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# Droplet Dynamics and Ionization Mechanisms in Desorption Electrospray Ionization Mass Spectrometry

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A droplet pickup and other mechanisms have been suggested for the ionization of biomolecules like peptides and proteins by desorption electrospray ionization. To verify this hypothesis phase Doppler particle analysis was used to study the sizes and velocities of droplets involved in DESI. It was found that impacting droplets typically have velocities of 120 m/s and average diameters of 2–4  $\mu\text{m}$ . Small differences in sprayer construction influence the operating conditions at which droplets of these dimensions are produced. Under these conditions, the kinetic energy per impacting water molecule is less than 0.6 meV and sputtering through momentum transfer during collisions or ionization by other electronic processes is unlikely. Droplets arrive at the surface with velocities well below the speed of sound in common materials, thereby excluding the possibility of ionization by shockwave formation. Some droplets appear to roll along the surface, increasing contact time and presumably the amount of material that is taken up into droplets during conditions typical of the DESI experiment.

Desorption electrospray ionization (DESI) is a mass spectrometry ionization technique that allows examination of compounds directly from ambient surfaces, reducing and often eliminating the need for sample preparation prior to analysis. DESI is one of a family of ambient ionization methods which share the advantage that ion production occurs in air where the sample is fully accessible during analysis.

As more applications of DESI-MS are developed<sup>1–6</sup> the need arises for a better fundamental understanding of the technique. During the DESI process an interrogating solution, often a water–alcohol mixture, sometimes with reagents such as acids or bases added, is electrohydrodynamically nebulized and directed at a

sample surface. High nebulizing gas velocities are used to accelerate the droplets. In contrast to electrospray ionization, the sample is not dissolved in the spray solvent; instead, analytes present in solid materials or liquids deposited and dried onto surfaces are directly analyzed, often without any sample preparation. During the interaction of the spray with the surface, analytes present on the sample exterior are ionized and transported from ambient pressure conditions into a standard atmospheric pressure interface mass spectrometer for mass analysis.

Impacting droplets and massive solvent clusters have previously been used for surface analysis by mass spectrometry. Massive cluster bombardment (MCI) with charged and accelerated glycerol droplets produced multiple charged ions of large biomolecules such as cytochrome *c* and bovine insulin.<sup>7</sup> In droplet impact/secondary ion mass spectrometry (EDI) experiments electrosprayed water droplets were accelerated in the vacuum system of a mass spectrometer and impacted on to a sample surface.<sup>8</sup> Since these processes take place in vacuum, the projectiles are subjected to fast evaporation, have high resultant surface field strengths, and are accelerated to high velocities by an electric field. The projectiles consequently have high kinetic energies, usually much higher in magnitude than chemical bonds. A shockwave theory is one of many proposed to account for the observation of multiple charging of large protein and peptide molecules by massive polymolecular solvent cluster bombardment in vacuum.<sup>9</sup> Large polyatomic ions such as  $\text{C}_{60}$  and metal clusters are also used for the production and subsequent mass analysis of secondary ions of solid-phase analytes examined in vacuum.<sup>10</sup> Secondary ion mass spectrometry (SIMS) analysis is characterized by damage to the surface and formation of fragments of analytes and substrate molecules. However, use of polyatomic clusters produces considerably less damage to substrates than traditional atomic ion SIMS, allowing for more accurate depth profiling of solid materials. In addition, a 2 orders of magnitude increase in ion intensity compared to atomic projectile SIMS has also been observed.<sup>11</sup> Simulations suggest that this increase is due to energy deposition into a larger number of upward moving substrate ions, distributed over a wider area, which leads to ejection of a greater

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number of intact molecules from the surface.<sup>12</sup> Simulations also indicate that molecules with multiple contact points with a surface are ejected intact by a cooperative uplifting of the molecule when the upward moving substrate atoms hit different parts of the molecule.<sup>13</sup>

In contrast to these techniques, DESI occurs at atmospheric pressure where fast moving droplets are slowed by aerodynamic drag forces due to their interaction with surrounding gas molecules. Many advantages have been noted for DESI and other related atmospheric pressure desorption ionization techniques. A key advantage is that surfaces are examined in the open atmosphere of the laboratory. This enables easy access to the sample during analysis for manipulation of the sample surface and allows for high-throughput,<sup>14</sup> nonproximate,<sup>15</sup> and other forms of analysis. DESI also causes no damage to polymeric or metallic surfaces, although physical ablation of soft sample material such as biological materials does occur.

