PRF #58113-DNI6

Project Title: Characterization of a Solution-Cathode Glow Discharge Source for Ionization, Tunable Fragmentation, and Rapid Identification of Molecules and Polymers with Mass Spectrometry

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Over the first year of this project, the capabilities of the atmospheric-pressure solution-cathode glow discharge (SCGD) as an ionization source for small molecules and biopolymers with mass spectrometry detected were further explored. In the initial studies, it was clear that a number of ionization and fragmentation processes exist within and near the SCGD plasma. Further, we found that these processes are dependent on a number of SCGD operating parameters. Therefore, a parametric evaluation of the SCGD coupled with mass spectrometry (MS) for detection and sequencing of peptides was conducted with angiotensin II as a model analyte. While many parameters were studied (e.g., source position, discharge gap, discharge current, solution flow rate, carrier solution composition, etc.), it was found that analyte signal was mostly heavily impacted by position of the SCGD in relation to the mass spectrometer inlet, the discharge current, and solvent composition of the cathode solution. For instance, a five-fold increase in signal for the doubly-protonated species ($MH_2^{2+}$) of angiotensin II was obtained with an optimized spatial position of the SCGD, a discharge current of 50 mA, and a support solvent composition of 0.1 M nitric acid. At the same time, these conditions also led to a decrease in signal for the protonated molecular ion ($MH^+$) and fragment ions. As a result, these conditions were used generate working curves for peptide quantification due to the low fragment-ion signal and simple spectra. Change in solvent composition cathode support solution was found to improve signal for molecular ions as well as fragment ions by more than two times (cf. Figure 1). Because all ion types exhibited enhanced signal in the presence, it is likely that the decreased surface tension and vapor pressure leads to more complete desolvation of analyte-containing charged droplets produced at the plasma/solution interface of the SCGD. A draft of a manuscript on this work has been completed and will be submitted soon.

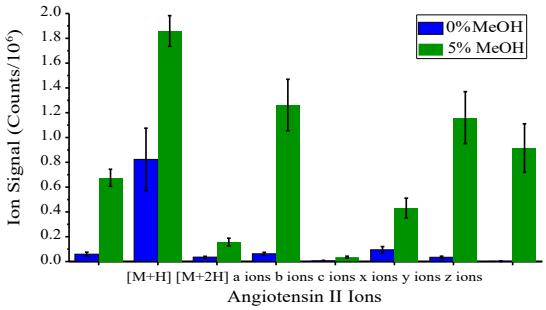


Figure 1. Effect of 5% methanol addition (green) to the support solution on signal for pseudo-molecular and fragment ions of angiotensin II, compared to a support solution of 0.1 M $HNO_3$ in water (blue).

For the detection and analysis of biopolymers and other precious samples, a nested-capillary geometry of the SCGD is used where the sample flows at a low flow rate (ca. 100 µL/min) through a fused-silica capillary that is fixed within a larger glass capillary where a support solution of 0.1-M nitric acid is pumped at a higher flow rate (ca. 1.5 mL/min) to sustain the discharge. Recent effort have focused on the chemistry of the sample-transfer fused silica capillary to minimize adsorption of sample onto the capillary walls. To explore the importance of capillary surface chemistry on SCGD-MS detection of biopolymers, an untreated fused-silica capillary, a commercially available methyl-deactivated silica capillary, and acid/base-treated capillaries (adapted from capillary electrophoresis protocols) were tested and compared with the peptide angiotensin I. A marginal improvement in peptide signal was observed for the methyl-deactivated capillary compared to the untreated capillary. However, a 1-M NaOH basic rinse, 0.2-M HCl acid wash, and equilibration step of the untreated silica led to an almost three-fold improvement in analyte ion signal (cf. Figure 2). We have since adopted this treatment method lab-wide all our fused silica capillary to be used for the analysis of peptide and proteins. Additionally, a draft of a communication is being prepared based on the findings from this work.

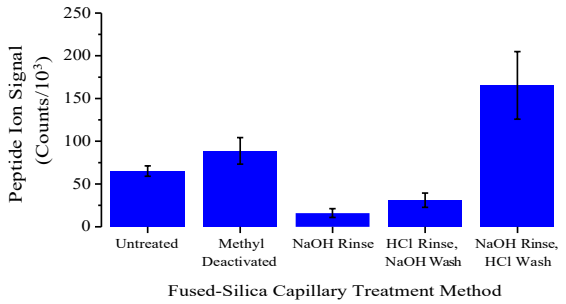


Figure 2. Comparison of angiotensin I molecular ion signal with SCGD-MS used untreated, methyl deactivated, and rinsed/washed fused-silica capillaries for sample transfer. NaOH rinse, followed by HCl wash and equilibration, yielded the lowest peptide adsorption.

