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Helium Assisted Desorption and Spray Ionization

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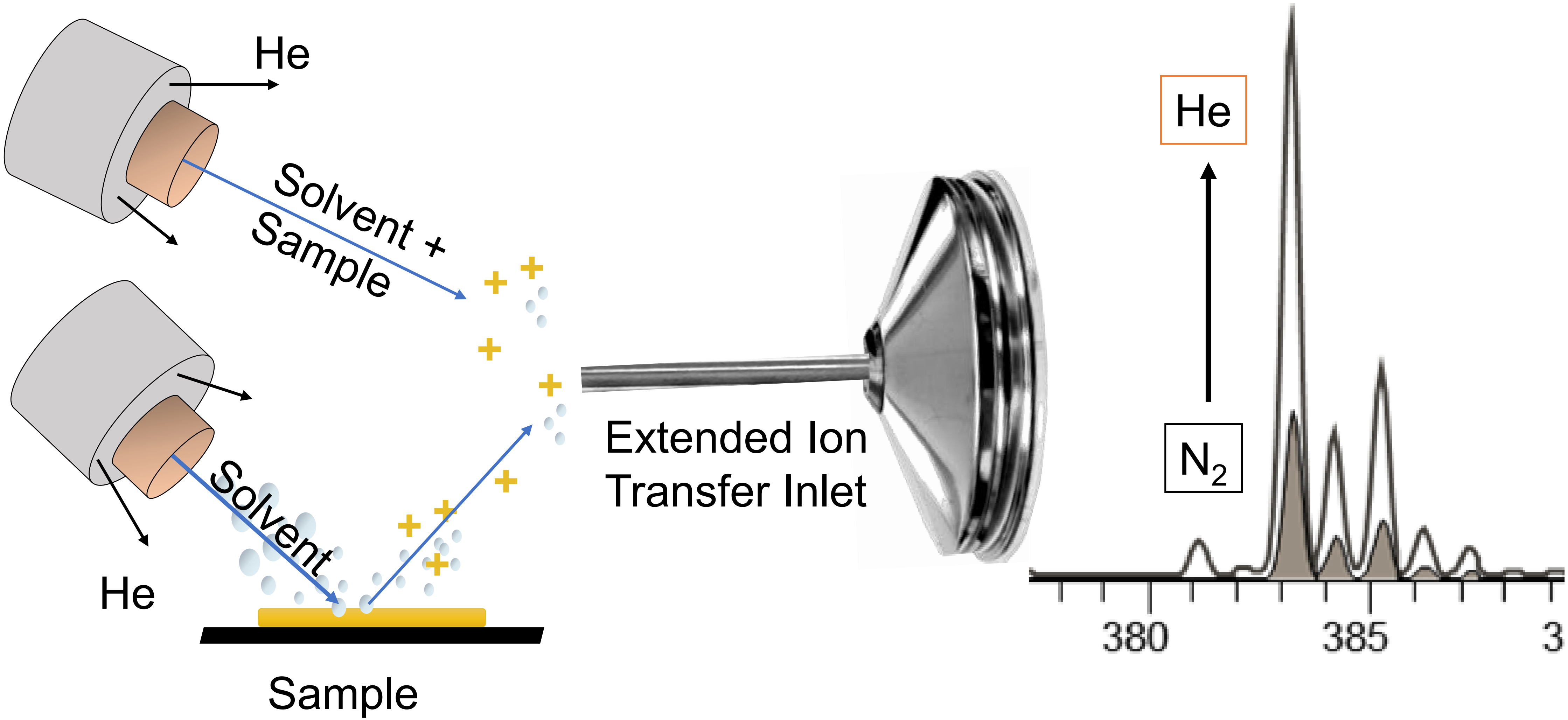
Response to Reviewers:	

Highlights

- Introducing Helium Assisted Desorption and Ionization (HADI) and Helium Assisted Spray Ionization (HASI).
- HADI and HASI allows for the simultaneous detection of low-polarity compounds and multiply-charged protein ions.
- Helium as the nebulizing gas, when using an electrosonic spray ionization source, increases analyte response in positive and negative mode.
- Helium as the nebulizing gas increases analyte response moderately in spray ionization but dramatically in desorption ionization methods.
- Helium nebulization decreases the desorption footprint in DESI, while increasing signal intensity.

Graphical Abstract



Helium Assisted Desorption and Spray Ionization

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Abstract

We introduce a new method, helium-assisted desorption ionization, that uses helium as nebulizing gas in desorption electrospray ionization (DESI). We also investigate Helium Assisted Spray Ionization, when helium is used with a micro-electrospray ionization source similar to electrosonic spray ionization (ESSI). In both methods, analyte ion signals are improved, while the undesirable effects of species created by the breakdown products of atmospheric gases and helium are suppressed. Restriction provided by the extended ion inlet capillary reduces the influx of helium, preventing a deleterious increase in operating pressure in the intermediate ion transfer region. We demonstrate significant improvements in signal intensities for analytes of different sizes and polarities, including xanthine alkaloids, lipids, and proteins. Compared to regular DESI, ion intensities for both protein and small molecules increased with helium addition, while less-polar small molecules were improved to a greater extent. The improvements in signal response with helium nebulization generally followed the same trend using either helium-assisted desorption or spray ionization, although the extent of improvement was more substantial in the desorption experiments. This observation suggests that desorption, ionization, and/or ion transport processes could be positively influenced when helium is used as nebulizing gas. Nebulization with helium also produces a smaller desorption footprint compared to nitrogen, promising benefits for chemical microscopy and other applications of DESI-MS.

