental experiments involving the treatment of cells and tissues opened the door of *in vivo* therapeutic applications. Several atmospheric pressure plasma devices, such as plasma jet, plasma needle, dielectric barrier discharge (DBD) and plasma torch [1-4], have already been used for sterilization of wounds and medical equipment, treatment of dental caries and faster coagulation of blood.[5-7]

Our plasma needle is a more advanced version of the one that was originally presented by Stoffels [8] and we have used it in several applications like sterilization of planktonic bacteria, differentiation of human periodontal stem cells into an osteogenic line and for plant stem cells – calli.[9-11]

Lately, low-temperature plasmas are attracting great attention in the field of oncology [12]. In this study we used the plasma needle for the treatment of two cancer cell lines (HeLa and A549). The plasma needle discharge is a mixture consisting of reactive species and radicals that are formed in contact with the surrounding air and the treated sample. The change of power delivered to the plasma and the exposure time create different amounts of ROS and RNS that are

delivered to the treated cells, so we observed the effect on cell survival for the used set of parameters.

2. EXPERIMENT

The construction of the plasma needle was designed to be suitable for applications in direct contact with the sample. The body of the plasma needle is made of Teflon. Inside the body we put a Pyrex glass tube (o.d. 6 mm and i.d. 4 mm) through which we released 1 slm of helium as the feeding gas. A tungsten wire was used as the central electrode powered with 13.56 MHz sine wave and it was placed within a ceramic tube inside the glass tube. The role of the ceramic tube is to prevent discharge between the central electrode and the glass tube. The ceramic electrode is sticking 1 mm outside of the ceramic and glass tubes so the discharge occurs on its tip as a weak glow.

A549 (lung adenocarcinoma) and HeLa (cervical cancer) cell lines were used as representative cancer cell lines. The cells were maintained in RPMI-1640 medium (Sigma-Aldrich, Co, USA) with 10% heat inactivated newborn calf serum (Sigma-Aldrich, Co, USA) at 37°C in 5% CO₂.

The cells were seeded in 96-well cell culture plates, A549 (7000 cells/well), HeLa (4000 cells/well). We have varied two experimental parameters: exposure time (10 s, 30 s and 60 s) and power delivered to the plasma (1.3 W and 2.2 W). The distance between the plasma needle tip and the sample surface was 5 mm and we kept it constant during all experiments. The experiments were performed in triplicates, and untreated cells and medium-treated cells were used as controls.

After the treatment we have measured the cytotoxic activity of plasma needle on HeLa and A549 cell lines using an MTT assay according to the method of Mosmann (1983) and modified by Ohno and Abe (1991).

3. RESULTS AND DISCUSSION

Several different factors can affect the death of cancer cells such as the type of the cell and the type of the treatment (direct and indirect contact with the sample). Here we used the direct contact method of discharge on the sample which is more effective than the indirect contact (discharge in contact with the medium without cells).

In Fig.1 we show images of microtiter plate wells observed by optical microscopy. One can notice a reduction in the number of A549 cells between the control (untreated samples) and the treated samples for different powers of plasma (1.3 W and 2.2 W) and different exposure times (10 s, 30 s and 60 s).

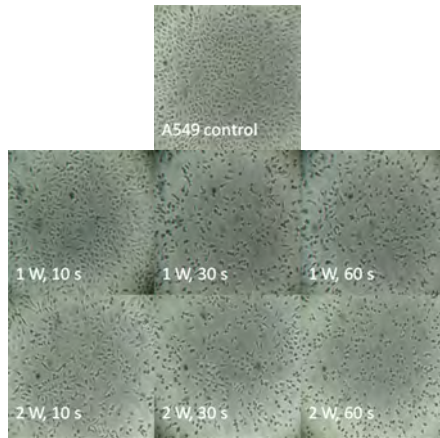


Figure 1. Images of microtiter plate wells obtained by optical microscopy for untreated A549 cells (control) and treated A549 cells for different powers of plasma and exposure times.

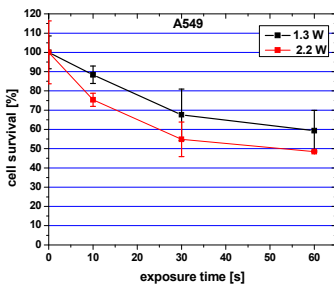


Figure 2. Cell survival comparison of A549 cells for two different applied powers and three different exposure times. The distance from plasma to sample was 5 mm.

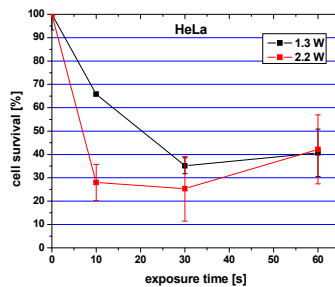


Figure 3. Cell survival comparison of HeLa cells for two different applied powers and three different exposure times. The distance from plasma to sample was 5 mm.

