International Journal of Mass Spectrometry 300 (2011) 167–172

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

An alternative ionization paradigm for atmospheric pressure mass spectrometry: Flying elephants from Trojan horses☆

Charles N. McEwen[a,*], Sarah Trimpin[b,*,1]

[a] Department of Chemistry and Biochemistry, University of the Sciences in Philadelphia, 600 South 43rd Street, Philadelphia, PA 19104, United States
[b] Department of Chemistry, Wayne State University, Detroit, MI, United States

ARTICLE INFO

Article history:
Received 1 April 2010
Received in revised form 14 May 2010
Accepted 18 May 2010
Available online 10 June 2010

Keywords:
Laserspray ionization
Atmospheric pressure
MALDI
Mass spectrometry
ESI
Desolvation ion transfer tube

ABSTRACT

We propose an alternative for production of multiply charged ions that is based on the recent discovery that laser ablation of certain common matrix-assisted laser desorption/ionization (MALDI) matrixes produce multiply charged ions at atmospheric pressure (AP) that are similar in charge state distribution to ions produced by electrospray ionization (ESI). In ESI and AP–MALDI, ions are primarily produced at AP before being transferred to the vacuum region of the mass analyzer. In this new laserspray ionization (LSI) method, the ionization mechanism is proposed to be similar to ESI, but with a solid rather than a volatile liquid solvent. Unlike in ESI, desolvation does not occur in the AP region, but in a heated AP to vacuum transfer region. Here we propose that highly charged droplets rather than naked ions pass the AP to vacuum entrance aperture before the multiply charged ESI-like ions are released into the viscous gas flow. Such a process offers a new and possibly highly sensitive method for ion formation in MS.

1. Introduction

John Fenn's Nobel Prize lecture "Electrospray, Wings for Molecular Elephants" [1] used the metaphor in reference to the ionization method that made it possible for ions of nearly unlimited mass to be analyzed on mass spectrometers with limited mass-to-charge (m/z) range. This development greatly extended the applications of mass spectrometry (MS). A key to success in interfacing electrospray ionization (ESI) to MS was the knowledge Fenn had gained studying molecular beams [2]. Interestingly, even though this knowledge was critical for the Fenn group, at the time, AP–MS instruments had been available for a decade [3,4] and shortly after Fenn's ESI–MS discovery a commercial version (Sciex TAGA 6000E) was shown to be capable of analyzing ions produced by ESI [5].

Gas-phase ion production, critical to the success of MS, occurs exterior to the mass analyzer, typically either near vacuum or near AP. In either case, the ions are produced in an external ion source and transferred to the vacuum conditions of the mass analyzer. Thus, electron ionization, chemical ionization, secondary ionization and matrix-assisted laser desorption/ionization (MALDI) are mass spectrometric methods that operate near vacuum conditions [6]. Atmospheric pressure chemical ionization (APCI), ESI, and (AP) MALDI are methods that operate at or near atmospheric conditions [6].

Recently, it was discovered that multiply charged analyte ions similar to ESI can be produced by laser ablation of a solid UV adsorbing matrix/analyte mixture even in the absence of an external voltage [7–10]. Thus, using MALDI conditions (laser ablation of a matrix) at AP, ESI-like multiply charged ions were observed for peptides, proteins, polymers, and other molecules capable of multiple charging. The success of this technique called laserspray ionization (LSI), originally discovered using transmission geometry and high laser fluence, was recently shown using a commercial AP–MALDI ion source to depend on neither [11]. Reflective geometry, various MALDI target surfaces, laser fluences normally used in AP–MALDI, and either a 337 nm or a 355 nm laser have all produced multiply charged ESI-like ions [7,11]. The voltage applied between the sample target plate and the ion entrance aperture of the ion transfer tube, unlike in AP–MALDI [12], produces the best results for observation of abundant highly charged ions when low or even zero. Further, the method has a strong first shot preference, at least for the matrix 2,5-dihydroxyacetophenone (2,5-DHAP) [11]. There also must be a heated region between the laser ablation and the vacuum of the mass analyzer [7,9,10].

☆ In recognition of John Fenn's achievements that made this work possible.

* Corresponding author. Tel.: +1 215 596 8552.

E-mail addresses: c.mcewen@usp.edu (C.N. McEwen), strimpin@chem.wayne.edu (S. Trimpin).

[1] Tel.: +1 313 577 9823.