A number of suggestions have been made to account for the desorption and ionization of molecules by DESI based on empirical data relating to the effects of operating conditions on the mass spectra. These mechanistic suggestions include droplet pick-up, chemical sputtering, evaporation followed by gas-phase ionization, and shockwave models.<sup>16</sup> Droplet pick-up is believed to take place during the brief contact time when droplets collide with sample surfaces and analytes are extracted into the departing droplet or droplet fragments. After droplet pick-up, electrospray ionization occurs by standard electrospray ionization processes, viz. either the field desorption (FDM)<sup>17</sup> or charge residue models (CRM).<sup>18</sup> Chemical sputtering refers to charge and momentum transfer during projectile/surface reactive collisions.<sup>19</sup> Compounds with a high vapor pressure may react with free ions above the surface of the sample or with droplets approaching or leaving the surface and be incorporated into the liquid droplet. There is experimental data that supports some of these mechanisms, although none is yet conclusive.<sup>20</sup> In this paper the validity of some of the proposed ionization mechanisms is evaluated by measuring the sizes and velocities of the inbound electrosprayed droplets. Measurements of droplets leaving the sample surface after a collision were also made.

Phase Doppler particle analysis (PDPA) was used to investigate the sizes and velocities of droplets and how these change with the operating parameters of the DESI experiment. Mass spectra of analytes desorbed from a sample surface were obtained, immediately followed by optical measurements using the same experimental conditions.

To make velocity measurements by PDPA, two crossing laser beams are used to create an interference pattern with a defined

beat frequency. Light is scattered as droplets move through the interference pattern in the measurement volume but with the beat frequency shifted due to the Doppler effect. This shift in frequency is used to calculate the component of velocity of the droplets orthogonal to the propagation direction of the light pattern. For spherical droplets Mie theory can be used to calculate the droplet diameters from the phase difference of the scattered light recorded by three detectors. Use of PDPA for droplet sizing in DESI can be compromised by the fact that electrosprayed droplets are not spherical during Rayleigh explosions or due to deformations caused by aerodynamic drag forces associated with very high nebulizing gas velocities or very large diameter drops. Droplets measured immediately after surface collisions are also expected to show some deformation. In addition to these concerns, the minimum size that can accurately be measured by PDPA is limited to droplets with diameters larger than 0.5  $\mu\text{m}$ . Despite these limitations PDPA produces valuable data and has previously been used to study electrospray ionization. For example, PDPA was used to establish that electrosprayed droplets would rupture when the charges on the droplet surface approach 70–80% of the Rayleigh limit,<sup>21</sup> when, depending on solvent composition, between 15% and 40% of the charges on droplets are lost, while only a small mass loss is experienced.<sup>22</sup> PDPA has also been used to compare droplets produced by different ESI source designs.<sup>23</sup> In this paper the droplet dynamics of an electrospray before and after collision with a surface is studied in an effort to better understand the underlying mechanisms of desorption electrospray ionization.

## EXPERIMENTAL SECTION

**DESI Source and Mass Spectrometry.** An electrospray emitter was prepared from a Swagelok T-piece and two coaxial sections of fused silica capillary tubing. The internal capillary had an inner diameter of 100  $\mu\text{m}$  and an outer diameter of 190  $\mu\text{m}$ . It extended through the T-piece and was connected to a syringe pump, which supplied solvent to the sprayer at 2  $\mu\text{L}/\text{min}$ . The outer tubing had an inner diameter of 250  $\mu\text{m}$  and an outer diameter of 400  $\mu\text{m}$  and was 15 mm in length. This was connected through the T-piece to a dry nitrogen tank supplying  $\text{N}_2$  at 1130 kPa (150 psi). The inner solvent capillary extended 0.3 mm beyond the outer gas capillary. A potential of 5 kV was applied from a high-voltage power supply (Spellman HVPS10, Spellman High Voltage Corp., Hauppauge, NY) to the stainless-steel needle of the solvent syringe. The electrospray that resulted was investigated for droplet size and velocity distributions. The spray was oriented to move vertically downward through the measurement volume, and the spray approaching the surface was measured at various distances from the spray tip. Measurements were also made on the spray after it collided with a PTFE surface. The impinging spray angle was fixed at 60° relative to the sample surface. The parameters were chosen to represent the typical operating conditions for those analytes that are believed to ionize following the proposed droplet pickup mechanism such as peptides, proteins, and other polar biomolecules. The DESI source was installed on a mass spectrometer (LTQ, Thermo-Finnigan, San Jose, CA), which has a heated capillary atmospheric pressure