The SCGD ionization source has shown to have some similar properties to electrospray ionization (ESI). At the same time, the sensitivity for peptides with SCGD-MS is markedly worse than ESI-MS. We hypothesized that many of the singly charged peptide ions were being neutralized by electrons and other negatively charged species produced in the plasma. To test this hypothesis, low-volatility reagents were added to the sample and sample carrier solution. With ESI-MS, these so-called "super-charging reagents", such as m-nitrobenzyl alcohol (m-NBA),

sulfonate, and glycerol 1,2-carbonate (GC), have been found to increase the charge state of peptides and proteins by slowing the droplet evaporation process. The three aforementioned super-charging reagents were tested with SCGD-MS for the detection and sequencing of various peptides. Surprisingly with the addition of super-charging reagents, the average charge state of all peptides tested decreased, while total ion signal decayed by at least an order of magnitude. Most interestingly, though, fragment-ion signal decreased significantly (sometimes altogether) in the presence of super-charging reagents (cf. Figure 3), while pseudo molecular ions were clearly observable. Furthermore, the loss of fragment-ion signal trended with increased boiling point of the super-charging reagent. These findings indicate that peptide excitation/fragmentation with SCGD occurs within or near the plasma. Addition of a slow-evaporating reagent effectively shielded the peptides in droplets from the harsh environment within and nearby the plasma. This information will be useful in future studies that will focus on fragmentation pathways and internal energy deposition during ionization.

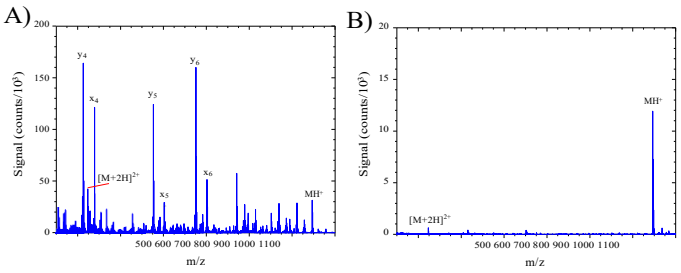


Figure 3. SCGD mass spectra for angiotensin II with 0% m-NBA (A) and 5% m-NBA (B) in the sample and sample-carrier solutions. Presence of 5% m-NBA resulted in ten-fold lower signals and near total loss of fragment ion signals.

In addition to the analyzing this larger biopolymers, the utility of SGCD-MS for the detection of small molecules, such as pesticides, was explored during this funding period. Initial efforts focused on the development of methods for the detection and quantification of organophosphate-based and carbamate-based pesticides. Chlorpyrifos was used to optimize SCGD-MS parameters and conditions for quantification with SCGD-MS. After optimization, the limit-of-detection (LOD) for chlorpyrifos was found to be ca. 10 fmol with a linear dynamic range of at least three orders of magnitude. Furthermore, SCGD-MS was interfaced with ultra-high performance liquid chromatography (UHPLC) for the analysis of pesticide mixtures. Baseline UPLC separation of a standard mixture of ten organophosphates was achieved in 15 minutes. Analysis of pesticide mixtures with UPLC-SCGD-MS is still a major focus with an emphasis on decreasing separation time and quantifying organophosphates and carbamates from the mixture. The optimized UPLC-SCGD-MS method will be combined with the QuEChERS ("quick, easy, cheap, effective, rugged, and safe") method of sample preparation and compared to a more-conventional ESI-MS method. It is likely this work with lead to a manuscript by the end of the second project year.

The information-rich peptide fragmentation spectra from SCGD-MS could make it difficult to distinguish multiple peptide signals within a mixture. As a result, UPLC coupled to SCGD-MS has been explored to minimize overlapping signal from different peptides. Furthermore, such a hyphenated approach could be readily adapted to very complex samples, such as petroleum. To achieve this, the effluent from the UPLC was connected to the inner fused-silica capillary of the nested-capillary geometry SCGD with a union. With UPLC connected to SCGD-MS, carrier solution composition and flow rate were optimized at a rate of 350 µL/min. A five-minute separation method was developed for a mixture of five peptides (bradykinin, methionine-enkephalin, leucine-enkephalin, angiotensin I and II) and used a gradient of 20% to 60% methanol over that time. Analytical working curves were obtained for all five peptides with detection limits of better than 10 ng for each peptide. Future efforts in this area will focus on improved ion-source design to enhance sensitivity and other analytical figures of merit for polymeric species.

In addition to furthering our understanding of SCGD as an ionization source, ACS PRF funds have had a notable impact on the PI and students working on this project. The primary graduate student on the project has produced enough results to draft two manuscripts as well as present a poster at the 2018 American Society for Mass Spectrometry (ASMS) conference. She was invited to give a lecture at the 2018 SciX meeting (to be held in late October) on her latest results. She is on pace to defend her Ph.D. dissertation at the end of the project period; it is anticipated that most, if not all, of the work in her dissertation will stem from this project. One undergraduate student worked on this project for her Senior Research requirement. Her experience with mass spectrometry, UPLC, pesticide detection, and proper analytical techniques in this project led her to pursue a graduate degree in environmental analytical chemistry. This student was accepted into every graduate program she applied to and she ultimately decided Colorado State University's program in Environmental Chemistry. Another undergraduate student started her independent reason on this project focused on the detection and identification of explosives with SCGD-MS. She was awarded an internal grant to support her research over the summer. She will be presenting a poster at the 2019 Pittsburgh Conference on this work and she will continue to perform research on SCGD-MS until her graduation. The PI of the project has presented this work at nine invited lectures at national and international conferences as well as two poster presentations.