# Study of Controlled Atmosphere Flexible Microtube Plasma Soft Ionization Mass Spectrometry for Detection of Volatile Organic Compounds as Potential Biomarkers in Saliva for Cancer

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**ABSTRACT:** A new soft ionization device for mass spectrometry is presented using the Flexible Micro Tube Plasma under controlled atmospheric conditions. The Controlled Atmosphere-Flexible Micro Tube Plasma consists of the plasma source itself, connected to a gas chromatograph and a mass spectrometer using a borosilicate glass cross piece. Controlled atmosphere, for example nitrogen and/or oxygen mixture is introduced to the system to create a clean ionization environment. Reproducibility issues are discussed and solutions are presented manipulating the gas flow in the cross piece. A proof of concept is shown using a ketone mixture introduced to the mass spectrometer to optimize atmospheric conditions. Furthermore, the application of the presented device for the sensitive and non fragmenting ionization of volatile organic biomarkers relevant for cancer is carried out. Sample treatment for human saliva is described and relevant candidate biomarkers are measured in the saliva matrix, showing very good ionization efficiency and neglectable matrix effects with limits of detection below 80 ppt

## Introduction

Within the last decade, atmospheric pressure plasmas are widely investigated within the research community because of their application as soft ionization sources for mass spectrometry (MS)[1-14]. Many different approaches to increase the sensitivity and quality of these plasma based ion sources have been proposed, such as manipulating the plasma gas[15], the geometry[16] or the distance of mass spectrometer to the ionization source[17]. For the dielectric barrier discharge ionization (DBDI) source we recently demonstrated a significant increase in sensitivity and subsequently significantly improved limits of detection when using a controlled atmosphere (CA) setup[18]. This previous study was carried out using fluorinated compounds as model analytes in order to compare the CA-DBDI with the DBDI at open atmosphere. Within this study, two advantages were found: The controlled atmosphere significantly decreased the chemical noise in the mass spectrometer and additionally the atmosphere could be tailored for a certain class of analytes, thus it could manipulate the chemical reaction pathways as it is beneficiary for the desired ionization process.

While plasma based ionization sources have been improved within the last decade, also the challenges in their applications have become more complicated. [19].In order to identify and measure biomarkers, e.g. for cancer, biological or biochemical samples have to be measured. These samples are usually very complicated biological matrixes, such as urine [20,21], blood[21] or saliva[22], leading to a complex mass spectrum because of interfering species. In the case of volatile organic compounds (VOCs), the standard method to identify and quantify biomarkers using a gas chromatography MS setup is electron ionization mass spectrometry (EI-MS). It covers a broad range of analytes with different polarities. Due to the high electron energy in the order of 70 eV, necessary for this method, the observed ions are usually fragmented. [23,24]. Although data bases are widely accepted for data analysis, soft ionization of these analytes is desirable to simplify data analysis. Analyzing different representative ketones with electron impact ionization for example can lead to the same fragmentation pattern for different analytes. Soft ionization mass spectrometry as it is presented in this publication may significantly simplify the identification of each analyte, leading to better and more reliable detection methods.

With this setup, observed analytes are marginally fragmented and therefore more easily identified. Because of the controlled atmosphere, the influence of the surrounding atmosphere can be neglected, leading to less matrix effects in the measurement. The use of FμTP[16]

allows to reduce the helium consumption by one order of magnitude and leads to an improvement in safe handling of the device because the high voltage electrode is fully covered by the tubing.

The development of this new setup is presented, including design issues for a long time reproducibility. This section is followed by an optimization part for the controlled atmosphere that is used. Finally, the application of this setup to a complicated matrix, as saliva, is shown measuring [22]The method presented in this work reaches limits of detection between 1.1 ppb and 80 ppt with negligible matrix effect.

**Experimental setup**[1616]

In this setup, a borosilicate glass cross piece is used to connect MS inlet, FμTP, CA and GC-column in a center interaction point as it is shown in figure 1a. The inner diameter of the cross piece is 2.4 mm with an outer diameter of 3.6 mm. The FμTP is driven with a rectangular voltage of 2.0 kV peak to peak with a frequency of 20 kHz. The cross piece is connected to FμTP, CA and GC-column using Swagelok connectors (PFA-420-6-2). This gives the possibility to easily adapt the setup to any necessary change. In order to create a gas tight atmosphere, the cross piece is installed on an in-house-built stage that is mechanically connected to the mass spectrometer. Using an XYZ-stage, the cross peace is pressed against the mass spectrometer inlet. This connection is made gas tight using an o-ring in the connection point. Helium flow through the FμTP is 100 sccm, CA can be varied between 0 and 2000 sccm. If not mentioned, CA-flow was always 500 sccm with a mixture of 80% nitrogen and 20% oxygen. GC conditions are explained in section Gas Chromatography. In figure 1a, a constriction between the gas flow crossing point and the mass spectrometer is shown. As this figure shows a cross section of the setup, the constriction in the cross point (z-axis) cannot be seen. The mentioned constrictions are explained in the next section.