Keywords: Helium, DESI, APCI, ESI, Ambient ionization, Protein, Lipid

1. Introduction

Ambient ionization mass spectrometry (AIMS) is a rapidly growing field focused on new sampling and ionization methods for the immediate analysis of samples.[1-3] In AIMS, samples in their native state are analyzed under atmospheric conditions, ideally with minimal to no sample pre-treatment prior to analysis. The revolutionary concept of AIMS was introduced by the development of solvent-based desorption electrospray ionization technique (DESI)[4] and plasma-based direct analysis in real-time (DART)[5] in the early 2000s. Currently, ambient ionization methods are approaching 100 variations and the applications of these methods are growing steadily.[1, 6-8] While there are many ways to classify such a large and diverse group of techniques, a straightforward approach is to categorize the techniques based on the primary ionization pathway[9] or by sampling/desorption method.[10] Three major classes of ionization methods can be defined: those based on electrospray ionization (ESI), those based on atmospheric pressure chemical ionization (APCI), and those based on direct ionization (e.g., Penning ionization).

APCI methods rely on the interaction of analytes with reaction products of atmospheric gasses created by their interaction with a variety of plasmas.[10] A drawback of plasma-based ionization techniques is that they generate a mass spectrum containing only a molecular or quasi-molecular ion peak. These techniques are also limited to low molecular weight compounds, usually ≤800 Da,[11] and are not capable of analyzing macromolecules such as biopolymers.

In ESI, production of multiply charged analyte ions of large biopolymers such as proteins enables analysis of very large molecules that would normally produce ions that fall outside of the mass range of typical mass spectrometers, with subsequent improvements such as an increase in mass resolution, identification of macromolecules by fragmentation, etc. On the other hand, ESI struggle to form ions from less polar or non-polar compounds. By extension, AIMS methods that rely on ESI for ion formation, such as most liquid-extraction based techniques, share challenges in ionization of low polarity compounds, in addition to matrix interferences, ion suppression, and adduct formation.

A prime example is the iconic liquid extraction-based AIMS method, DESI, that relies on solvent extraction followed by droplet desorption and subsequent electrospray ionization to detect molecules from a surface. The mass spectra acquired by DESI are very similar to ESI,[12-13] but not necessarily identical.[14] An important similarity is the ability to produce multiply charged analyte ions of large biopolymers such as proteins, although usually with lower sensitivity compared to ESI. DESI also suffers from low signal response of less polar compounds due to limitations imposed by ESI.

There have been many attempts to extend the range of compounds directly analyzable by AIMS. Often these approaches include the use of a combination of complimentary ion sources. One example is the *multimodal desorption electrospray/metastable-induced ionization* technique[15] and another is the *hybrid nanoelectrospray and dielectric barrier discharge ionization* source.[16]

Here were describe the use of helium as nebulizing gas with ESI and DESI, and show that a significant improvement in signal for low-polarity compounds can be obtained, while simultaneously allowing for the analysis of multiply charged protein ions. We will refer to the improved techniques as helium-assisted spray ionization and helium-assisted desorption ionization.

Our method is different from the techniques known as helium ionization mass spectrometry (HIMS)[17] or helium plasma ionization (HePI).[18] In HIMS and HePI, an electrospray ionization source is converted into a helium plasma ionization source by passing helium at 20-30 ml/min through the solvent metal capillary held at a high potential, without any other significant modifications of the source.[18-23] The methods described in this paper also differ from desorption atmospheric pressure chemical ionization (DAPCI).[24] With DAPCI, air and dopants pass over a corona discharge needle creating reagent ions. These gaseous chemical reagents then desorb and ionize compounds from sample surfaces to produce singly charged analyte ions.

We demonstrate that when helium is used as nebulizing gas, significant improvements in signal intensity for a wider variety of molecules, from small, polar compounds such as xanthine alkaloids, to moderately nonpolar compounds such as steroids and phospholipids are achieved. These improvements in signal for small compounds are achieved without a deleterious effect on detecting large, highly polar molecules such as proteins. Solution-phase additives, namely ammonium bicarbonate and formic acid, can easily be used in combination with He as needed, and provide significant additional improvement in the analysis of especially less-polar compounds that typically do not ionize efficiently by ESI. These new methods require using an electrosonic spray ionization source[25] and an extended ion inlet capillary. Under these conditions the user can easily switch between DESI and microESSI, or the novel ionization modality of *helium-assisted desorption ionization* and *helium-assisted spray ionization* by selecting the appropriate nebulization gas.