1387-3806/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2010.05.020

Our interpretation is that LSI involves an ionization mechanism in which laser ablation produces charged clusters (molten droplets) that must be desolvated (evaporation of matrix) until a surface charge is reached in which the droplet surface becomes unstable and fission to smaller charged droplets results [7]. Repeating this process, just as in ESI, leads to ion production by either the charge residue model [13] or by ion evaporation [14]. The mechanism is similar to the final stages of ESI in that multiply charged ions are produced from liquid droplets charged near the Rayleigh limit [15]. However, in order to produce ESI-like ions, it is also necessary that the clusters or molten droplets be highly charged. Karas proposed such a model for MALDI ionization in which the observed singly charged ions are the 'lucky survivors' from the originally produced multiply charged ions [16,17]. In this model, droplet charging was suggested to be from either a charge separation process or from photochemical processes. Producing charges on droplets is not unusual and occurs in clouds [18], waterfalls [19], volcanos [20], sandstorms [21], and sonic spray ionization (SSI) [22], as examples. A number of mechanisms for droplet charging in clouds have been proposed [18]. Some of the suggested mechanisms for liquid droplet and ice particle charging could be applicable to LSI, but clearly, even after great experimental effort over many years, no single mechanism for droplet charging in clouds, volcanos and sandstorms has been fully accepted [18,21], so it might be a stretch to propose a charging mechanism for LSI.

The proposed LSI ionization process presents an interesting new concept for ion production in MS, and because the laser beam can be well focused, presents new possibilities for tissue imaging at AP using high performance mass spectrometers [8,23]. We propose here, not an ionization mechanism different from ESI, but a different place to give Fenn's elephants wings. The highly charged matrix clusters from laser ablation of a solid matrix, similar to the Trojan horse in Virgil's poem, the Aeneid, carry the analyte past the AP to vacuum aperture where the naked multiply charged ions are released in a viscous gas flow for efficient transfer to vacuum and mass analysis.

2. Experimental

2.1. Materials

The matrixes, 2,5-dihydroxybenzoic acid (2,5-DHB), 2,5-dihydroxyacetophenone (2,5-DHAP), and α-cyano-4-hydroxycinnamic acid (CHCA), as well as the peptides angiotensin I and bovine insulin, were obtained from Sigma–Aldrich (St. Louis, MO) and used as received; 2,5-DHAP was MALDI grade. Solvents were obtained from Fisher Scientific (Fair Lawn, NJ). Gold Seal (80% transmission) microscopy glass slides (Gold Seal Products, Portsmouth, NH) were used as the MALDI target plate (76.2 mm × 25.4 mm × 1 mm in dimensions).

2.2. Sample preparation

The matrix was prepared as a concentrated solution in 1:1 acetonitrile:water. The angiotensin I used for the temperature study was a 2 pmol μL^{-1} solution diluted 1:1 with 2,5-DHB matrix solution and 1 μL was placed on the glass slide for each analysis. For the temperature study using bovine serum albumin (BSA) tryptic digest, 1 μL of a 1:1 matrix solution:digest (1 pmol μL^{-1}) was placed on the glass slide and dried. For the insulin sensitivity study, 0.5 μL of a 40 fmol μL^{-1} solution of bovine insulin in 2,5-DHAP was placed on the glass slide and dried before analysis. The sample deposition in each case used the dried droplet method [24].

2.3. LSI–MS

LSI mass spectra were acquired on a Thermo Fisher Scientific Corporation (Bremen, Germany) Orbitrap Exactive modified by removal of the ion source and overriding interlocks. A Spectra Physics VSL-337ND-S nitrogen laser was aligned so that the focused laser beam (<100 μm) was aimed at, and in-line with the ion entrance aperture (transmission mode) of the ion transfer tube as previously described [25]. The matrix/analyte on the glass slide was moved by hand through the focused laser beam at a distance of 1–3 mm from the ion entrance aperture while using normal data acquisition parameters. All data were acquired at 100,000 mass resolution (FWHH, *m*/*z* 200).

2.4. Microscopy

Optical microscopy (Nikon, ECLIPSE, LV 100) was performed to obtain qualitative information on the physical changes of the sample caused by the laser fluence. Two glass microscope slides were held *ca.* 2 mm apart with a layer of 2,5-DHB matrix applied solvent-based on the inside of the parent glass slide. As with LSI–MS, the laser penetrated through the glass slide with the sample in transmission mode and the ablated material was collected on the opposing slide. The optical microscopy data was obtained for both the parent and the collecting glass slide. Magnification conditions ranged from 5× to 100×, providing detailed views down to <1 μm resolution.

3. Results and discussion

Laser ablation of samples prepared identically to those for vacuum MALDI can produce mass spectra essentially indistinguishable from those produced with ESI. Just as in ESI, we have proposed that desolvation must occur to observe multiply charged ions [7]. The difference is that LSI occurs with solid matrixes without application of an electric field, and the conditions to achieve desolvation require more aggressive heating. We also know from molecular dynamic modeling that laser ablation in MALDI should produce matrix clusters above a threshold laser fluence [26,27], and that clusters have been observed experimentally [27,28]. Additionally, it has been proposed that ions should be observed from charged clusters in MALDI, similar to ESI [16,17].