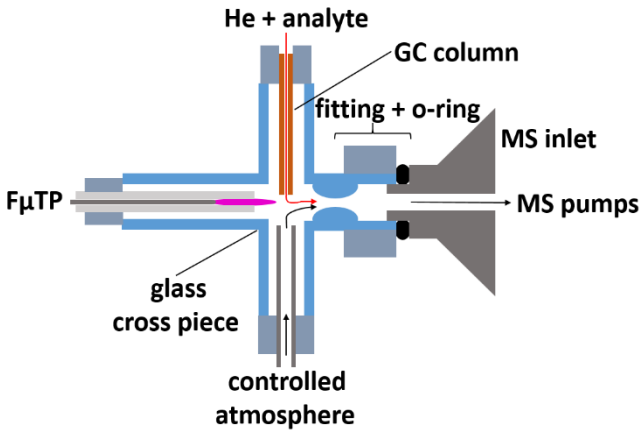


**Figure 1. a**Scheme of glass cross piece connecting FμTP, GC-column and mass spectrometer under controlled atmospheric conditions. Analytes introduced by GC-column are ionized by FμTP and measured by MS.

Reproducibility of the system

When we first carried out measurements under controlled atmospheric conditions, using a symmetric glass cross piece, reproducibility issues were found after running the setup for a longer period of time. After several hours, the measured signal intensity decreased significantly. In order to overcome this problem, it was crucial to understand the gas flow physics in the presented system: Three different gas flows have to be mixed together that have significantly different orders of magnitude: 2 sccm, 100 sccm and 500 sccm. This results in many different possibilities for the gas flow: An appropriate mixing might not be possible without turbulences or the flow of the controlled atmosphere might block the flow from the GC column. Keeping in mind that the flow from the CA is two orders of magnitude higher than the GC flow. Fluid dynamic simulations as presented in figure 2 can explain the problem of the gas flow dynamics: in figure 2.a and 2.b it is clearly shown, that the controlled atmosphere blocks the flow from the GC column. Due to the high pressure caused by the CA flow directly at the exit of the GC column, the flow of the GC is significantly reduced and the gas from the GC cannot enter the mass spectrometer inlet immediately. Figure 2.c and 2.d show the difference in this flows when the cross piece is constricted in the cross point (z-axis in the picture) and in the connection to the mass spectrometer (y-axis). These changes have a high influence on the gas dynamics in the system and the GC flow is no longer blocked by the CA flow and is directly reaching the mass spectrometer.

Operating the controlled atmosphere FμTP setup using the constricted cross piece led to a highly reliable reproducibility in all conducted measurements. Therefore, the presented setup in figure 1 shows the constriction in the connection to the mass spectrometer. The constriction of the crossing point perpendicular to the glass tubes is not shown in figure 1.