2. Experimental

Chemicals

High purity nitrogen and helium (>99%) were purchased form Airgas (PA, USA). Equine muscle myoglobin, hydrocortisone, cortisone acetate, caffeine and theobromine were purchased from Sigma-Aldrich (MO, USA). Over-the-counter pharmaceutical tablets and hydrocortisone ointment (1%) were purchased from a local pharmacy. BioUltra grade ammonium bicarbonate (ABC), HPLC-MS grade methanol (MeOH), LC-MS grade formic acid (FA), 3-aminobenzoic acid ethyl ester (MS222) were purchased from Sigma-Aldrich (MO, USA). Milli Q water (18 $\mathrm{M\Omega\ cm^{-3}}$) was obtained from a Thermo-Barnstead Water Polisher. Fused silica capillaries were purchased from Trajan Scientific (Ringwood, Australia). PTFE plates were purchased from Prosolia Inc. (IN, USA).

Sample preparation

Sample solutions were either sprayed on the surface or deposited as droplets. Samples were sprayed with a pneumatically-assisted nebulizer spray made of two coaxial fused silica capillaries.[4] The sprayer was orthogonally positioned at 2 cm above the surface. Nebulizing gas pressure and solvent flow rate were 40 psi and 3 µl/min, respectively. The stage moved at 200 µm/s, resulting in long homogenous protein lines deposited with 10 ± 0.1 mm widths. For PTFE slides, 2 µl of solutions were pipetted on the slides and dried under vacuum for 30 minutes.

Adult zebrafish (Danio rerio) were anesthetized and euthanized in 0.03% MS222 until opercular motion ceased and fish no longer responded to a tail pinch. The brain was immediately dissected out and smeared onto a glass slide before being flash-frozen on dry ice. Brain samples were stored at -80°C until DESI-MS analysis. Before analysis, samples were placed in a vacuum chamber to thaw for approximately 10-15 minutes. All experimental and animal care protocols have been approved by the Institutional Animal Care and Use Committee (IACUC).

Instrumentation

A linear ion trap LTQ mass spectrometer (Thermo Scientific, Waltham, MA, USA) was combined with a 3-dimensional translational stage (Purdue University, West Lafayette, IN, USA) for DESI and ESI analyses. An extended ion sampling capillary with a 5 cm extension was purchased from Adaptas Scientific Instrument Services, MA, USA. Electrospray emitters and desorption sprayers were prepared from Swagelok T-pieces and pieces of coaxial fused silica capillary tubing.[4] The outer capillary (for sheath gas) was approximately 15 mm in length with an inner diameter of 320 μm. The internal capillary (for solvent) had an outer diameter of 220 μm, and inner diameter of 50 μm. The solvent capillary extended through the T-piece and was connected to a syringe pump which delivered the solvent. Better helium consumption and equivalent results were obtained with a sprayer built from a gas capillary with an inner diameter of 250 μm, and a solvent capillary with outer diameter of 190 μm and inner diameter of 75 μm. This reduced the volumetric helium flow, measured by liquid displacement, from around 6 L/min to below 2 L/min for helium, compared to 2 L/min and 1 L/min for nitrogen at 120 psi supply pressures.

DESI-MS and ESI-MS parameters

DESI sprayer incident angle was 54°. The nominal distances between the desorption sprayer and heated extended capillary and desorption spray from surface were 4 and 1 mm, respectively. ESI experiments were performed under the same conditions using the DESI sprayer, but sample solutions were directly sprayed towards the heated extended capillary. Spray voltage was 4 kV, capillary temperature was set at 250°C, and capillary voltage and tube lens were optimized between 20-40 V and 90-175 V, respectively.

Mass spectra were collected and viewed in Xcalibur Qual Browser (2.0.7). Signal intensities were calculated as the average of three trials and error bars represent ± mean standard deviation.

3. Results and Discussion

Helium as nebulizing gas in ESI has been mostly disregarded due to the gaseous breakdown and electrical discharge with helium even at low voltages, compared to air and N_2.[26-28] Once the gaseous breakdown occurs, it can lead to arc discharge that seriously affects the electric field near the ESI emitter and degrades the performance of electrospray.[29] This is demonstrated by data in the Supplemental Information (Figure S1 and S2) where helium replaced nitrogen gas in two commercial ESI ionization sources. Strong corona discharge leads to low m/z peaks completely dominating the spectra and suppressing the protonated analyte signal. Preliminary data suggests that the improvement observed with the helium-assisted methods is a nuanced but complicated balance of several different properties, the mechanism of which is still under investigation, but discussed later on in this manuscript.

The usefulness of helium-assisted desorption ionization is shown in Figure 1. An equimolar sample of hydrocortisone and myoglobin was spray-deposited onto a glass microscope slide. Exchanging the nebulizing gas from N_2 to He improved the signal intensity of protonated hydrocortisone, a steroid, more than 20-fold, while still allowing detection of multiply charged apo-myoglobin and increasing its signal intensity 5 times.

