Analyst

Interdisciplinary detection science

Nanospray-DESI

Nanospray Capillary

Primary Capillary

Liquid Bridge

Sample

MS
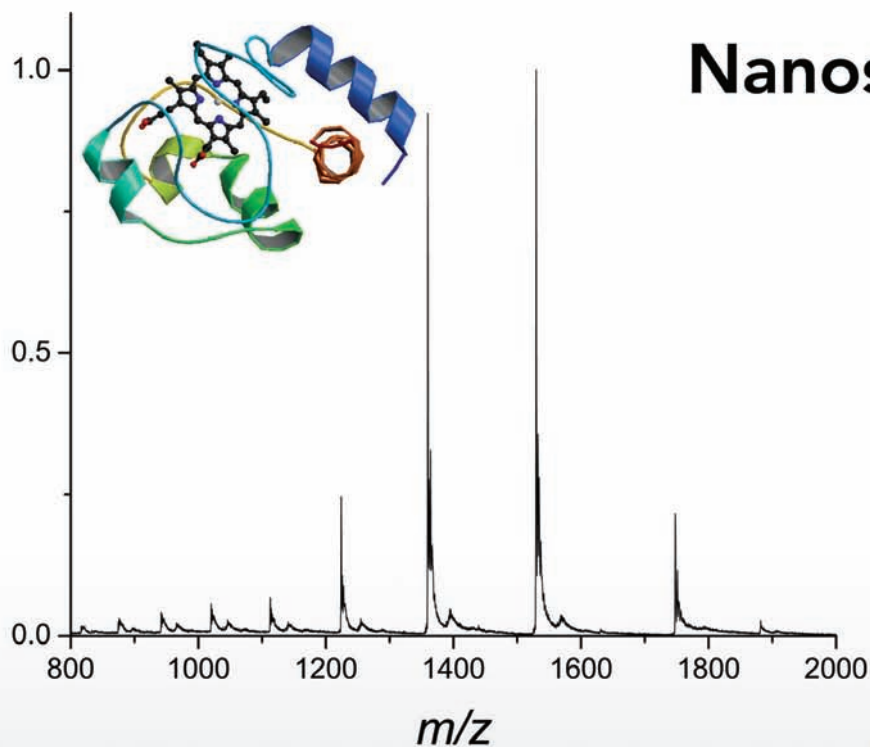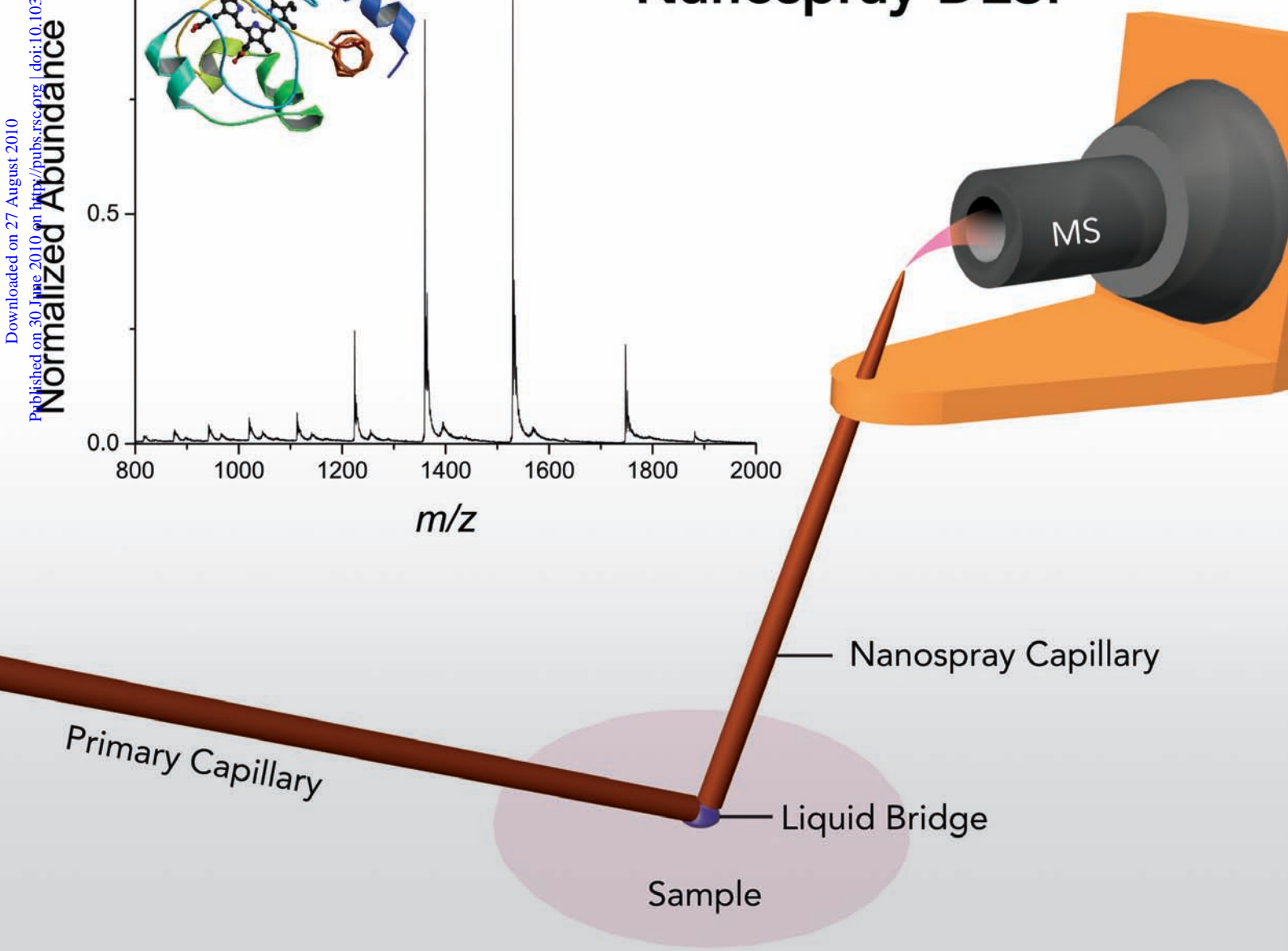
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Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry