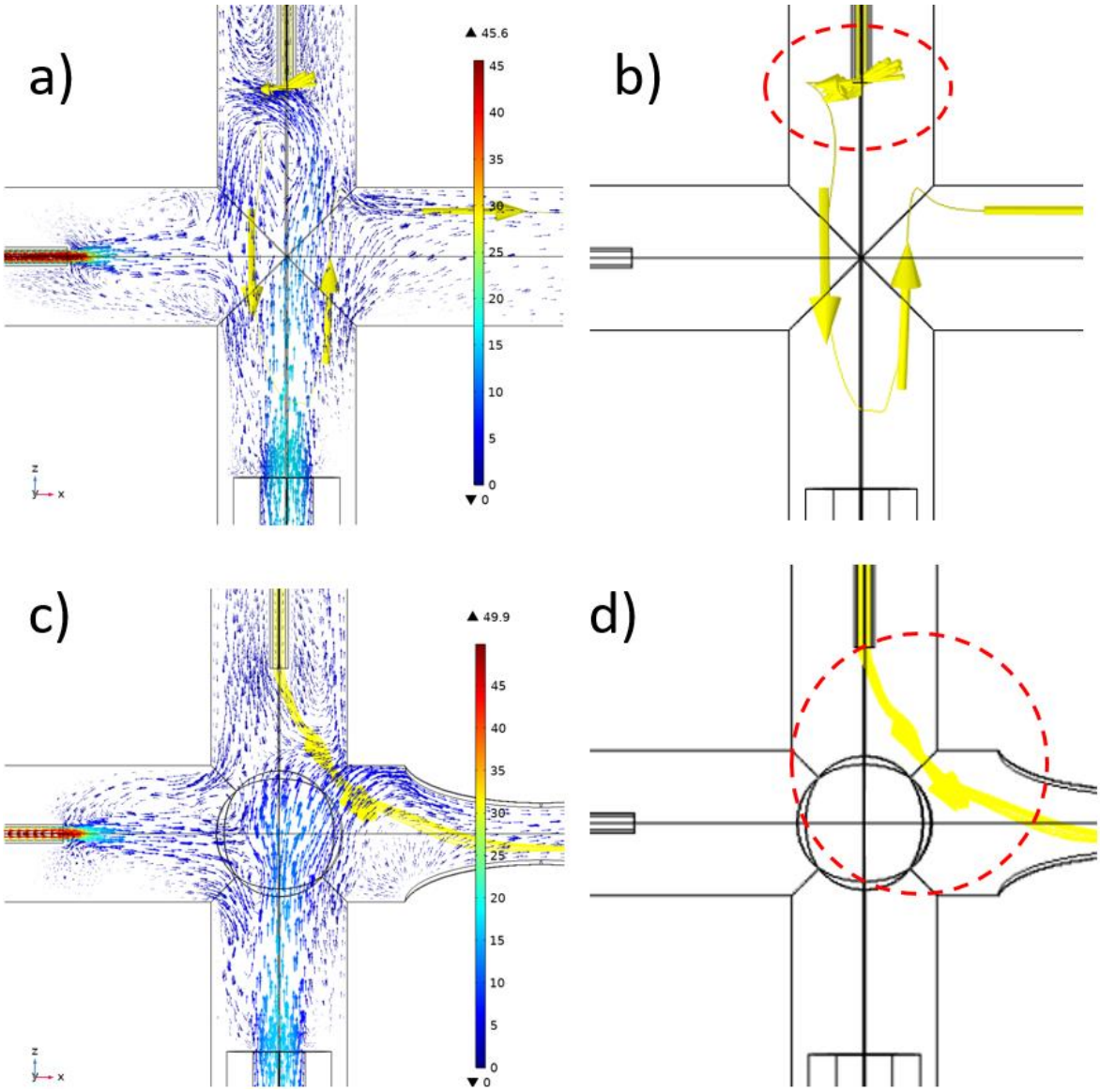


**Figure 2.** Fluid dynamics simulation of the cross pieces. a) Showing velocity distribution of the gas flows (arrows) and distribution of the analyte from the GC column (yellow) for the cross piece without any constrictions. b) Same as figure 2.a without velocity arrows to clearly demonstrate that analyte flow is nearly blocked by CA-flow. c) Showing velocity distribution of the gas flows (arrows) and distribution of the analyte (yellow) for the cross piece with constrictions. d) Same as figure 2.c without velocity arrows to clearly demonstrate that analyte flow is no longer blocked because of the made changes.

**Gas Chromatography-Mass Spectrometry**

An ion trap mass spectrometer with an atmospheric pressure inlet (Thermo Finnigan LTQ) was coupled to a gas chromatograph (Agilent Technologies 6890N) with a Restek gooseneck splitless liner (ID = 4 mm, OD = 6.5 mm, L = 78.5 mm) and 30 m Rxi-5ms standard column ( ID=0.25 mm inner diameter, 0.25 µm film thickness). 2 µL were injected by splitless mode with the injector temperature maintained at 200°C and split opened after 1 min with a purge flow of 50 mL/min. The gradient profile was as follows: the oven temperature was held at 40 °C for two minutes and then heated to 160 °C in 10 minutes. The helium flow was 2 mL/min.

**Chemicals**

*N*-hexane (purity >98%) and 2-propanol (LC grade) were obtained from Merck KGaA (Darmstadt, Germany). Analytical standard of Mesityl oxide, 1-Hexanole, 4-Methylphenole, 3-Heptanone, 1-Octen-3-ol, Phenethyl alcohol. Indole; Dimethyl sulfone, 2-Methoxythiopen, 2-

Hexanone, 3-Octanone, 2-Nonanone, and 1,2,4-Trimethylbenzene were purchased from Sigma Aldrich (ST. Louis, MO, USA). Individual standard solution of each compound was prepared in *n*-hexane or 2-propanol. Working standard solutions containing all compounds were prepared in *n*-hexane by proper dilution of the stock standard solutions.

**Sample collection and treatment**

Fresh human saliva samples were obtained from a not smoker male volunteer in our laboratory. eachand mixed 24 h period The sample was kept in the fridge at 4°C until their analysis. In order to prevent sample degradation, the sample treatment was carried the after the sample collection.

Sample treatment was as follows: 1 mL of saliva was placed on an Eppendorf tube. Then, 100 µL of *n*-hexane was added to carry out the extraction and this mixture was ultra-sonicated for 10 min. To obtain a full separation of both phases, the mixture was centrifuged at 5000 rpm for 5 min. Finally, 2 µL of the organic layer was collected and directly injected into the GC-MS system. Using this procedure, a pre-concentration factor of 10 was achieved.

**Results and discussion**

Optimization of atmospheric conditions

As many of the biomarkers mentioned by Shigeyama et al.[22] are ketones or other hydrocarbons, we decided to use a ketone mixture as model analyte in order to test the system and to optimize atmospheric conditions. Different gas mixtures were tested, such as helium/nitrogen, helium/oxygen and nitrogen/oxygen.