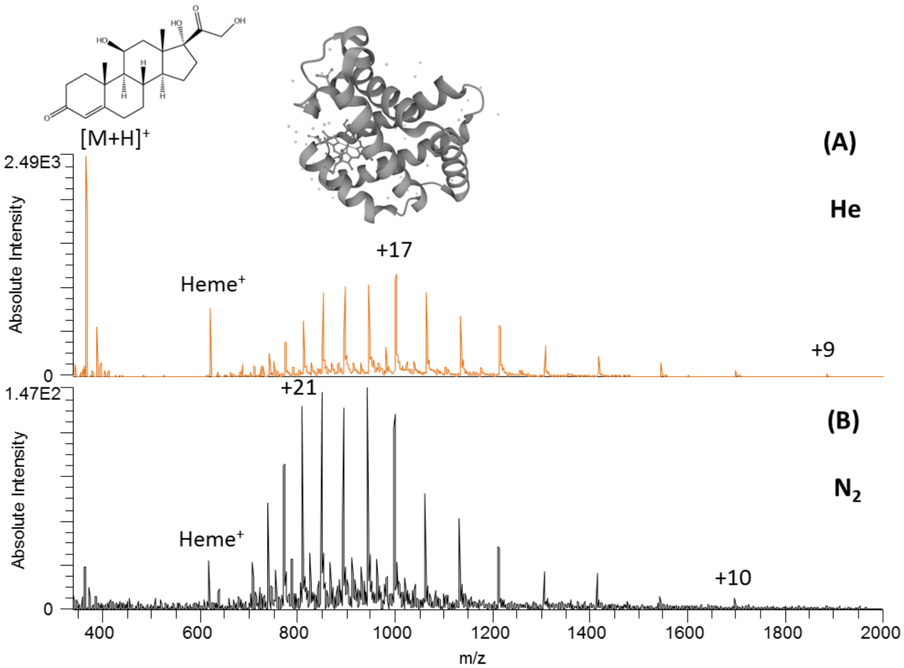

Figure 1. DESI-MS spectra of 10 pmol/mm^2 myoglobin and hydrocortisone desorbed from a glass microscopic slide using 0.2% formic acid in 50% MeOH as desorption solvent and (A) He and (B) N_2 as nebulizing gas.

Direct detection of active ingredients under ambient conditions, such as detection of pharmaceuticals from tablets and creams were some of the first reported applications of ambient ionization techniques, in particular DART and DESI-MS. To further demonstrate the application of helium-assisted desorption ionization for small molecule analysis, multiple pharmaceutical active ingredients under ambient conditions (directly from tablet or topical cream) were analyzed with 50% MeOH as desorption solvent.

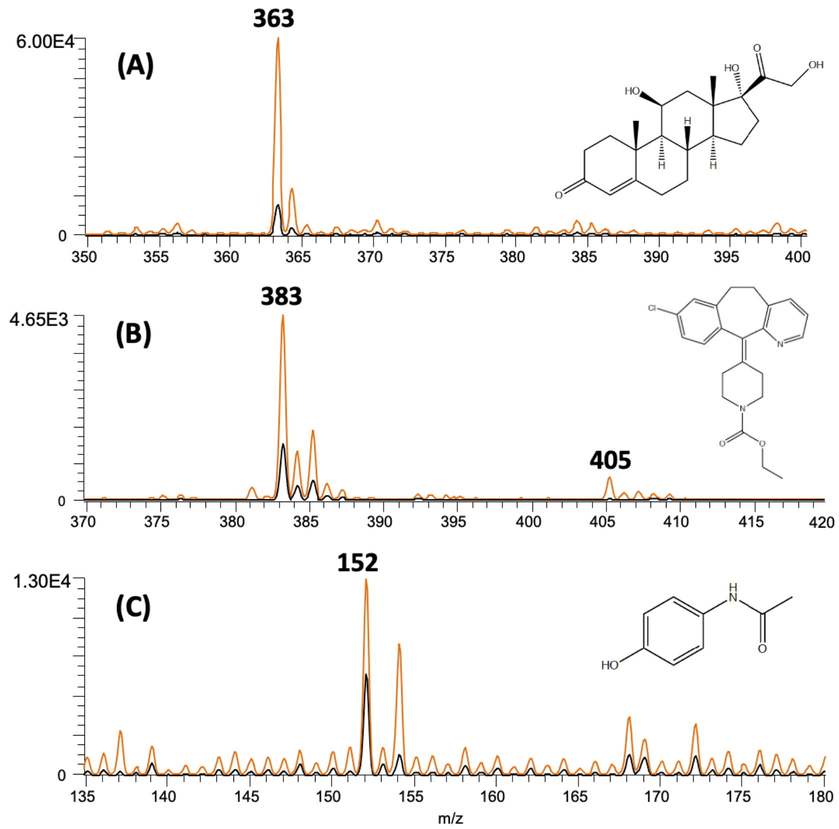

Figure 2 . Direct detection of active ingredients in pharmaceuticals under ambient conditions, using DESI (black spectra) or helium-assisted desorption ionization (orange spectra). (A) Hydrocortisone from 1% hydrocortisone ointment (B) Loratadine from an allergy tablet, C) Tylenol from a tablet.

In Figure 2, we demonstrate that using He as nebulizing gas can increase the DESI-MS signal intensity of several active ingredients directly detected from the tablet or cream by using MeOH:H_2O as desorption solvent without any additives such as formic acid. For example, hydrocortisone was easily detected from 1% hydrocortisone cream using helium-assisted desorption ionization, with a 10-fold increase in signal intensity compared to standard N_2 nebulized DESI. Previously, DESI-MS has been reported to perform poorly for nonpolar compounds such as hydrocortisone compared to DAPCI.[30] Similarly, Loratadine and Tylenol from the tablets were also directly detected by DESI-MS using 80% MeOH as desorption solvent. Switching the nebulizing gas from N_2 to He increased the absolute signal intensity of these active ingredients by approximately 4 and 3 times, respectively.