Fig. 2 represents a graphic view of the results of A549 cells already showed in Fig 1. and here one can notice an exponential drop caused by a longer exposure time for two different applied powers of discharge (1.3 W and 2.2 W). From the given chart it is obvious that higher power is more efficient in reducing cancer cell survival.

HeLa cells displayed greater vulnerability to the impact of plasma than A549 cells, especially for the higher power output (2.2 W) where a significant drop in cell survival (Fig. 3.) at the shortest exposure time (10 s) was observed. In both cases (1.3 W and 2.2 W), the effect of reducing cell survival was higher than 50%.

4. CONCLUSION

In this study we used our version of plasma needle for the treatment of two different carcinoma cell lines (HeLa and A549) in order to observe the effect on cell survival. Due to the different nature of the investigated cancer cell lines, which is reflected in a number of differences, among them and in the cells defense system (e.g the cell response to oxidative stress.) [13], these two lines showed different sensitivity to plasma treatment. The HeLa cells were more sensitive than A549 cells on the plasma treatment which was manifested in higher reduction of the cell survival determined by MTT assay.

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AXIAL PROFILES OF PLASMA BULLET WITH DIFFERENT ELECTRODE GAPS

Nenad Selaković¹, Dejan Maletić¹, Nevena Puač¹, Saša Lazović¹,
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Abstract. We used fast ICCD imaging in order to record time evolution of plasma bullet formation. Plasma jet was made of Pyrex glass tube with two transparent electrodes made of polyester (PET) foil whose position can be easily adjusted. The excitation voltage was approximately 10 kVpp at frequency of 80 kHz. The power transmitted to the plasma in all measurements was 4 W. The working gas was helium with constant flow rate of 4 slm. In this paper we shall show the total axial light emission profiles of plasma jet bullets obtained for three different electrode gaps, of 10, 15 and 20 mm.

1. INTRODUCTION

One of the main reasons for the development of new low temperature plasma sources which are working at atmospheric pressure is their simple, non-expensive design with great potential for possible applications. These plasma sources are used for treatment of polymers, cells, tissues, etc. [1-3]. One of the newest scientific fields developed from this high-end research is plasma medicine [4]. Achieving high temperatures of electrons, much higher than the temperatures of ions and ambient gas, is crucial for sustaining stable non-equilibrium plasmas. In order to reduce the breakdown voltage in these discharges, noble gases are used, usually helium or argon [5]. For powering plasma jets, various types of signals at high frequencies are used [6], as well as different geometries [7, 8]. By choosing different materials for the electrodes, different electrode gaps and sizes, one can significantly change the behavior of plasma jets and control if the plasma jet will form bullets or not. The optimal geometry parameters can be found in order to maximize the distance that plasma bullets can reach. In this paper we present axial profiles for several gaps between the powered and the grounded electrode.

2. EXPERIMENTAL SETUP

The atmospheric pressure plasma jet used in these experiments is shown in Fig. 1. Its body was made of a Pyrex glass tube (O. D. of 6 mm and I. D. of 4 mm) with two transparent thin conductive PET foil electrodes (width of 15 mm) wrapped around the glass tube. The copper foil was used to connect electrodes to an external electrical circuit. The electrode closer to the edge of the glass tube was the powered one, and the other electrode was grounded (see Fig. 1). A resistor of 100 k Ω between the grounded electrode and the ground was connected for current measurements. During all measurements, the helium flow rate was 4 slm. As the power supply, a signal generator was used, and it was connected to a home-made amplifier. The excitation signal was sinusoidal and the working frequency was 80 kHz. Since the maximum value of the voltage at the output of the amplifier is around 1 kV, it was necessary to make a high-voltage transformer to increase the signal from the amplifier up to 10 kV peak-to-peak. The calculated power transmitted to plasma in all measurements was 4 W. The gap between electrodes was 10, 15 and 20 mm. The distance between the powered electrode and the edge of the glass tube was kept constant at 7 mm for all measurements.

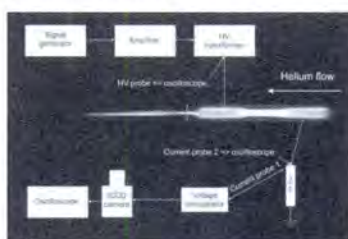


Figure 1. Experimental setup

3. RESULTS AND DISCUSSION

Signals of current and voltage, with trigger positions, are presented in Fig. 2 (left). The voltage signal has pure sine waveform, while the current signal is a somewhat deformed. Three triggering positions of ICCD imaging were selected to present behavior of plasma at different time positions of the whole cycle. The first trigger position is close to the minimum, the second one is near the zero and the third is right after the maximum of the voltage signal. Axial (along the glass tube axis) profiles of plasma emission were calculated from the obtained ICCD images (see Fig. 2 (right)-3). The presented profiles are calculated as a sum of the emission intensity coming from plasma along the axis of the glass tube diameter. In Fig. 2 (right)-3, the right edge of the grounded electrode is chosen to be the reference (zero) position, the positions of the powered and the grounded electrode are marked by vertical solid lines and the edge of the glass tube by a vertical dashed line. The direction of helium flow is from the grounded toward the powered electrode (as shown in Fig. 2 (right)-3).