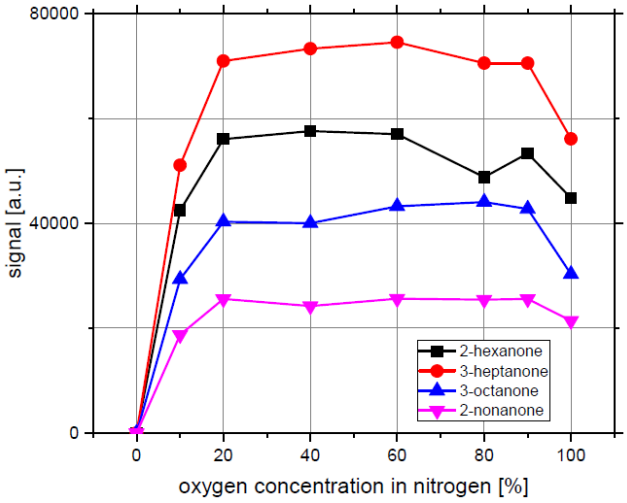


**Figure 3.** Variation of the oxygen concentration in a controlled atmosphere using ketones as model analytes to find optimal artificial atmospheric conditions for efficient soft ionization. 2 µl of a 100 ppb dilution of ketones in hexane were injected for every given oxygen concentration

A nitrogen/oxygen mixture was found to be the best for ketones. In order to find the best mixing ratio between nitrogen and oxygen, a 100 ppb ketone mix was injected into the gas chromatograph and the signal intensities for the measured ion peaks in the chromatogram were evaluated. Different mixing ratios were tested, starting at 100 % nitrogen and then successively increasing the oxygen amount up to 100 % oxygen. The results are shown in figure 3. Surprisingly, using pure nitrogen, no ions were measured in the experiment. Adding a small amount of oxygen directly led to a significant ionization rate, resulting in a saturation effect at approximately 20 % of oxygen in nitrogen. For a pure oxygen atmosphere the ionization efficiency decreased slightly compared to lower oxygen amounts. As ketones are measured as $[M+H]^+$, the reaction pathway is probably dominated by the known protonation process: Following the ionization process for atmospheric pressure chemical ionization (APCI) sources, the main species involved in the production of positive ions using plasma based ionization sources are nitrogen and water, forming protonated water clusters[25].

$$N_2^+ + 2N_2 \rightarrow N_4^+ + N_2$$

$$N_4^+ + H_2O \rightarrow H_2O^+ + 2N_2$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^\bullet$$

$$H_3O^+ + H_2O + N_2 \rightarrow H^+(H_2O)_2 + N_2$$

These water clusters then collide with an analyte, resulting in a proton transfer reaction as follows:

$$M + H^+(H_2O)_n \rightarrow MH^+ + (H_2O)_n$$

All this equations do not require oxygen for the ionization process. In contrast to these equations, our measurements shown in figure 3 show clearly, that there is no ionization without adding oxygen to the controlled atmosphere. For a better understanding of figure 3 and the processes responsible for the ionization, low mass ions produced by the plasma without adding any analyte were measured while varying the oxygen concentration in the nitrogen based controlled atmosphere. The dependency of the signal on the oxygen concentration is shown in figure 4 for $NO^+$ at m/z = 30 and $(H_2O)_2H^+$ at m/z = 37. Neither $NO^+$ nor $(H_2O)_2H^+$ was produced without adding oxygen to the controlled atmosphere, which is in very good agreement with the measurements presented in figure 3.

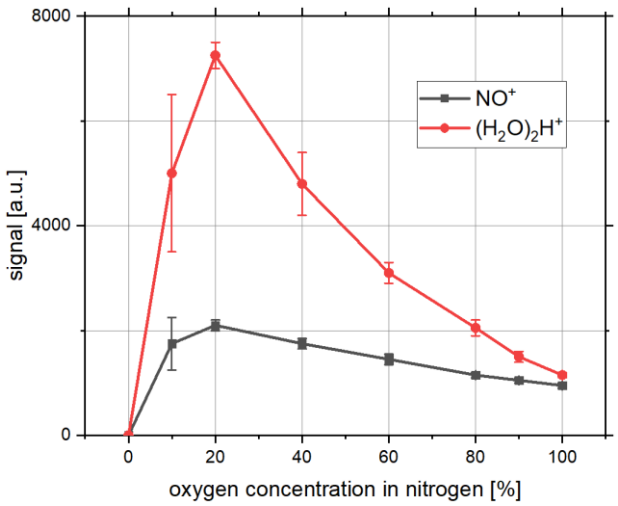


**Figure 4.** Measurement of ionized nitric oxide and protonated water clusters depending on the oxygen concentration in the nitrogen based controlled atmosphere.