Patrick J. Roach,*[a] Julia Laskin[a] and Alexander Laskin[b]

Nanospray desorption electrospray ionization (nano-DESI) mass spectrometry is presented as an ambient pressure liquid extraction-ionization technique for analysis of organic and biological molecules on substrates. Analyte is desorbed into a solvent bridge formed between two capillaries and the analysis surface. One capillary supplies solvent to create and maintain the bridge, while the second capillary transports the dissolved analyte from the bridge to the mass spectrometer. A high voltage applied between the inlet of mass spectrometer and the primary capillary creates a self-aspirating nanospray. This approach enables the separation of desorption and ionization events, thus providing independent control of desorption, ionization, and transport of the analyte. We present analytical capabilities of the method and discuss its potential for imaging applications.

1 Introduction

Ambient pressure (AP) surface ionization mass spectrometry (MS) allows chemical analyte to be sampled from interfaces without special sample preparation.[1–4] Desorption electrospray ionization (DESI), first introduced by Cooks and co-workers,[5] is a powerful ambient ionization technique. DESI (Fig. 1A) is accomplished by directing charged droplets from an electrosonic spray ionization source[6] towards a surface with a proximal atmospheric pressure mass spectrometer inlet. DESI is undergoing continued development; notable examples include high throughput analysis,[4,7–9] rapid sampling,[10] imaging,[11–17] and online liquid sampling.[18]