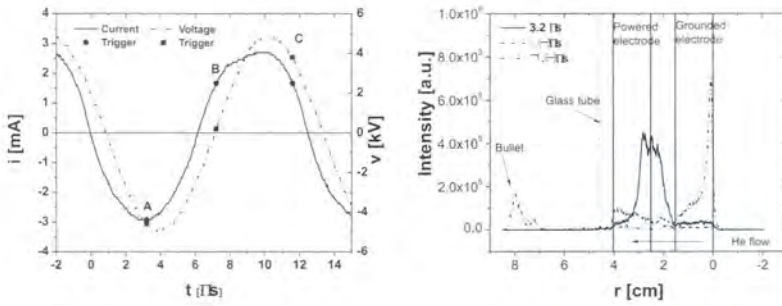


Figure 2. Current and voltage signals with trigger positions for 15 mm electrode gap (left); Axial light emission profiles for 10 mm electrode gap for three different trigger delays (3.2 μ s, 7.2 μ s and 11.6 μ s), 4 slm flow of helium and power of 4 W

Axial profiles of light emission for the gap of 10 mm between electrodes are shown in Fig. 2 (right). For the minimum of the voltage and current signal, the maximum emission intensity coming from the discharge is on the right edge of the powered electrode (Fig. 2 (right), black solid curve). At the same time, the emission intensity in other areas is several orders of magnitude smaller. With increase of the voltage and current signals, plasma is moving towards the left edge of the powered electrode and the emission intensity decreases.

For the delay of 7.2 μ s (Fig. 2 (right), black dashed curve) the intensity is very small with the maximum near the left edge of the powered electrode. A plasma bullet is formed at the maximum values of current and voltage signals and it reaches the maximum distance of 3.5 cm from the edge of the glass tube for the delay time 11.6 μ s. Also, there is another well-defined maximum at the right edge of the grounded electrode.

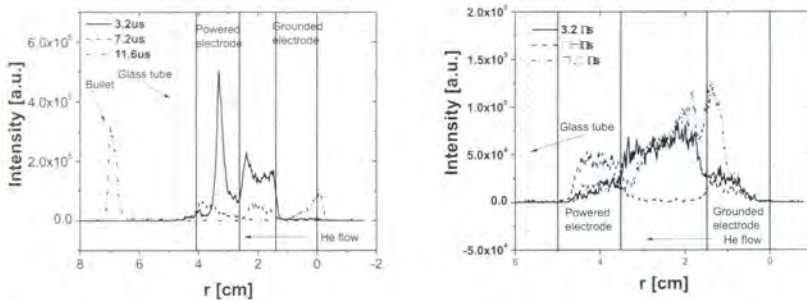


Figure 3. Axial light emission profiles for 15 mm (left) and 20 mm (right) electrode gap for three different trigger delays (3.2 μ s, 7.2 μ s and 11.6 μ s), 4 slm flow of helium and power of 4 W

Axial profiles for the gap of 15 mm are presented in Fig. 3 (left). A well-defined peak of light emission can be seen in the powered electrode, as

well as significant emission between electrodes (for the delay of 3.2 μs , solid black curve). For the delay of 7.2 μs , the intensity significantly drops in and between the electrodes (dashed black curve). For the delay of 11.6 μs , there are two distinct peaks: one inside the grounded electrode and the second one outside the tube approximately at 2.2 cm from the edge of the glass tube. For the 15 mm gap, the bullet is better defined than in the case of other electrode gaps used in this paper.

For the largest electrode gap in Fig. 3 (right), the emission intensity is much smaller than in other two cases. For the delays of 3.2 μs and 7.2 μs , there are no well-defined peaks. For the delay of 11.6 μs , there is no light emission outside the glass tube. This electrode gap is not suitable for treatment of surfaces because plasma does not leave the glass tube.

4. CONCLUSION

Using ICCD imaging it has been shown that the light emission is highly dependent of the electrode geometry. In this study it has been shown that if it is needed to obtain a well-defined plasma bullet, the most suitable configuration is a gap of 15 mm between the electrodes. In other two configurations, the plasma bullet is distorted or it does not leave the glass tube at all. One of the directions for future research could be to record emission profiles by using filters for different wavelengths.

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