Both measured ions show similar behavior depending on the oxygen concentration. Due to the fact, that nitric oxide is most probably formed by reactions with oxygen and nitrogen and the protonated water cluster ion peak shows exactly the same dependency, we suggest nitric oxide and as conclusion also oxygen to be an important species for the production of protonated water clusters in the presented setup. Comparing figure 3 with figure 4, the main difference in the curve progression is the saturation up to a concentration of 90% in figure 3 and the signal decrease in figure 4. A possible reason for this difference is very probably the low concentration of analyte injected in the measurements of figure 3: Adding a small amount of oxygen to the controlled atmosphere creates already a sufficient amount of protonated water species to ionize most of the analyte, so that a further increase of protonated water species or a decrease does not affect the measurements anymore.

Due to the maximum of both $NO^+$ and protonated water cluster production for 20% of oxygen concentration in nitrogen we decided to choose this as optimum condition for further experiments.

Analytical performance

In order to show the capability and performance of the presented setup for the determination of cancer biomarkers, standard and matrix-matched calibration curves were prepared. Concentrations from 1 to 1000 and 0.1 to 100 ppb were measured in the case of standard calibration and matrix-matched calibration, respectively. A chromatogram representing the total ion count of the mass spectrometer can be seen in figure 5, showing both the biomarkers in standard dilution and in a saliva matrix.

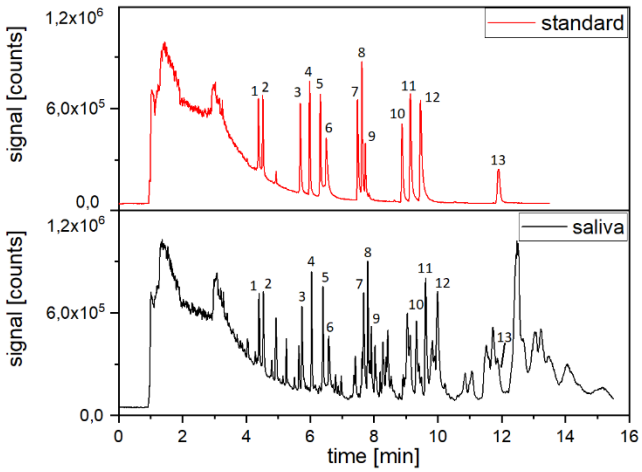


**Figure 5.** Comparison of total ion count chromatogram of 1 ppm biomarkers in hexane (standard) and 0.1 ppm of VOC potential biomarkers in a saliva matrix (1 ppm in the final extract because of the pre-concentration factor of 10). Peaks 1-13 correspond to the following analytes: 1. 2-hexanone; 2. . mesityl oxide ; 3. 1-hexanole; 4. 3-heptanone; 5. 2-methoxythiophen; 6. dimethyl sulfone; 7. 1-octen-3-ol; 8. 3-octanone; 9. 1,2,4-trimethylbenzene; 10. 4-methylphenole, 11. 2-nonanone; 12. phenethyl alcohole; 13. indole

Matrix effect (%)was calculated as Matrix effect (%) = [(slope of matrix matched calibration / slope of external standard calibration)-1] × 100..A matrix effect of zero means that signal or suppression or enhancement has not been observed. All measured compounds showed negligible matrix effects with values from -5% to -1% in all cases. Thus, high reproducibility of the system even for different sample matrixes has been shown. Also retention times are not influenced by the saliva matrix. Although many additional peaks occur for the measurements in the saliva matrix, this has hardly an influence on the detection, identification and quantification of the observed biomarkers because of the soft ionization technique resulting from the FμTP.This benefit results in a more straight forward identification of the measured m/z signals with no data bases required for data analysis. LODs are shown in table 1 with instrumental LODs from 10 ppb for 2-hexanone to 700 ppt for 3-octanone and 2-nonanone.

| Analyte |
|---|
| |
| Mesityl oxide |
| 1-Hexanole |
| 4-Methylphenole |

| | | | |
|---|---|---|---|
| 3-Heptanone | 9 | 0.7 | 2 |
| 1-Octen-3-ol | 10 | 0.7 | 3 |
| Phenethyl alcohol | | | |
| Indole | | | |
| Dimethyl sulfone | | | |
| 2-Methoxythiopen | | | |
| 2-Hexanone | | | |
| 3-Octanone | | | |
| 2-Nonanone | | | |
| 1,2,4-Trimethylbenzene | | | |

Table 1 Limits of detection for biomarkers in both standard dilution and saliva matrix.

Although 2-hexanone, 3-heptanone, 3-octanone and 2-nonanone are all ketones, the instrumental LODs for 2-hexanone and 3-heptanone are significantly higher compared to 3-octanone and 2-nonanone. This is due to the fact that the solvent hexane causes a broad background in the signal from approximately 1 minute to 5 minutes. Most relevant retention times for this background are between 1 and 3 minutes, but between 3 and 5 minutes the noise of this background still affects LODs for compounds that have an $[M+H]^+$ in the range of 80-120 amu, as it is the case for 2-hexanone and 3-heptanone. As 3-octanone and 2-nonanone have retention times higher than 5 minutes and are measured at 129 m/z and 143 m/z respectively, they are not influenced by this effect and show excellent limits of detection in the ppt range.