One of the more important applications of DESI-MS is the detection of lipids directly from animal tissue, often for the purposes of chemical microscopy. Figure 3 shows the improvement obtained when exchanging nitrogen with helium for the detection of phospholipids from zebrafish brain. For this analysis, in positive mode using 50% ACN:DMF as the desorption solvent, the majority of peaks corresponded to phosphatidylcholines (PC) as sodium or potassium adducts in the m/z 750-850 range. Other phospholipid types were also detected as sodium adducts including sphingomyelin (SM), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and triglyceride (TG). Peaks were tentatively identified from available literature, LipidBlast [31], and LipidMaps libraries.

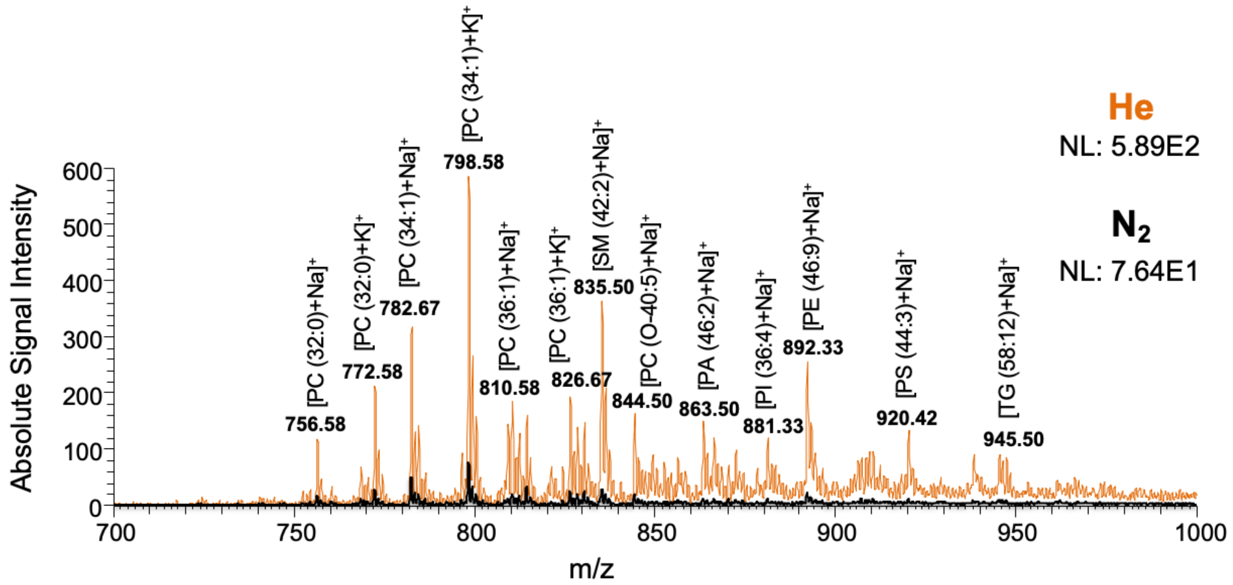

Figure 3. Detection of phospholipids in zebrafish brain on a microscopic glass slide analyzed by 50% ACN:DMF in positive mode by helium-assisted desorption ionization vs. regular DESI.

Spectra collected with He and N_2 were normalized to the highest peak in the scan range obtained with helium and are colored in orange for helium nebulization and black for nitrogen (DESI), respectively.

All lipids increased with helium nebulization. For example, the absolute signal intensity of m/z 798, tentatively identified as $[PC (34:1)+K]^+$, increased by approximately 8 times when He was used as nebulizing gas, compared to N_2. Lipid signal intensities also increased with helium when analyzed in the negative ionization mode (Figure S3).

Preliminary investigation into mechanistic aspects of the observed signal improvements with helium as nebulizing gas.

The observed increase in signal intensity when helium is used as nebulization and desorption gas in DESI could potentially be the consequence of improvements in desorption and ion transport, or changes in the ionization mechanism. To investigate this phenomenon, we compared the use of helium and nitrogen side by side, as desorption and/or nebulization gas in DESI and ESI, while all other conditions remained constant. The preserved conditions included using the same electrosonic ionization source, extended ion inlet capillary, mass spectrometry conditions, and solvent systems. For DESI experiments, hydrocortisone, and equimolar mixture of theobromine and caffeine were spotted on glass microscope slides at 20 pmol/mm^2 for each analyte. The spots were desorbed with 50% MeOH:H_2O, or 50% MeOH:H_2O containing either 200 mM ammonium bicarbonate (ABC) or 0.2% formic acid (FA). For the ESI experiments, the electrosonic spray ion source was turned towards the ion inlet capillary and mixtures containing 100 nmol of hydrocortisone, or the equimolar mixture of theobromine and caffeine were electrosprayed out of 50% MeOH:H_2O, and 50% MeOH:H_2O with either 200 mM ammonium bicarbonate or 0.2% formic acid added.