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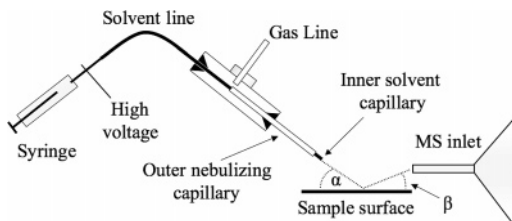
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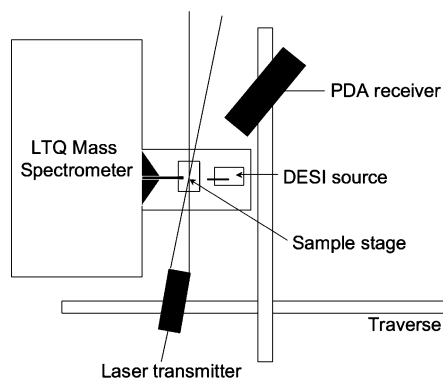
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**Figure 1.** DESI sprayer. The inner, solvent, capillary extends through the T-piece, while the other end is connected to a syringe pump. The outer capillary extends half-way through the T-piece and delivers the nebulizing gas from the third port of the T-piece to the spray tip. See text for capillary dimensions.  $\alpha$  = incident angle =  $60^\circ$ ;  $\beta$  = collection angle =  $10^\circ$ .



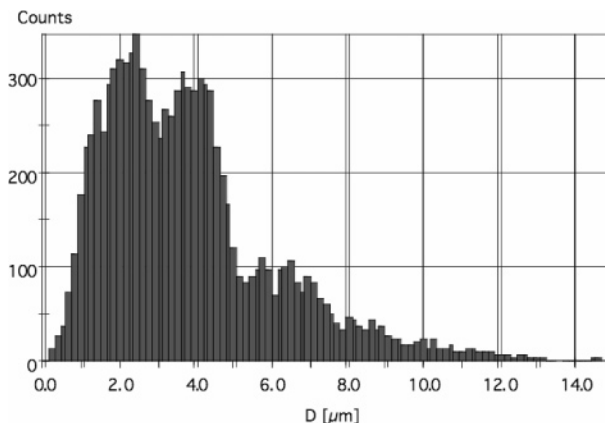
**Figure 2.** DESI-MS experiment with online PDPA droplet analysis.

interface, and the ion currents and droplet sizes and velocities were investigated as a function of various operating conditions, Figure 1.

**Phase Doppler Particle Analyzer (PDPA).** Phase Doppler anemometry measurements were made using a commercial 2D system (Dantec Dynamics Inc., Ramsey, NJ) providing velocity measurements in the vertical and one horizontal dimension. A 633 nm HeNe laser and a frequency doubled Nd:YAG laser beam at 532 nm wavelength were passed through a phase separator and beam splitter. The beams were refracted by an 80 mm lens to produce a dirigible-shaped measurement volume of  $1 \text{ mm}^3$  at the crossing point. The detector, containing three photomultiplier tubes, allowed diameter measurements to be obtained concurrently on each detected droplet. The detector array was placed at a  $30^\circ$  scattering angle relative to the axis through the measurement volume, and the traverse containing the lens and detectors was placed in front of the LTQ mass spectrometer so as to make measurements of the spray impinging on a DESI surface (Figure 2).

## RESULTS AND DISCUSSION

The DESI experiment is a multistep process involving (i) formation of charged primary droplets by an electrospray source, (ii) flight of the droplet projectiles toward a surface, (iii) collision of charged droplets (depending on operating conditions free gas-phase ions) with the surface, producing smaller droplets through collision-induced breakup, (iv) takeoff of secondary droplets, ablated material, and free ions, as well as (v) transport of these through the atmospheric pressure interface and ultimately into the mass spectrometer. During the journey toward and in to the mass spectrometer droplets shrink as solvent evaporates, and if the Rayleigh limit is reached, electrostatic fission events take place, finally producing free ions.



**Figure 3.** Diameters of droplets at 5 mm from the sprayer tip when a 50% methanol/water solution was sprayed using a supply pressure of 1130 kPa and a liquid flow rate of  $2 \mu\text{L}/\text{min}$ .