Soft ionization compared to electron impact ionization

As it was already mentioned in the introduction of this publication, the main advantage of soft ionization methods compared to the standard procedure for GC-MS measurements that is currently EI ionization is to avoid the fragmentation of analyte ions. To demonstrate this advantage of the presented system, EI mass spectra from mesityl oxide found in the literature[27] is compared to the mass spectra obtained by using the presented CA-FμTP ion source. The CA-FμTP spectra for mesityl oxide is shown in figure 6.

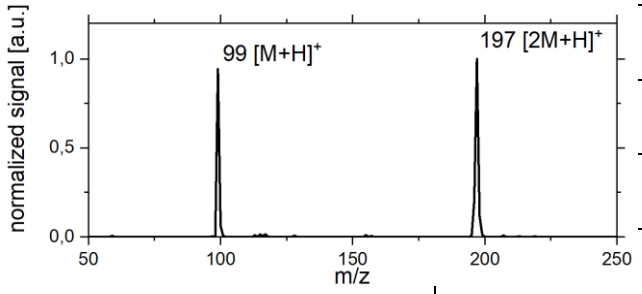


**Figure 6.** Soft ionization spectra of the CA-FμTP for 1 ppm mesityl oxide showing the $[M+H]^+$ and the $[2M+H]^+$ peak.

Comparing figure 6 to the spectra obtained in the literature[27] or that can be found in common EI MS data bases such as NIST, the potential of plasma based soft ionization becomes clear. Because there is no fragmentation, analyte identification and also quantification and sensitivity is significantly improved compared to electron impact ionization methods. In the case of mesityl oxide, the presented method is approximately 2.5 times more sensitive than the cited method taking into account that the referenced method uses a 1 μL injection and a 1:1 split ratio while the manuscript uses 2 μL injection and splitless injection. eses

Comment on application of obtained results

In the optimization section of the presented work, an optimum for soft ionization efficiency of the setup was found at a mixture of 80 % nitrogen with 20 % oxygen. As this is approximately the mixture of the ambient air, further experiments were carried out to understand the significant improvements of controlled atmosphere setups compared to ionization sources at ambient air. Ambient air consists of approximately 78 % nitrogen, 21 % oxygen, 0.9 % argon and 0.1 % other gases. Synthetic air (commercial 80 % nitrogen, 20 % oxygen) mixture was tested as well. In a next step, 1 % of Argon was added to this commercially available synthetic air and the experiment was repeated. As a last step, ambient air from the laboratory was used instead of synthetic air. Flows were always kept constant at a flow rate of 500 sccm. Results are shown in figure 7 for 100 ppb biomarker standard dilution. The 80 % nitrogen with 20 % oxygen mixture obtained by two mass flow controllers, synthetic air (only one MFC necessary) and synthetic air with Argon show similar results. All analytes could be detected. It is important to mention, that analyte 6 is not seen in the total ion count chromatogram, but can easily be detected by observing m/z=95. Using ambient air from the laboratory, biomarkers could no longer be measured at this concentration. Comparing the base line of ambient air with the base line in the other chromatograms, the base line for the ambient air measurement is 5 times higher. As a conclusion, ambient air is polluted by many different species resulting in a high chemical background that reduces the sensitivity of the system for the analytes that have to be measured. in the case of air ambient This shows the advantage of controlling

the atmosphere: A clean ionization environment reduces the chemical noise, leading to a significantly more efficient and therefore more sensitive detection of the analytes.