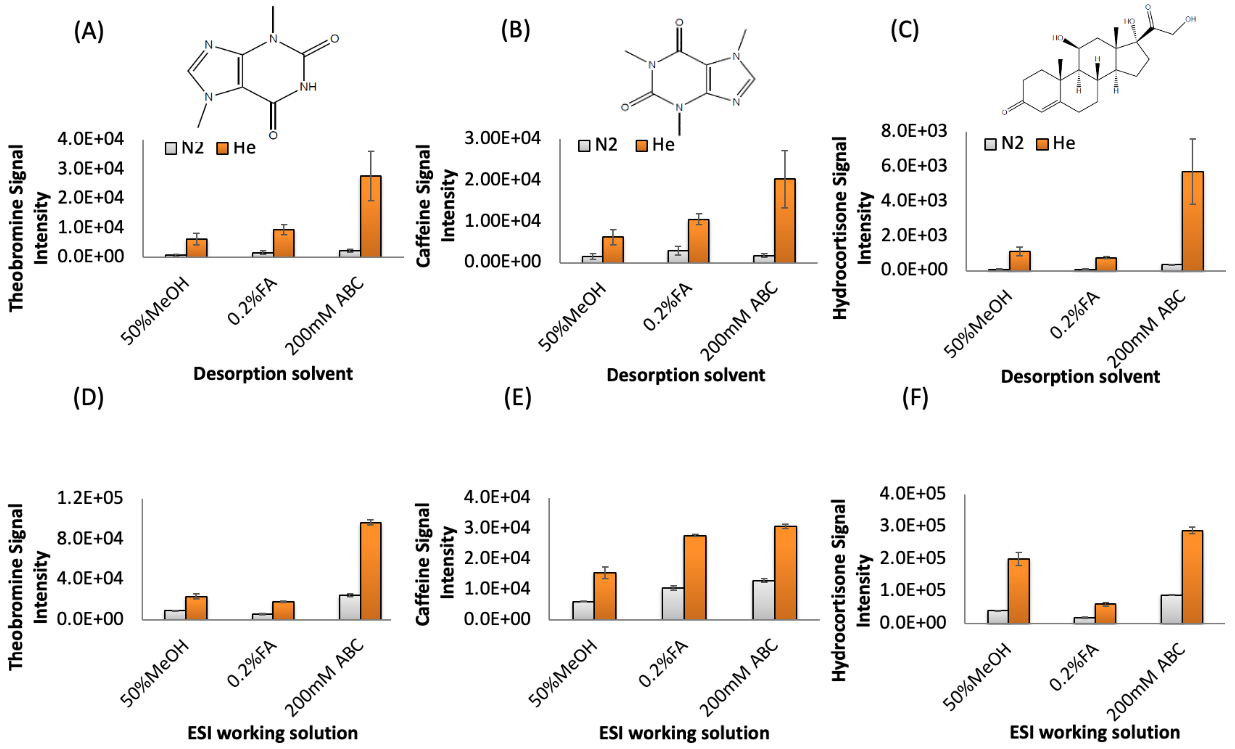

Figure 4. Comparison between signal response of (A, D) theobromine, (B, E) caffeine and (C, F), hydrocortisone in (A, B, C) DESI and (D, E, F) ESI in 50%MeOH:H_2O or this solvent system with either formic acid or ammonium bicarbonate added. Results with He as nebulizing gas shown in orange and with N_2 in grey.

As shown in Figure 4 for both DESI and ESI analyses, the response of all three compounds increased when using helium as nebulizing gas. The improvements were also observed with all three solvent systems. Interestingly, the presence of ammonium bicarbonate during helium-assisted analysis increased the absolute signal intensity more, especially in helium-assisted desorption ionization.

With helium-assisted desorption ionization, the signal intensity recorded from analyzing the spots of the three compounds on PTFE slides increased between 4 and 15 times with 50% MeOH in the desorption solvent. When adding 0.2% formic acid into this desorption solvent, the observed improvement was slightly lower and improved between 4 and 10 times with helium nebulization. Interestingly, by using 200 mM ammonium bicarbonate (ABC) in the desorption solvent and using helium for nebulization, the absolute signal intensity of all three compounds increased between 12 and 16 times.

Comparing regular ESSI and helium-assisted spray ionization, the absolute signal intensities of the three compounds increased between 2 and 5 times respectively when 50% MeOH was used as working solution with helium nebulization. The signal increased between 2 and 3 times when 0.2% formic acid was added to the working solution. The relative signal increase with helium nebulization and 200 mM ammonium bicarbonate added to working solution was between 2 and 4 times.

Since both ESI and DESI signal intensities increased when using helium as opposed to nitrogen, it appears that improvements in ionization processes and/or ion transfer efficiencies are affected. On the other hand, considering that helium assisted desorption produced larger improvements relative to helium assisted spray ionization, it is possible that in addition to improved ionization, desorption efficiency also increased with helium nebulization.