Basic mechanisms for analyte desorption and ionization have been discussed in early DESI publications.[19,20] Later, experimental[21] and theoretical[22,23] investigations have examined the "droplet pick-up" mechanism, wherein momentum transfers from incoming droplets to liquid at the surface to create secondary droplets containing charged analyte. The secondary droplets are sampled into the mass spectrometer as well as being splashed onto the surrounding area,[24,25] a process that has been termed indirect transfer. Splashing of the solvent and the analyte caused by collisions of the incoming droplets and neutral gas molecules with the liquid film of the substrate results in transport of the analyte on the surface. Transport of the analyte on the surface is undesirable in specific applications such as chemical imaging and results in decreased detection efficiency if the charged

analyte first encounters an alternate counter electrode that is not the mass spectrometer inlet. This process was directly observed in microscopic imaging experiments reported by Farnsworth and co-workers. They showed that if the momentum transfer mechanism is hindered, rivulets form from charged droplets moving across the surface and emit ions from a multitude of Taylor cones, thus preserving the DESI signal.[25]

An alternate approach to indirect transfer is to directly transport the analyte from the surface within a confined liquid flow through the use of a surface probe capillary. Examples of techniques which utilize direct transport are liquid microjunction surface-sampling probe (LMJ-SSP),[26] scanning mass spectrometry (SMS) probe,[27] and probe electrospray ionization (PESI).[28] LMJ-SSP employs direct liquid extraction using a dual coaxial tube
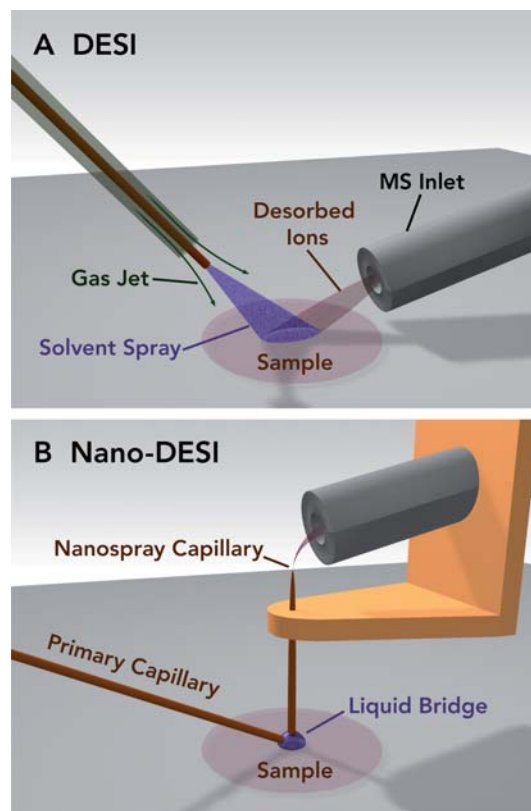

Fig. 1 Experimental configuration of DESI and nano-DESI. (A) Traditional DESI setup. Solvent is electrosprayed with aid of nebulizer gas jet. Solvent and analyte are removed from the surface. (B) Nano-DESI setup. A solvent bridge formed between the primary and nano-spray capillaries contacts the analysis surface. Analyte-containing solvent is removed from the surface by self-aspirating nanospray.

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configuration to supply solvent to a surface and transport dissolved analyte. SMS is used for sampling from bulk liquids through a self-aspirating capillary. PESI exploits the advantages of separating the sampling from the ionization processes through batch sampling of liquids using a solid needle.

Here we show a novel AP surface ionization method that utilizes the beneficial features of DESI and the direct transport approaches. We call the modified source configuration, shown in Fig. 1B, nanospray DESI (nano-DESI) because it uses a self-aspirating nanospray[29] capillary to directly transport and ionize analyte that is desorbed from the surface into liquid. The use of a nanospray capillary here exploits the natural rivulet phenomena observed in DESI experiments by providing the charged liquid with one lowest energy path to the mass spectrometer inlet. This approach separates desorption and ionization events, eliminates splashing, and minimizes analyte transport on the substrate, which allows the direct user specification of the area that is sampled, and is expected to yield improved sampling efficiency.

2 Materials and method

2.1 Source description

A primary capillary supplies solvent to a second nanospray capillary, which fills initially by capillary action resulting in a solvent "bridge"[30] between the two. A several keV potential difference is created between the primary capillary and the MS inlet. The circuit provided by the continuous liquid causes self-aspirating nanospray that pumps volume from the solvent bridge. Liquid removed by the nanospray process is replenished continuously to maintain the bridge. The bridge is brought into contact with an analyte-containing substrate allowing analyte to be dissolved from the substrate and transported with the solvent into the mass spectrometer. When operated in positive ion mode, the source creates intact even-electron ions, typically produced by conventional electrospray ionization (ESI) and DESI.