3.1. The role of desolvation

Assuming that the matrix/analyte clusters are charged, a major difference in LSI and SSI [22] or ESI is that the charged droplets in LSI are made up of a 'solvent' that is a solid at room temperature, and thus not as easily vaporized to produce the desolvation conditions necessary for successful ionization. In LSI, ions are postulated to be produced after the charged clusters entered the heated ion transfer tube [7]. As shown in Fig. 1 for angiotensin I in 2,5-DHB at low ion transfer tube temperature few multiply charged ions are observed. However, as the temperature increases, multiply charged ions observed by MS increase rapidly until at 350 °C, the slope of the line showing the rate of increase of the +3 ions decreases. Charge stripping in ESI can be achieved by application of a voltage at the atmosphere/vacuum interface [29], but only heat was applied here resulting in lower charge states. Note in Fig. 1 that the +1 ions produced from the solvent-based dried droplet preparation, unlike the +2 and +3 ions, only increase slowly with increasing temperature. The +1 ions exclusively produced by the solvent-free MALDI sample preparation method [30] shown in Fig. 1 stay relatively constant with increasing temperature.

Increasing the ion transfer desolvation temperature is a means of converting peptides that are doubly charged to singly charged

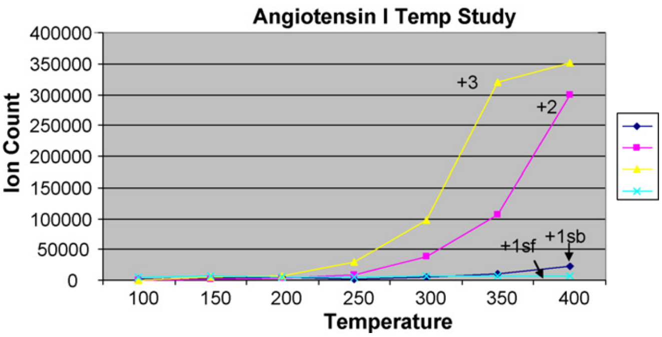

Fig. 1. A plot of the relative ion count for the +1 through +3 charge states of angiotensin I prepared solvent-based (sb) with 2,5-DHB as matrix and using LSI is shown for ion transfer tube temperatures ranging from 100 °C to 400 °C. The rapid increase in abundances with increasing temperature moderates for the +3 charge state after about 350 °C but the +2 and +1 charge states continue to increase in abundance. For comparison, the +1 charges state of angiotensin I obtained using solvent free (sf) preparation which does not produce multiply charged ions is shown.

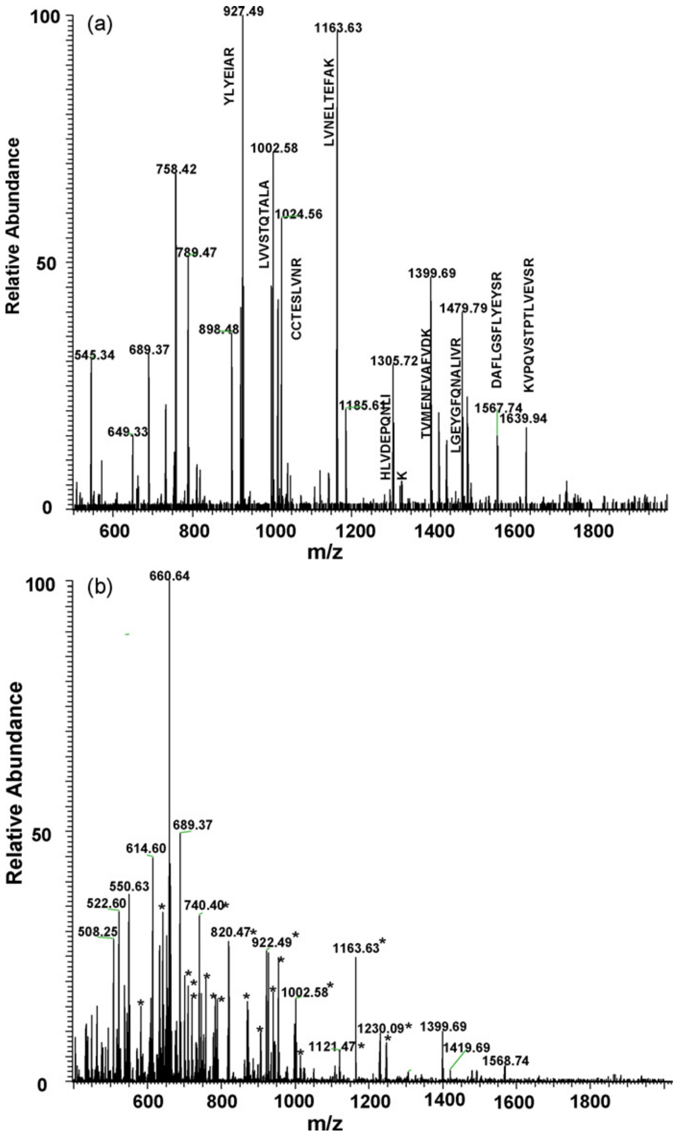

Fig. 2. LSI of a BSA tryptic digest using 2,5-DHAP as matrix at (a) an ion transfer tube temperature of 450 °C showing predominately singly charged ions and (b) an ion transfer tube temperature of 350 °C showing numerous doubly charge ions denoted by a*. The singly charged ions produced in this way are very similar to MALDI produced spectra of BSA tryptic digests [45].

ions when using 2,5-DHAP as matrix. This is demonstrated for a bovine serum albumin (BSA) tryptic digest (Fig. 2), where Fig. 2a is obtained at a capillary temperature of 450 °C and Fig. 2b at 350 °C using 2,5-DHAP as matrix. At 350 °C, numerous doubly charged ions are observed as denoted by the asterisks, but at 450 °C, the +2 ions have mostly been converted to +1 ions presumably because of droplet turbulence caused by the high desolvation temperature, sub-AP conditions, and a more volatile matrix interfering with the ionization mechanism for production of multiply charged ions. Further study is needed to elucidate the mechanism for production of the singly charged ions.