Desorption Footprint investigation

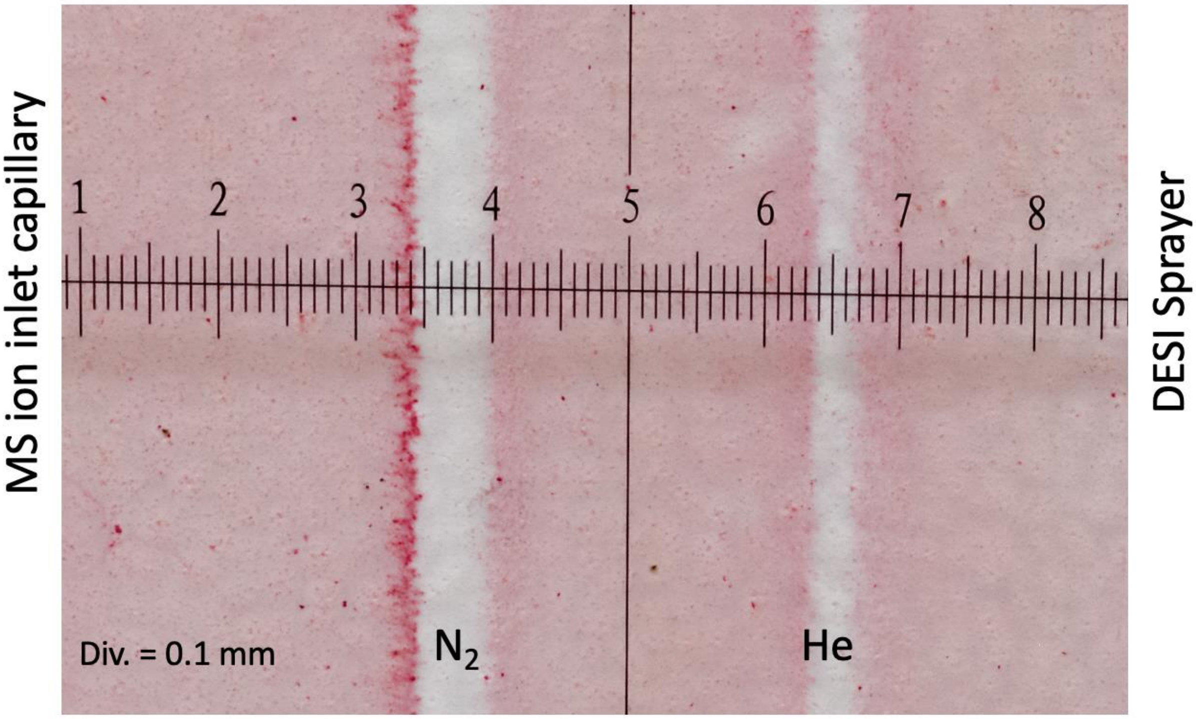

Figure 5. A photograph of a frosted microscope slide containing Rhodamine 6G after desorption by DESI (nebulization by N_2) vs. nebulization by He. The sample surface was overlaid with a calibration slide during photography where the smaller divisions are equal to 0.1 mm.

To investigate the effect of an increased signal with helium-assisted desorption ionization compared to regular DESI, we measured the difference in desorption footprint obtained with nitrogen and helium nebulization. Surprisingly, as shown in Figure 5, the desorption profile reduces from approximately 500 µm with nitrogen to around 200 µm with helium. (DESI desorption footprints have an ellipsoidal profile,[32] and here for a scanning experiment, we are reporting the width of the desorption band created by the major axis of the ellipse, as opposed to previous measurements reporting the smaller minor axis.) The desorption footprint is nearly twice as large with nitrogen compared to helium, which should translate into a larger amount of sample desorbed, yet the signal obtained with nitrogen nebulization is lower. A contributing factor is likely the redeposition of desorbed material observed in the direction of the ion inlet capillary with nitrogen nebulization compared to helium.

Previously, in a comparison between argon and helium as nebulization gas, phase Doppler particle anemometry measurements showed that helium produces faster moving, larger droplets, of a more uniform size distribution.[33] However, these results were contradicted by other studies, finding slower moving droplets when helium was used at the same volumetric gas flow,[34-35] highlighting the potential importance of nebulizer design. (Similar comparisons between N_2 and He could not readily be found, but the argon comparisons are notable for having an even larger mass difference than nitrogen relative to helium.) While the momentum transfer per helium atom collision is lower than for the heavier gasses, with sufficient gas pressure droplets should reach terminal velocities equal to the gas carrier stream. Under constant supply pressure conditions, twice the volumetric gas flow rate is obtained for helium compared nitrogen, further increasing the likelihood of faster moving droplets. The interplay between pressure, volumetric flow, nebulizer design, and linear velocity are complex however, and care should be taken with interpretation of these previous studies until comparative droplet dynamic measurements are made under these conditions. Still, it is not unreasonable to hypothesize that due to higher droplet kinetic energy, the heavier, and potentially faster droplets produced by helium should be more efficient at releasing secondary droplets from the micro-localized liquid layer on the sample surface,[36] likely producing smaller secondary droplets with sufficient energy to prevent redeposition soon after lift-off, and with a better trajectory towards the ion transfer tube.

Another consequence of the small mass of helium atoms is that ions and droplets should experience less scattering due to gas collisions along the trajectory which could explain the more focused droplet beam and smaller desorption profile, assuming that the stagnant atmosphere surrounding the ionization plume was sufficiently replaced by helium. Similarly, less scattering of the secondary droplets and spray plume could also lead to better ion sampling, which would contribute to the higher intensities observed with helium even though the spray footprint is smaller.