2.2 Samples and reagents

Water, methanol (both HPLC grade), glacial acetic acid, bovine heart cytochrome c and reserpine (all from Fischer Scientific, Inc.) were used in nano-DESI experiments. Cytochrome c was dissolved in a mixture of water, methanol, and acetic acid (50 : 48 : 2, v/v) to a concentration of 19 ng μl^{-1}. Reserpine was dissolved in a mixture of methanol and acetic acid (10 : 1, v/v) to a concentration of 0.7 ng μl^{-1}. A 2 μl aliquot of cytochrome c solution and a 1 μl aliquot of reserpine solution were pipetted onto an Omnislide™ hydrophobic array (Prosolia, Inc., Indianapolis, USA) and allowed to dry before analysis. A film of rhodamine dye from a red Sharpie® permanent marker (Sanford) was created on a plain microscope slide (Fischer Scientific) by coloring the slide and allowing the deposited dye film to dry. Methanol (rhodamine and reserpine) or methanol and water (50 : 50, cyt c) were used as spray solvents. The flow rate of the solvent was matched to the self-aspiration rate of the probe capillary and was typically 0.6 μl min^{-1}.

2.3 Apparatus

Samples were analyzed using a Finnigan LTQ/Orbitrap™ mass spectrometer (Thermo Electron, Bremen, Germany) equipped with a 2D DESI source (Prosolia, Inc., Indianapolis, USA) modified for nano-DESI experiments as illustrated in Fig. 1B. Fused Silica capillaries (50 μm ID, 184 μm OD, Polymicro Technologies, L.L.C., Phoenix) were used to create supply and nanospray capillaries. The nanospray capillary is mounted in a 1/16″ OD capillary PEEK tubing (Upchurch Scientific, Oak Harbor, USA) and affixed to an extended ion transfer tube (Prosolia, Inc., Indianapolis, USA) using a custom PEEK holder. Images of the droplet imprints left in the rhodamine dye film were taken using a Nikon Eclipse LV150 microscope with a 20×/0.45 final objective and NIS Elements Imaging Software.

3 Results and discussion

3.1 Source operation

The contact area between the solvent bridge and surface is controlled by changing the volume of liquid forming the bridge by means of the supply flow rate, and by varying the diameter of the capillaries used. Thus, the size of the probe droplet can controllably be made smaller to minimize contact area and provide better spatial resolution, or made larger to sample from a larger area when desired, without affecting the ionization process. The source configuration allows operation with typical constant bridge volume flow rates of 600 nl min^{-1}. This process does not require momentum transfer, thereby eliminating nebulizer gas and reducing splashing or undesirable analyte transport. Positioning of the emitter-end of the nanospray capillary with respect to the mass spectrometer inlet, the voltage applied across the circuit, and the composition of the solvent mixture affect the rate of self-aspiration and are important for optimizing the source performance. The achievable ion current is stable and easy to maintain so long as the liquid circuit is maintained and analyte is present.

The collection capillary must be filled prior to application of a spray voltage, else the desired circuit is not established, due to phenomenon presumably related to electro-wetting.[31] Bubbles introduced into the collection capillary are detrimental to operation (either because of the Jamin effect[32] or the electrical resistance of bubbles) because outside forces are not available to clear the line. Bubbles are the most probable cause of source malfunction. While bubbles can be removed by heating the nanospray capillary, the most practical solution has been to simply replace the clogged capillary with a new one.

3.2 Rhodamine dye on glass

To demonstrate analytical capabilities, nano-DESI analysis was performed on a film of rhodamine dye on a glass slide to characterize the area sampled, as has been previously performed in related surface-sampling experiments.[33,34] Time dependent ion signal of rhodamine at m/z 443.5 and optical images of the contact area are presented for two experiments conducted at 0.5 s "tapping" (Fig. 2A) and 25 min "extended" (Fig. 2B) contact times. The tapping experiment resulted in a sharp Gaussian peak with a full width half maximum (FWHM) of 0.8 s. An averaged mass spectrum, shown in the inset of Fig. 2A, has signal-to-noise ratio (S/N) of 330. The optical image of the contact area is less than 100 μm in diameter.