The desolvation temperature requirement for desolvation is different for different matrix materials. For example, excellent quality mass spectra are obtained using 2,5-DHAP with a capillary temperature below 250 °C [10,11]. On the other hand, CHCA was believed not to produce any multiply charged ions in LSI [7]. However, recent experiments demonstrated that this was a function of the transfer tube temperature and that at 400 °C doubly and triply charged ions of angiotensin I are observed using CHCA, although in low abundance relative to 2,5-DHB at that temperature. Thus, one might expect to get equivalent results with 2,5-DHAP at about 100 °C, 2,5-DHB at about 225 °C, and CHCA at about 400 °C.

Unlike with APCI, ESI, and SSI, where the heated ion transfer capillary can be replaced by a simple skimmer without substantial loss of observed ions, in LSI, as shown in Fig. 1, a heated region is essential for observation of multiply charged ions. In ESI even under poor desolvation conditions, less than an order of magnitude increase in ion abundance is observed over an ion transfer capillary temperature range of 50–450 °C with the Orbitrap Exactive, but in LSI, using 2,5-DHB as matrix, essentially no multiply charged ions are generated with low temperature, demonstrating that these ions are not efficiently produced prior to the ion transfer capillary.

3.2. The role of voltage

No electric field in the AP region is required to produce multiply charged ions and is in fact counterproductive [11,31]. Thus, conversely to the results with increasing transfer tube temperature, the opposite trend was observed with increasing voltage applied between the metal sample plate and the ion entrance aperture in that the +1 ions increase in abundance while the multiply charged ions decrease. This has implications relative to ion transport from AP to vacuum, but also may be important in understanding the LSI mechanism. Modeling of ion transfer from AP to vacuum has shown that in the presence of an electric field >99% of the ions may be lost, many at the rim of the ion entrance orifice ('rim loses') [32,33]. Thus, in addition to the low sampling efficiency of AP ionization processes [34] such as APCI and ESI, those ions passing through the ion entrance orifice suffer major loses at the rim. Without the electric field in LSI, there are no field lines to cause rim losses and ions entrained in the viscous gas flow will enter the ion transfer capillary unimpeded. Nevertheless, the efficiency of sampling singly charged ions in the absence of an electric field is poor, possibly because of the low sampling efficiency caused by diffusion spreading of the ion cloud before the ions reach the area dominated by viscous flow. On the other hand, the efficiency of production of multiply charged ions in the absence of a focusing field can be quiet good, producing the mass spectrum shown in Fig. 3 for just 20 fmol of insulin applied to a microscopy glass slide in the matrix 2,5-DHAP.

We originally proposed that in the absence of an electric field the rapidly expanding matrix plume resulting from the explosive deposition of laser energy would more efficiently propel clusters, having higher momentum, toward the ion entrance aperture relative to the singly charged, lower momentum ions, created very near the matrix surface [7]. Entrapment of the clusters in the viscous gas

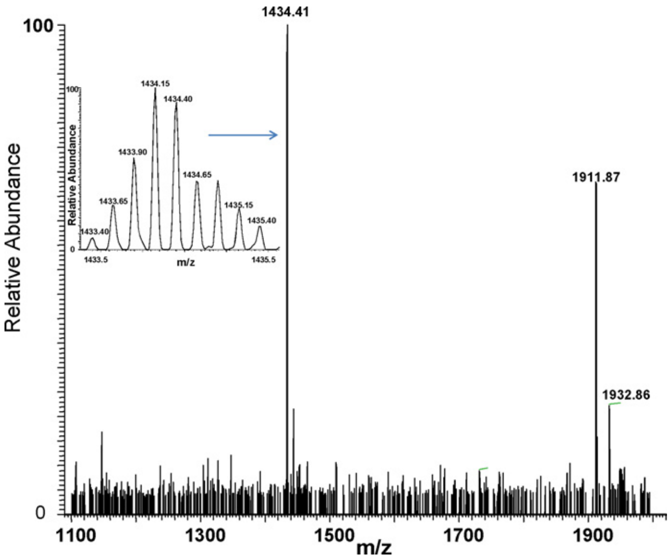

Fig. 3. LSI mass spectrum of 20 fmol of bovine insulin applied to the glass slide using 2,5-DHAP as matrix by the dried droplet method.

flow would aid cluster transfer into the ion transfer capillary. Unlike the dominant process in ESI, where the multiply charged ions are mostly produced at AP and then enter the vacuum of the mass spectrometer, here, the charged clusters, like Trojan horses, pass the ion entrance aperture before the naked ions are released. Thus, herding 'winged elephants' through the gate using field lines is replaced with the concept of fewer but the more massive matrix droplets (Trojan horses) directed toward the gate with high momentum. Desolvation and multiply charged ion formation occur in the heated region inside the viscous gas flow within the ion transfer tube, potentially releasing many analyte ions for each charged matrix droplet.