Figure 3 is a histogram of primary droplet sizes produced under typical experimental conditions by the sprayer described in Figure 1. Measurements were made 5 mm downstream of the sprayer tip from which a 50% methanol/water solution was sprayed using a supply pressure of 1130 kPa and a liquid flow rate of  $2 \mu\text{L}/\text{min}$ . Small droplets are produced when the solvent mixture exits from the fused silica capillary aided by the high nebulizing gas flow rate. High gas velocities shear off liquid from the stream at the capillary exit, preventing formation of the stable Taylor cone that is usually produced when a high potential is applied to a conducting solution flowing from a capillary.<sup>24</sup> This shearing of the liquid and production of multiple small and intermittent spray-producing filaments leads to formation of a spray with a heterogeneous distribution of droplet sizes. Droplet diameters appear to be distributed across a number of populations centered at 2, 4, and  $6 \mu\text{m}$ . This is likely caused by the design of the sprayer where the internal solvent capillary is not centered in the nebulizing gas capillary but tends to lie against one side of it, causing nonuniformity in the interaction of the solvent film and the nebulizing gas.

The high nebulizing gas pressure leads to a high initial droplet velocity. This high velocity may aid in droplet break-up by accelerating the droplets, increasing the aerodynamic shear forces, and thereby leading to secondary droplet formation. However, calculation of the Weber number ( $W < 8$ ) indicates that the aerodynamic drag alone does not induce secondary droplet formation.<sup>25</sup> Deformation of the droplet surface causes perturbations in the charge distribution over the droplet surface, which may increase local charge concentrations to the Rayleigh limit leading to fission.

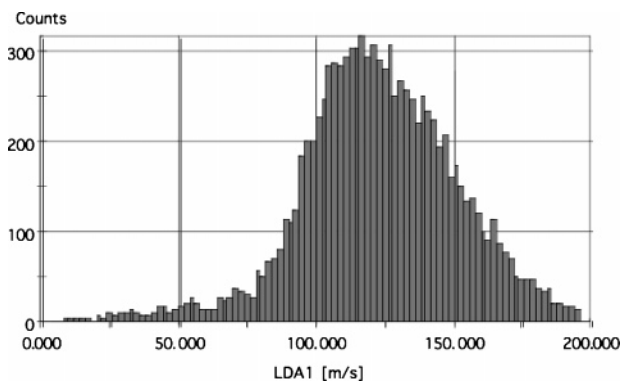
At 2 mm from the sample surface the average droplet velocity was measured to be 120 m/s. Figure 4 shows the distribution of velocities observed at this distance. The velocities are distributed over a wide range between 90 and 200 m/s.

As expected, droplet velocities decrease with distance from the sprayer due to aerodynamic drag forces (Figure 5). Most DESI experiments are performed between 2 and 10 mm from the spray tip. Typical droplet mean velocities of 120 m/s at 2 mm and 40 m/s at 10 mm were measured.

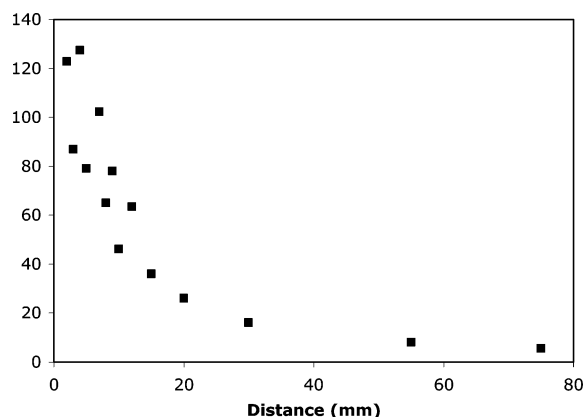
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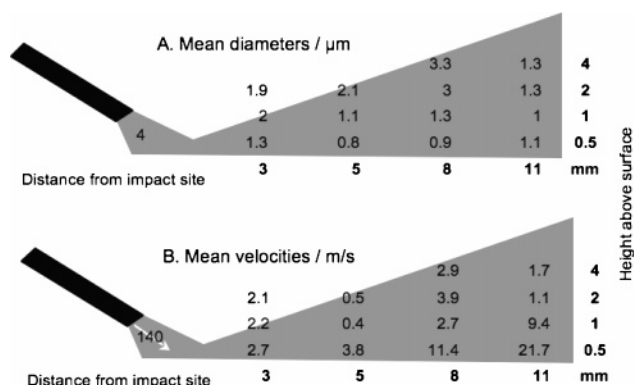
**Figure 4.** Velocities of droplets at 2 mm from the sprayer tip when a 50% methanol/water solution was sprayed using a supply pressure of 1130 kPa and a liquid flow rate of 5  $\mu\text{L}/\text{min}$ .