A "chip" in the rhodamine film in the center of the contact area, shown in the image (Fig. 2A), occurred as a result of physical contact between the nanospray capillary and the surface. The extended experiment resulted in an ion signal that increased to maximum
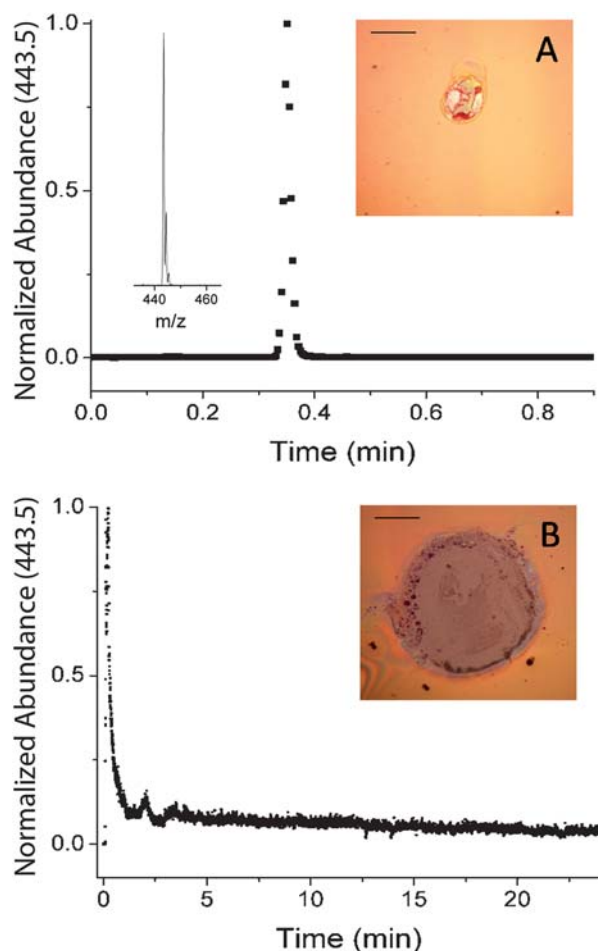
Fig. 2 Nano-DESI analysis of rhodamine film on glass. Time dependent signal of rhodamine at (A) *m/z* 443.5 from 0.5 s tapping interaction with an averaged mass spectrum (left) and optical image of the resulting sampling spot (right); (B) *m/z* 443.5 from 25 min interaction time with optical image of the resulting sampling spot (right). Scale bars on the images are 100 μm. The zero of the time axis corresponds to the start of data recording.

intensity by 7 s and fell to half-maximum after 16 s. A slowly decaying shoulder continued throughout the experiment. We attribute the initial intensity spike and the extended shoulder to initial desorption of the analyte over the entire contact area and subsequent dissolution at the circumference, comparable to previous observations by Van Berkel *et al.*[26] Dissolution at the circumference can also explain the increased spot size with extended contact. Mass spectra at the maximum abundance and at the 20th minute have *S/N* of 500 and of 20, respectively. The optical image shows a resulting sampled spot in the rhodamine film of 300 μm in diameter.