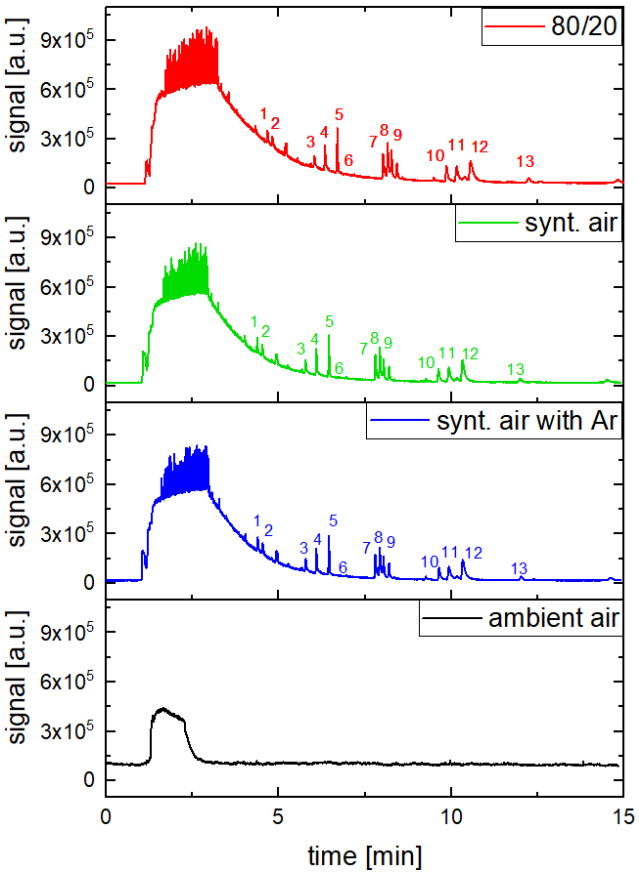


**Figure 7.** Comparison of total ion count for different controlled atmospheric conditions measuring 100 ppb of biomarker standard dilution. On-line mixing (80/20), usage of synthetic air and synthetic air with 1 % argon show similar results. Using a controlled amount of ambient air from the laboratory, a significant decrease in signal appears. Analytes could no longer be quantified or detected under these conditions. Numbers in diagram belong to the analytes as follows: 1. mesityl oxide; 2. 2-hexanone; 3. 1-hexanole; 4. 3-heptanone; 5. 2-methoxythiopen; 6. dimethyl sulfone; 7. 1-octen-3-ol; 8. 3-octanone; 9. 1,2,4-trimethylbenzene; 10. 4-methylphenole, 11. 2-nonanone; 12. phenethyl alcohole; 13. indole

## Conclusions

The CA-FμTP was presented as a further development of the CA-DBDI ion source for mass spectrometry. Understanding and controlling the gas flows was found to be a crucial parameter for reproducible long time measurements. Following previous publications, oxygen was found to be an important part of the ionization atmosphere for high sensitivity measurements. Optimal nitrogen/oxygen mixture was measured using ketones as a model analyte for volatile organic compounds as biomarkers relevant for oral squamous cell cancer. Using this optimized conditions for the detection of biomarkers, resulted in limits of detection in the range of 0.7-10 ppb for all analytes without any form of preconcentration techniques. Comparing the LODs of the presented work with LODs of other methods in the literature, depending on the analyte, an improvement of 2.5 times were found. Due to the soft ionization technique, matrix effects resulting from human saliva can be neglected, because of the nearly vanishing fragmentation of analyte ions. It was demonstrated that the use of ambient air for the detection of the analytes led to a significant decrease of the signal, resulting in a worse sensitivity of the system. Therefore the CA-FμTP ionization source is a very promising tool for robust and sensitive analysis of biological samples for the detection of biomarkers.

## Acknowledgements

The financial support from the Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen, the Senatskanzlei des Landes Berlin and the Bundesministerium für Bildung und Forschung is gratefully acknowledged. This project has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement 810686 and under the Marie Sklodowska-Curie grant agreement number 840743.

The authors want to gratefully acknowledge the highly competent support from the scientific glass workshop of the Technische Universität Dortmund in combination with the construction and design of the glass cross piece.

## References

1. Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M., Atmospheric pressure chemical ionization source. 2. Desorption-ionization for the direct analysis of solid compounds. Anal Chem 2008, 80 (8), 2654-63.

2. Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M., Atmospheric pressure chemical ionization source. 1. Ionization of compounds in the gas phase. Anal Chem 2008, 80 (8), 2646-53.

3. Badal, S. P.; Michalak, S. D.; Chan, G. C. Y.; You, Y.; Shelley, J. T., Tunable Ionization Modes of a Flowing Atmospheric-Pressure Afterglow (FAPA) Ambient Ionization Source. Anal Chem 2016, 88 (7), 3494-3503.

4. Badal, S. P.; Ratcliff, T. D.; You, Y.; Breneman, C. M.; Shelley, J. T., Formation of Pyrylium from Aromatic Systems with a Helium:Oxygen Flowing Atmospheric Pressure Afterglow (FAPA) Plasma Source. J Am Soc Mass Spectr 2017, 28 (6), 1013-1020.

5. Bregy, L.; Sinues, P. M.; Nudnova, M. M.; Zenobi, R., Real-time breath analysis with active capillary plasma

ionization-ambient mass spectrometry. J Breath Res 2014, 8 (2), 027102.

6. Cody, R. B.; Laramee, J. A.; Durst, H. D., Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem 2005, 77 (8), 2297-2302.

7. Gyr, L.; Wolf, J. C.; Franzke, J.; Zenobi, R., Mechanistic Understanding Leads to Increased Ionization Efficiency and Selectivity in Dielectric Barrier Discharge Ionization Mass Spectrometry: A Case Study with Perfluorinated Compounds. Anal Chem 2018, 90 (4), 2725-2731.

8. Hagenhoff, S.; Franzke, J.; Hayen, H., Determination of Peroxide Explosive TATP and Related Compounds by Dielectric Barrier Discharge Ionization-Mass Spectrometry (DBDI-MS). Anal Chem 2017, 89 (7), 4210-4215.

9. Hagenhoff, S.; Korf, A.; Markgraf, U.; Brandt, S.; Schutz, A.; Franzke, J.; Hayen, H., Screening of semifluorinated n-alkanes by gas chromatography coupled to dielectric barrier discharge ionization mass spectrometry. Rapid Commun Mass Sp 2018, 32 (13), 1092-1098.