**Figure 5.** Velocities measured at increasing distances from the spray tip when a 50% methanol/water solution was sprayed using a supply pressure of 1130 kPa and a liquid flow rate of 2  $\mu\text{L}/\text{min}$ .

Basic physics calculations were used to estimate the kinetic energy of the droplets hitting the surface. They indicate that droplets of 3  $\mu\text{m}$  arriving at the surface at 100 m/s have 500 MeV. However, this energy is distributed across the large contact area of the impacting droplet. When the kinetic energy per impacting water molecule is calculated, the energy is found to be 0.6 meV. In Table 1 the projectile sizes, velocities, and kinetic energies of the impacting droplets in DESI are compared to those of related techniques in various forms of secondary ion mass spectrometry.

The size and velocity distributions of droplets after impact at various distances from and heights above the impact site were also measured (Figure 6). Light scattered by the surface interferes with the laser measurements, precluding measurements less than 0.5 mm above the surface. There is a trend toward higher droplet velocities measured at greater distances from the impact site. This is assumed to be a consequence of the droplets moving close to the surface after impact, below the minimum measurement height,



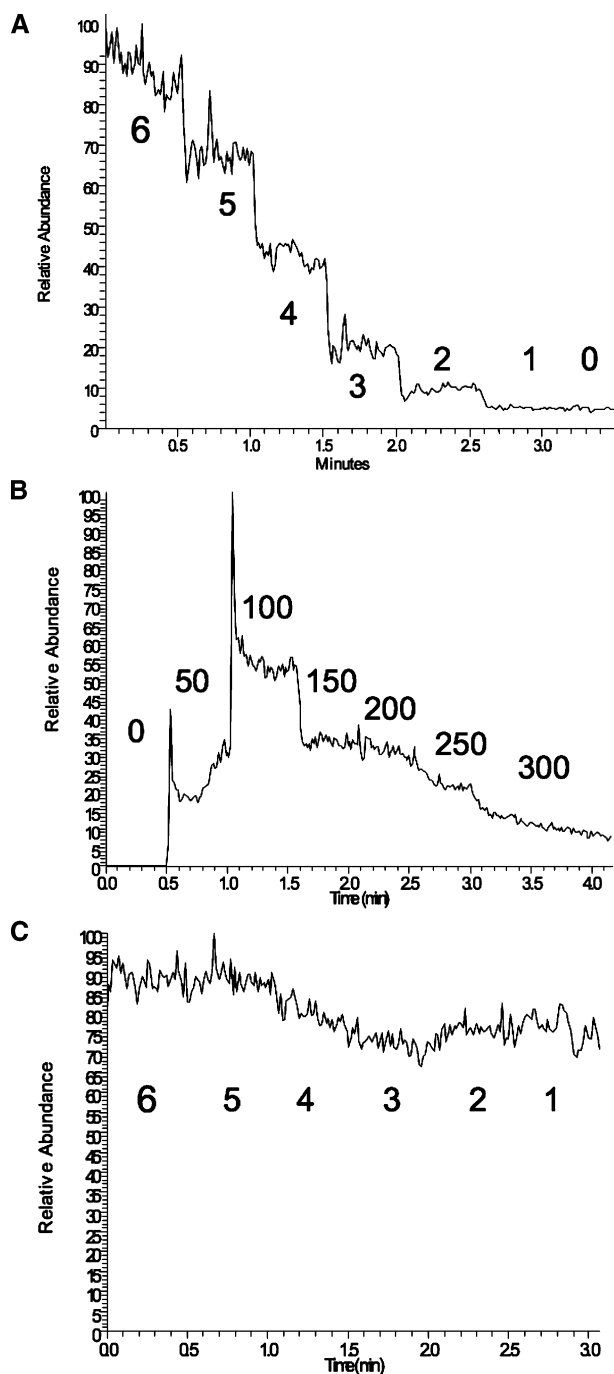
**Figure 6.** Measurement of droplet sizes and velocities at increasing distances from the impact site and heights above the sample surface after collisions when a 50% methanol/water solution was sprayed at 2  $\mu\text{L}/\text{min}$  using a supply pressure of 1130 kPa.