3.3 Reserpine

Analysis of organic material was demonstrated with 10 pmol (0.7 ng) of reserpine deposited onto an Omnislide™ substrate (Prosolia) containing polytetrafluoroethylene (PTFE). A recent study reporting a *S/N* ratio of 5 from 10 ng of reserpine was performed on a similar substrate.[35] A time dependent signal of the protonated reserpine at *m/z* 609.5 is presented in Fig. 3. A single scan mass spectrum taken at
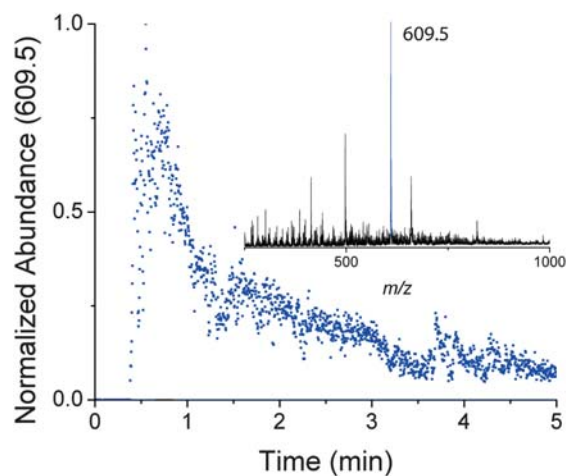

Fig. 3 Nano-DESI analysis of 10 pmol (0.7 ng) of reserpine on the Omnislide™ substrate showing time dependent signal at *m/z* 609.5. Inset plot shows single scan mass spectrum taken at maximum intensity.

the maximum abundance has *S/N* of 20, which decreases to *S/N* of 5 taken at 2 min. The relative abundance increased to a maximum in 10 s and decayed to half maximum in 34 s.

3.4 Cytochrome c

Fig. 4 shows a 15 min extended analysis of 3 pmol (38 ng) of cytochrome c. A time dependent signal of the +8 charge state, [C + 8H]⁸⁺, at *m/z* 1534.4 and an averaged mass spectrum are presented. The mass spectrum is comparable to that previously obtained using DESI.[36] The abundance increased in the first 1.5 min and decayed to half maximum by 4 min. The slower rise and decay times, with respect to rhodamine and reserpine, are attributed to different rates of analyte dissolution. Abundance fluctuations at 6 min and 11 min are attributed to nonlinearities in the supply pumping rate or splashing caused by bubbles in the solvent supply line that changed the contacted area.
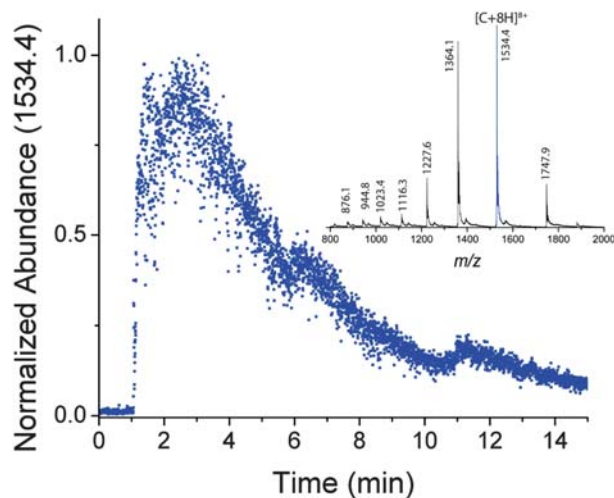

Fig. 4 Nano-DESI analysis of 3 pmol (38 ng) of bovine heart cytochrome c on Omnislide™ substrate showing time dependent signal at *m/z* 1534.4. Inset plot shows averaged mass spectrum.

4 Conclusions

Nano-DESI is a new ambient desorption ionization method wherein desorption and ionization mechanisms have been engineered to allow controllable, stable, and reliable operation while minimizing the number of relevant adjustable parameters. We discuss the mechanism for analyte desorption and ionization that generally resembles the droplet pick-up mechanism in DESI, but does not require momentum transfer from incoming spray droplets, allowing the elimination of nebulizing gas. In nano-DESI the analyte is ionized through self-aspirating nanospray, which eliminates the dependence of the sampling efficiency on the dynamics and velocity distribution of secondary droplets. Similar to DESI, this approach enables sampling from a variety of surfaces including glass, PTFE, aluminium foil, and paper. The size of the sampled area can be directly varied by manipulating the solvent flow rates or changing the capillary diameter, promising improved spatial resolution in imaging applications. Future studies will be directed towards exploiting the improved detection efficiency demonstrated herein to develop a higher-resolution probe for chemical imaging applications.

Acknowledgements

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