10. Klute, F. D.; Michels, A.; Schutz, A.; Vadla, C.; Horvatic, V.; Franzke, J., Capillary Dielectric Barrier Discharge: Transition from Soft Ionization to Dissociative Plasma. Anal Chem 2016, 88 (9), 4701-5.

11. Klute, F. D.; Schutz, A.; Brandt, S.; Burhenn, S.; Vogel, P.; Franzke, J., Characterization of dielectric barrier discharges for analytical chemistry. J Phys D Appl Phys 2018, 51 (31).

12. Lara-Ortega, F. J.; Robles-Molina, J.; Brandt, S.; Schutz, A.; Gilbert-Lopez, B.; Molina-Diaz, A.; Garcia-Reyes, J. F.; Franzke, J., Use of dielectric barrier discharge ionization to minimize matrix effects and expand coverage in pesticide residue analysis by liquid chromatography-mass spectrometry. Anal Chim Acta 2018, 1020, 76-85.

13. Mirabelli, M. F.; Wolf, J. C.; Zenobi, R., Atmospheric pressure soft ionization for gas chromatography with dielectric barrier discharge ionization-mass spectrometry (GC-DBDI-MS). Analyst 2017, 142 (11), 1909-1915.

14. Shelley, J. T.; Chan, G. C. Y.; Hieftje, G. M., Understanding the Flowing Atmospheric-Pressure Afterglow (FAPA) Ambient Ionization Source through Optical Means. J Am Soc Mass Spectr 2012, 23 (2), 407-417.

15. Schütz, A.; Lara-Ortega, F. J.; Klute, F. D.; Brandt, S.; Schilling, M.; Michels, A.; Veza, D.; Horvatic, V.; García-Reyes, J. F.; Franzke, J., Soft Argon–Propane Dielectric Barrier Discharge Ionization. Anal Chem 2018, 90 (5), 3537-3542.

16. Brandt, S.; Klute, F. D.; Schutz, A.; Marggraf, U.; Drees, C.; Vogel, P.; Vautz, W.; Franzke, J., Flexible microtube plasma (FmicroTP) as an embedded ionization source for a microchip mass spectrometer interface. Anal Chem 2018.

17. Pu, J.; Dai, J.; He, F.; Zhu, S.; Zhao, Z.; Duan, Y., Interpretation of Ionization Mechanism Responsible for Reagent Ion and Analyte Formation in Microwave-Induced Plasma Desorption Ionization Mass Spectrometry. J Am Soc Mass Spectrom 2020.

18. Vogel, P.; Marggraf, U.; Brandt, S.; Garcia-Reyes, J. F.; Franzke, J., Analyte-Tailored Controlled Atmosphere Improves Dielectric Barrier Discharge Ionization Mass Spectrometry Performance. Anal Chem 2019, 91 (5), 3733-3739.

19. Henry, N. L.; Hayes, D. F., Cancer biomarkers. Mol Oncol 2012, 6, 140-146.

20. Silva, C. L.; Passos, M.; Camara, J. S., Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of potential urinary cancer biomarkers--a powerful strategy for breast cancer diagnosis. Talanta 2012, 89, 360-8.

21. Filipiak, W.; Mochalski, P.; Filipiak, A.; Ager, C.; Cumeras, R.; Davis, C. E.; Agapiou, A.; Unterkofler, K.; Troppmair, J., A Compendium of Volatile Organic Compounds (VOCs) Released By Human Cell Lines. Curr Med Chem 2016, 23 (20), 2112-31.

22 Shigeyama, H.; Wang, T.; Ichinose, M.; Ansai, T.; Lee, S. W., Identification of volatile metabolites in human saliva from patients with oral squamous cell carcinoma via zeolite-based thin-film microextraction coupled with GC-MS. J Chromatogr B Analyt Technol Biomed Life Sci 2019, 1104, 49-58.

23 Portolés, T.; Mol, J.G.J.; Sancho, J.V.; Hernández, F., Advantages of Atmospheric Pressure Chemical Ionization in Gas Chromatography Tandem Mass Spectrometry: Pyrethroid Insecticides as a Case Study. Anal Chem 2012, 84, 9802–9810.

24. Mirabelli, M.F.; Wolf, J.C.; Zenobi, R., Atmospheric pressure soft ionization for gas chromatography with dielectric barrier discharge ionization-mass spectrometry (GC-DBDI-MS).Analyst, 2017,142, 1909-1915.

25. Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M., Atmospheric pressure chemical ionization source. 1. Ionization of compounds in the gas phase. Anal Chem 2008, 80 (8), 2646-2653.

26. Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M., Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 2003, 75 (13), 3019-30.

27. Peng, M.; Wen, H.; Le, J.; Yang, Y., Trace-level analysis of mesityl oxide in enalapril maleate by gas chromatography with electron ionization mass spectrometry. Analytical Methods 2012, 4 (12), 4063-4067.