3.3. *Formation of liquid droplets*

Formation of liquid droplets in the laser ablation process is therefore essential for multiply charged ion formation in LSI. Modeling of droplet formation is limited by computational power to observation of clusters formed very near the matrix surface and having only hundreds of matrix molecules [27]. Experimental collection of laser ablated (nitrogen 337 nm) matrix/polymer material under vacuum conditions and using atomic force microscopy (AFM) to measure the collected cluster sizes shows particles in the 200 nm range [28]. A laser fluence of 120 J m^{-2} produced a lower ratio of individual molecules to clusters than a fluence of 40 J m^{-2}. The results presented required 10,000–20,000 laser shots [28].

Optical microscope images shown in Fig. 4 (as well as the supplemental section) of laser ablation of a 2,5-DHB MALDI matrix at AP using *ca.* 1000 J m^{-2}, similar to LSI experiments, showed on the parent slide (Fig. 4a) what appears to be melted matrix in the area ablated by 10 laser shots. On the collecting slide (Fig. 4b) numerous crystals and droplets were observed ranging in diameter from submicron to a few microns. Also observed on the collecting slide are droplets that hit the surface with an outer liquid layer and a solid core. A solid film is evident which is strongly suggestive of liquid droplets hitting the collection slide with some force. These results clearly show that laser ablation of 2,5-DHB at AP produces rather large quantities of liquid droplets. The number of analyte molecules will be reflected in the droplet volume which increases by the radius cubed. However, a higher charge state of the droplet as well as better desolvation will be necessary to ionize all of the analyte from the larger droplets. A possible interpretation of the temperature graph in Fig. 1 is that effective desolvation of ever larger matrix droplets require higher desolvation temperature. Interestingly, 2,5-DHAP collected in a similar manner produces much smaller liquid droplets than 2,5-DHAP.

3.4. *Droplet charging*

As noted, a major unknown in LSI is the mechanism of droplet charging. It may be a daunting task to determine the source of droplet charging in LSI as the laser energy may be critical in producing the initial droplet charges [16]. In support of this concept are reports that infrared (IR) laser ablation of solid matrixes produces matrix clusters but not multiply charged ions in the absence of an electric field, or with dry solid matrixes even in the presence of an electric field [35–37]. One possible explanation of these results is that insufficient energy is available with IR lasers to produce the droplet charging [28]. Of course, an alternative explanation is that the right matrix for producing multiply charged ions using IR lasers in the absence of an electric field has yet to be used. Nevertheless, if UV, but not IR laser ablation, produces multiply charged ions, it is an indicator that the charges may be related to the laser energy. Notably, volcanos produce copious quantities of positively charged ions [38] from what would appear to be a purely thermal process which could also be the function of a laser beam striking the matrix.

However, it is possible that the charges on the molten droplets are not all produced at the moment of droplet formation, but are altered by, for example, the heated ion transfer capillary. Jarrold et al. [39] have measured an increase in charge for sonic spray produced droplets passing through such a capillary. These authors proposed a bag mechanism in which aerodynamic breakup within the viscous gas flow produces charge separation resulting in preferentially positively charged droplets being detected by a charge detector on the low-pressure side of the capillary. The distortion of the droplets within the gas flow and the tendency for negative charge to preferentially reside at the air–liquid interface produces droplets with either more positive or more negative charges [39]. It was proposed that the negatively charged droplets were too small, and thus did not carry enough charge to be observed using a charge detector. This explanation would suggest that in their experiment negative ions would be observed by MS, however, only positive multiply charged ions have thus far been observed with LSI. This is clearly an area needing further research.

We know that there must be substantial charges generated in LSI because the analyte signal continues to increase and maintain the multiply charged ion charge state distribution, similar to ESI, at least to concentrations of 25 μM analyte in matrix solution. If charge limitation were an issue, first the charge state distribution would be skewed to lower charge states and then a plateau in ion signal would occur as the analyte concentration increased [15,40]. Further, over 20 charges have been observed to occur on a single molecule.