Regardless of the origin of the more efficient desorption profile, the increased signal and reduced footprint is likely to be very beneficial for mass spectrometry imaging applications.

Changes in ionization efficiency

Ionization efficiencies increase due to improved nebulization or desolvation processes. The thermal conductivity of helium is approximately 8 times higher than argon[38] and estimated about 5 times higher than nitrogen,[39] resulting in a more efficient desolvation with helium, even as larger initial droplets are produced as discussed above, potentially contributing to higher signal intensities.

Ion current measurements was made using the power supply readback of the LTQ instrument while performing ESI experiments. Above 2 kV, ion current measurements were consistently higher with helium compared to nitrogen, as shown in Figure S4. Interestingly the increase in ion current when changing to helium remained nearly constant as the voltage increased. The current difference between helium and nitrogen was much higher in ammonium bicarbonate compared to formic acid containing solvent systems. The current measurements using the readback when the solvent contained only 50% MeOH and no additives were noisy and of too low intensity to interpret. Interestingly, the solvent system containing ammonium bicarbonate also showed the largest improvement in signal intensities, as shown in Figure 4.

The increase in spray current is a likely consequence of helium producing larger glow discharge current compared to nitrogen. A low-current atmospheric pressure glow discharge is an intrinsic property of the electrospraying process[40] and should occur in nitrogen and helium gas streams as they exit the nebulization capillary, and out over the Taylor cone. These discharges are nonequilibrium, low-temperature plasmas which generate such chemically active species as metastable neutrals, high-energy electrons, and radical ions.[41]

Glow discharge by helium is also more likely to lead to subsequent analyte ionization. With helium, glow discharge primarily produces long-lived ("metastable") helium electronic excited state atoms with internal energies of 19.8 eV. We believe that further downstream, after mixing with water vapor from the spray solvent and atmospheric gasses, it is likely that metastable helium will react to produce protonated water clusters similar to those in microplasma sources and DART.[5, 42] On the other hand, when glow discharge takes place in N_2 atmosphere, short-lived excited state nitrogen molecules are produced with internal energies, at most, up to 12.3 eV. This excess energy is not enough to ionize ambient moisture, methanol and other common polar electrospray solvents.[43] The water cluster ions produced in a helium nebulizing gas should be advantageous for efficient proton transfer to neutral analyte molecules desorbed from the sample surface in the case of helium-assisted desorption ionization or evaporating from sample droplets as they approach the ion inlet in both helium-assisted desorption and ionization. The advantage of the coupled electrospray-plasma process described in this manuscript using helium as nebulizing gas is that the liquid atomization is combined with plasma chemical processes within the same device, by using the same power supply applied to the ion source.

Changes in ion transmission

Volumetric gas intake by the mass spectrometer and flow dynamics inside the ion transfer tube are major factors in transmission of ions and droplets through the ion sampling inlet. For turbulent flow inside ion transfer tubes,[44] the volumetric flow into the MS is dependent on the molar mass of the gas atom/molecule as well as its dynamic viscosity. Thus, the volume flow through the ion transfer tube would increase substantially with helium versus heavier gases such as nitrogen. Since the volumetric flow of He is more than double, compared to nitrogen, either when measured at atmospheric pressure for the same supply gas pressure, or when calculated for flow through the ion transfer tube,[44] some contribution to the observed ion signal intensity in both desorption and spray ionization could be due to increased ion/droplet sampling by the MS using helium compared to nitrogen.

However, this effect is unlikely the sole reason for the observed higher signal intensities with He, since the magnitudes of improvements are analyte sample dependent, and influenced by solvent systems, suggesting that some of the other factors described above also contribute.

Helium Consumption

Helium is a limited natural resource and increasingly expensive. Other ambient ionization methods that rely on helium as carrier gas, such as DART and LTP, generally use similar flow rates of 1.0 - 6.0 L/min[45-47] and 0.005 – 1.0 L/min[48] respectively. Similar to strategies employed for these techniques, helium consumption in these experiments can be reduced by further modifications to the source such as miniaturization[49] and pulsed analysis instead of continuous flow.[50] It is also worth noting that while pressures up to 140 psi was used for the data presented here, recent designs of the commercially available DESI source operates at substantially lower gas pressures, down to as low as 15 psi N_2.

4. Conclusion

The combination of helium gas with DESI and ESSI, referred to as helium-assisted desorption ionization and helium-assisted spray ionization, improved the simultaneous detection of both small, lower-polar compounds while also allowing detection of large polar analytes such as proteins. The signal intensities of small, low-polarity compounds were improved to a higher extent, while large biopolymers were still detected as multiply charged ions. Furthermore, the improvements were observed with a variety of solvent systems and additives, and especially substantial improvements were observed with ammonium bicarbonate containing solvent systems in helium-assisted desorption ionization. Preliminary results indicate that both desorption and ionization efficiencies were improved leading to a larger overall improvement in desorption electrospray ionization compared to electrospray ionization. It is further quite likely that ion transmission is improved through the heated capillary interface when helium is used as opposed to nitrogen.

5. Conflict of interest

The authors have no conflict of interest.

6. Acknowledgment

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Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Andre R Venter has patent #Application No. 63/306,350 pending to Western Michigan University.

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Helium Assisted Desorption and Spray Ionization

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