3.5. *Alternative paradigm*

The multiply charged ions released from the highly charged matrix/analyte droplets within the ion transfer tube experience field free conditions and are entrained in a viscous gas flow. In the viscous gas flow, ions can be lost by diffusion to the capillary walls, with lower molecular weight ions expected to have a higher diffusion and increased losses vs. higher mass ions and molten droplets which diffuse more slowly and are expected to experience fewer losses. It is not known if the liquid droplets striking the tube surface will be lost to further ion production, or if they simply bounce off the hot wall similar to a drop of water bouncing on a hot skillet surface. It is also not unreasonable to expect that droplet surface collisions might even increase the droplet positive charge by neutralization

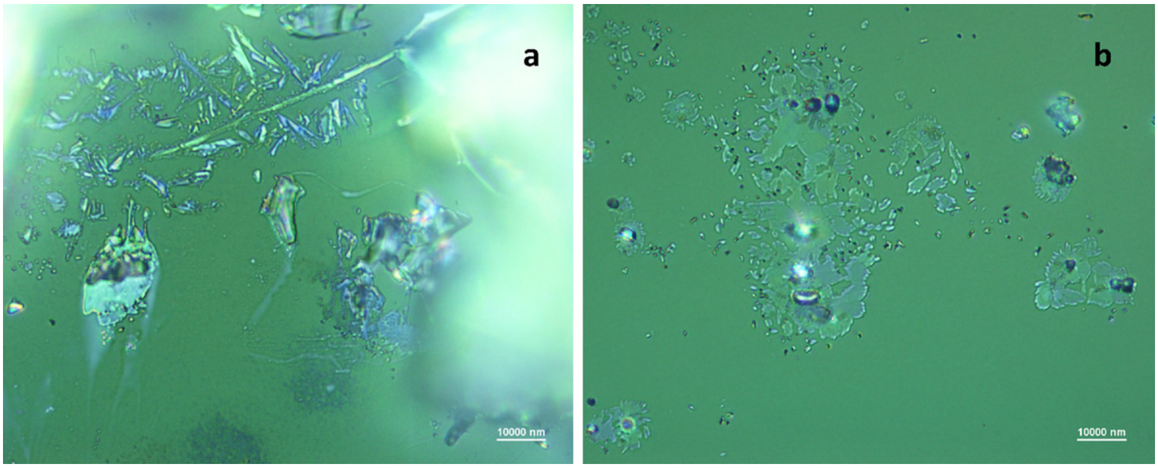

Fig. 4. Optical microscope image of the impact area on a glass slide held 2 mm from an opposing glass slide with 2,5-DHB applied solvent-based and ablated by 10 laser shots from the back in transmission geometry using a nitrogen laser focused to ∼75 μm. (A) The image is of the parent slide showing a 10 μm bar for reference. The light areas to the right and upper left corner are undisturbed matrix and the center is the ablated region showing what appears to be some melting of the matrix. (B) Shows the collected matrix plume, again with a 10 μm bar. Clearly, liquid droplets striking the surface have left a solid film. Some clusters, as in the lower left corner, were only partially liquid when they landed. Numerous submicron droplets and crystals are collected.

of negative charges that under certain circumstances preferentially resides at the droplet liquid–air interface [39]. Ion loss under viscous flow is also proportional to the tube length so that short ion transfer tubes provide fewer losses. Thus, production of ions from charged droplets within a heated ion transfer tube should, in principle, be tunable to maximize droplet desolvation and minimize losses of ions originating from the charged clusters. We have experimented with cone shaped ion transfer tube entrances in order to increase droplet capture with modest success.

Further, ion funnel technology [41,42] can be applied at the low-pressure end of the ion transfer tube to increase the efficiency of ion transfer into the high vacuum region for mass analysis. Recently, it was demonstrated that ESI operated at reduced pressure within an ion transfer ion funnel is an effective means of transferring a higher percentage of ESI generated ions for mass analysis [43]. However, ESI at reduced pressure increases the difficulty of complete desolvation because of evaporative cooling. Because there is a rapid pressure drop within the ion transfer tube used with LSI, evaporative cooling could also be an issue. Desolvation is most effective at higher pressure where sufficient collisions occur to overcome the affects of evaporative cooling, therefore, it is expected that an effective ion transfer tube should be efficiently heated close to the AP inlet.

Until now, heated AP to vacuum ion transfer tubes have been thought of more as a means of transferring the ions produced at AP into the mass analyzer while providing a region to evaporate any residual solvent droplets before they reach the high vacuum of the MS. Of course, desolvation that occurred within the transfer tube with ESI or SSI produces ions, but relative to LSI, only small differences in ion current are observed with a cold vs. a hot ion transfer tube demonstrating that most ions are formed in the AP region [44]. In LSI, essentially all ions are produced in this heated region between AP and vacuum. Thus, optimization of this transfer region for droplet capture and desolvation, ion transmission, and possibly enhanced droplet charging could result in considerable sensitivity enhancement. Not only is this applicable to LSI but possibly to nanospray ESI where the ESI droplets are produced close to the ion entrance orifice.

4. Conclusion

An alternative paradigm for production of multiply charged ions is to produce the ions in the transfer region between AP and vacuum. In this scenario, the charged droplets are not desolvated prior to, but within, the transfer capillary. Thus, the transfer capillary becomes the ionization chamber producing ions from highly charged droplets that are entrained in a viscous gas flow. Considering that LSI, which uses this process, is already highly sensitive at such an early stage in its development, it seems likely that further improvements will ensue.

Acknowledgements

The authors are appreciative of support from the Richard E. Houghton fund at the University of the Sciences in Philadelphia (CNM) as well as the Wayne State University Start-up funds (ST), the National Science Foundation CAREER Award 0955975 (ST), the American Society for Mass Spectrometry Research Award supported by the Waters Corporation (ST) and the DuPont Company Young Investigator Award (ST). The authors also gratefully acknowledge the assistance of Ellen Inutan in the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.05.020.

References

[1] J.B. Fenn, Electrospray wings for molecular elephants, Angew. Chem. Int. Ed. 42 (2003) 3871–3894.
[2] M. Yamashita, J.B. Fenn, Electrospray ionization. Another version of the free-jet theme, J. Phys. Chem. 88 (20) (1984) 4451–4459.
[3] D.I. Caroll, I. Dzidic, R.N. Stillwell, M.G. Horning, E.C. Horning, Subpicogram detection system for gas-phase analysis based upon atmospheric pressure (API) mass spectrometry, Anal. Chem. 46 (1974) 706–710.
[4] D.I. Caroll, I. Dzidic, K.D. Haegele, R.N. Stillwell, E.C. Horning, Atmospheric pressure ionization mass spectrometry corona-discharge ion source for use in liquid chromatography–mass spectrometry computer analytical system, Anal. Chem. 47 (1975) 2369–2373.
[5] A.P. Bruins, T.R. Covey, J.D. Henion, Ion spray interface for combined liquid chromatography/atmospheric pressure ionization mass spectrometry, Anal. Chem. 59 (1987) 2642–2646.
[6] M.P. Balogh, The Mass Spectrometry Primer, 2009, ISBN: 1-879732-04-1.
[7] S. Trimpin, E.D. Inutan, T.N. Herath, C.N. McEwen, Laserspray ionization – a new AP–MALDI method for producing highly charged gas-phase ions of peptides and proteins directly from solid solutions, Mol. Cell. Proteomics 9 (2010) 362–367.
[8] F. Zydel, S. Trimpin, C.N. McEwen, Laserspray ionization using the atmospheric solids analysis probe for sample introduction, J. Am. Soc. Mass Spectrom. (in press).

[9] S. Trimpin, E.D. Inutan, T.N. Herath, C.N. McEwen, Matrix-assisted laser desorption/ionization mass spectrometry method for selectively producing either singly or multiply charged molecular ions, Anal. Chem. 82 (2010) 11–15.

[10] E.D. Inutan, S. Trimpin, Laserspray ionization (LSI) ion mobility spectrometry (IMS) mass spectrometry (MS), J. Am. Soc. Mass Spectrom. 21 (2010) 1260–1264.

[11] C.N. McEwen, B.S. Larsen, S. Trimpin, Laserspray ionization on a commercial AP–MALDI mass spectrometer ion source: selecting singly or multiply charged ions, Anal. Chem. 82 (2010) 4998–5001.

[12] V.V. Laiko, M.A. Baldwin, A.L. Burlingame, Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry, Anal. Chem. 72 (2000) 652–657.

[13] M. Dole, L.L. Mack, R.L. Hines, R.C. Mobley, L.D. Ferguson, M.B. Alice, J. Chem. Phys. 49 (1968) 2240–2249.

[14] J.V. Iribarne, B.A. Thomson, On the evaporation of small ions from charged droplets, J. Chem. Phys. 64 (1976) 2287–2294.

[15] M.A. Tarr, J. Zhu, R.B. Cole, Atmospheric pressure ionization mass spectrometry, Encyclopedia Anal. Chem. (2010) 1–34, Mass Spectrometry.

[16] M. Karas, M. Gluckmann, J. Schafer, Ionization in matrix-assisted laser desorption/ionization: singly charged molecular ions are the lucky survivors, J. Mass Spectrom. 35 (2000) 1–12.

[17] M. Karas, R. Kruger, Ion formation in MALDI: the cluster ionization mechanism, Chem. Rev. 103 (2003) 427–439.

[18] H.R. Pruppacher, J.D. Klett, Microphysics of clouds and precipitation (2000) 792–852.

[19] A. Luts, T.-E. Parts, L. Laakso, A. Hirsikko, T. Gronhohm, M. Kuhmala, Some air electricity phenomena cause by waterfalls: correlative study of the spectra, Atmos. Res. 91 (2009) 229–237.

[20] E.R. Williams, S.R. McNutt, Total water contents in volcanic eruption clouds and implications for electrification and lightning, in: Research Signpost, Recent Progress in Lightning Physics, 2005, p. 13, ISBN: 81-308-0016-0.

[21] T. Pahtz, H.J. Herrmann, T. Shinbrot, Why do particle clouds generate electric charges? Nat. Phys. (2010), doi:10.1038/nphys1631, 11 April 2010.

[22] A. Hirabayashi, M. Sakairi, H. Koizumi, Sonic spray mass spectrometry, Anal. Chem. 67 (1995) 2878–2882.

[23] S. Trimpin, A perspective on MALDI alternatives – total solvent-free analysis and electron dissociation of highly charged ions by laserspray ionization, J. Mass Spectrom. 45 (2010) 471–485.

[24] M. Karas, F. Hillenkamp, Laser desorption ionization of proteins with molecular masses exceeding 10,000 Da, Anal. Chem. 60 (1988) 2299–2301.

[25] S. Trimpin, T.N. Herath, E.D. Inutan, S.A. Cernat, J. Wager-Miller, K. Mackie, J.M. Walker, Field-free transmission geometry atmospheric pressure matrix-assisted laser desorption/ionization of unadultrated tissue samples, Rapid Commun. Mass Spectrom. 23 (2009) 3023–3027.

[26] L.V. Zhigilei, B.J. Garrison, Microscopic mechanism of laser ablation of organic solids in the thermal and stress confinement irradiation regimes, J. Appl. Phys. 88 (2000) 1281–1298.

[27] L.V. Zhigilei, Dynamics of the plume formation and parameters of the ejected clusters in short-pulse laser ablation, Appl. Phys. A 76 (2003) 339–350.

[28] M. Handschuh, S. Nettesheim, R. Zenobi, Laser-induced molecular desorption and particle ejection from organic films, Appl. Surf. Sci. 137 (1999) 125–135.

[29] C.N. McEwen, W.J. Sinomsick, B.S. Larsen, The fundamentals of applying electrospray ionization mass spectrometry to low mass poly(methyl methacrylate) polymers, J. Am. Soc. Mass Spectrom. 6 (1995) 906–911.

[30] S. Trimpin, A.C. Grimsdale, H.J. Raedar, K. Muellen, Characterization of an insoluble poly(9,9-diphenly-2,7-fluorene) by solvent-free sample preparation for maldi-tof mass spectrometry, Anal. Chem. 74 (15) (2002) 3777–3782.

[31] M.C. Galicia, A. Vertes, J.H. Callahan, Atmospheric pressure matrix-assisted laser desorption/ionization in transmission geometry, Anal. Chem. 74 (2002) 1891–1895.

[32] E.W. Sheehan, R.C. Willoughby, Ion enrichment aperture arrays. US Patent 7,060,976 B2 (2006).

[33] R.C. Willoughby, How do we eliminate ion losses at AP? Keeping the barn doors open, LCMS.COM 1 (1) (2006).

[34] J.S. Page, I. Marginean, E.S. Baker, R.T. Kelly, K. Tang, R.D. Smith, Biases in ion transmission through an electrospray ionization–mass spectrometry capillary inlet, J. Am. Soc. Mass Spectrom. 20 (2009) 2265–2272.

[35] S. Konig, O. Kollas, K. Dreisewerd, Generation of highly charged peptide and protein ions by laser desorption/ionization ion trap mass spectrometry, Anal. Chem. 79 (2007) 5484–5488.

[36] J.S. Sampson, D.C. Muddiman, Atmospheric pressure infrared (10.6 μm) laser desorption electrospray ionization (IR-LDESI) coupled to a LTQ Fourier transform ion cyclotron resonance mass spectrometer, Rapid Commun. Mass Spectrom. 23 (2009) 1989–1992.

[37] J.S. Sampson, A.M. Hawkridge, D.C. Muddiman, Development and characterization of an ionization technique for analysis of biological macromolecules: liquid matrix-assisted laser desorption electrospray ionization, Anal. Chem. 80 (2008) 6773–6778.

[38] R.J. Thomas, P.R. Krehbiel, W. Rison, H.E. Edens, G.D. Aulich, W.P. Winn, S.R. McNutt, G. Tytgat, E. Clark, Electrical activity during the 2006 Mount St. Augustine volcanic eruptions, Science 315 (2007) 1097 (23 February).

[39] L.W. Zilch, J.T. Maze, J.W. Smith, G.F. Ewing, M.F. Jarrold, Charge separation in the aerodynamic breakup of micrometer-sized water droplets, J. Phys. Chem. A 112 (2008) 13352–13363.

[40] J.B. Fenn, Ion formation from charged droplets: the roles of geometry, energy, and time, J. Am. Soc. Mass Spectrom. 4 (1993) 524–535.

[41] K. Tang, V. Aleksey, A.V. Tolmachev, E.N. Nikolaev, R. Zhang, M.E. Belov, H.R. Udseth, R.D. Smith, Independent control of ion transmission in a jet disrupter dual-channel ion funnel electrospray ionization ms interface, Anal. Chem. 74 (2002) 5431–5437.

[42] Y. Ibrahim, K. Tang, A.V. Tolmachev, A.A. Shvartsburg, R.D. Smith, Improving mass spectrometer sensitivity using a high-pressure electrodynamic ion funnel interface, J. Am. Soc. Mass Spectrom. 17 (2006) 1299–1305.

[43] J.S. Page, K. Tang, R.T. Kelly, R.D. Smith, Subambient pressure ionization with nanoelectrospray source and interface for improved sensitivity in mass spectrometry, Anal. Chem. 80 (2008) 1800–1805.

[44] C.N. McEwen, T. Wang, Unpublished results.

[45] http://www.neb.com/nebecomm/products/productP8108.asp.