before lifting off to a measurable height at greater distances from the spray impact point on the surface. It is unknown from these measurements but reasonably assumed that even faster moving droplets are present closer to the surface. The droplet velocity data (Figure 6B) also show that at a given distance from the impact point, droplets scattered through larger angles have lower velocities, a simple kinematic effect. Shown in Figure 6A are diameter measurements indicating that larger droplets have a steeper lift-off angle than smaller droplets. Large droplets have more momentum and are able to leave the surface at an angle closer to their specular reflection, while smaller droplets are swept along with the gas flow that tends to follow the plane of a large flat obstruction in its path. As a consequence, when other electrosprayers producing larger primary droplets are used for DESI experiments, the optimum collection angle may be greater than the 10° angle typically used for the sprayer described in this paper. Microphotography<sup>26</sup> of the impact site shows streams of solvent rolling along the surface for a distance equal to about twice the spray diameter (Supporting Information). Yet another possible ionization mechanism can be construed from this phenomenon where ions are perhaps evaporated directly from the charged surfaces of the solvent streams.

Changes in the sizes and velocities of droplets were measured as a function of operating parameters in an attempt to explain the changes in the ion currents observed when conditions are changed (Figures 7–9). The parameters studied were spray potential, nebulizing gas pressure, spray solvent flow rate, and distance of the sprayer from the sample surface. Both Rhodamine B and mellitin were analyzed. Small differences in the capillary tubing dimensions used for the sprayers in the experiments led to differences in the velocity and diameter measurements. For the Rhodamine experiments a sprayer with a smaller internal diameter for the solvent line than described in Figure 1 (50 vs 100  $\mu\text{m}$ )

**Table 1. Comparison of Velocities, Sizes, and Kinetic Energies of Projectiles Used in Desorption Electrospray Ionization (DESI) Compared to the Vacuum Methods of Massive Cluster Ionization (MCI),<sup>7</sup> Electrospray Droplet Impact (EDI),<sup>8</sup> and C<sub>60</sub> Secondary Ionization Mass Spectrometry (SIMS)<sup>28</sup>**

	projectile velocity	projectile size	projectile kinetic energy	kinetic energy per nucleon
DESI	0.1 km/s	$\sim 10^{13}$ u (3 $\mu\text{m}$ )	500 MeV	0.6 meV/u
MCI	6 km/s	$10^8$ u ( $\sim 80$ nm)	1 MeV	1 eV/u
EDI	11 km/s	$10^5$ u ( $\sim 20$ nm)	1 MeV	1 eV/u
molecular SIMS	10–100 km/s	$\sim 10^3$ u (1 nm)	5–20 keV	80–300 eV/u



**Figure 7.** Intensities of ions of Rhodamine B observed by DESI-MS as a function of operational conditions: (A) spray voltage in kV; (B) gas line pressure in psi; (C) solvent flow rate in  $\mu\text{L}/\text{min}$ .

was used and the solvent capillary was also retracted closer to the outer nebulizing gas capillary. This resulted in formation of smaller primary droplets with a narrower distribution of diameters than those obtained for the mellitin analyses and the data presented in Figure 3. A shorter section for the outer gas capillary also resulted in higher maximum velocities as this resulted in a smaller restriction in the gas flow. While the absolute values obtained for the velocities and diameter measurements were

different, they are quite close, and similar trends were observed for the sets of experiments

Increasing the voltage applied to the sprayers produced moderately smaller droplets. For the Rhodamine experiments droplets decreased in size from 2.2 to 1.5  $\mu\text{m}$ . No clear relationship was apparent in the case of mellitin. In both cases a small increase in velocities of droplets impacting the surface was observed as the electrostatic field between the sprayer and the inlet increased with increasing applied voltage (Figure 9C,D). However, changes in this parameter had a large effect on the intensities of ions observed. This is likely the result of increasing the number of charges on the droplet surface, leading to improved efficiency in ionization during the final stages of the desolvation process inside the heated atmospheric pressure interface of the mass spectrometer. For the Rhodamine B analysis (Figure 7A) the intensities continued to increase up to the highest applied voltage of 6 kV. In the case of the mellitin analysis (Figure 8A) the  $(M + 4H)^{+4}$  ion intensity continued to increase with voltage while the intensities of the  $(M + 5H)^{+5}$ ,  $(M + 3H)^{+3}$ , and  $(M + 2H)^{+2}$  ions decreased when voltages higher than 3 kV were applied. The sum of the intensities of all the charge states stabilized after increasing with applied potential up to 3 kV. In a previous study,<sup>16</sup> where only the intensity of the  $(M + 3H)^{+3}$  ion was reported, the signal was shown to increase up to 5 kV before stabilizing. Analyzing the intensity of a single charge state may lead to loss of information as the distribution of charges across the various states may change with variation in the applied voltage.

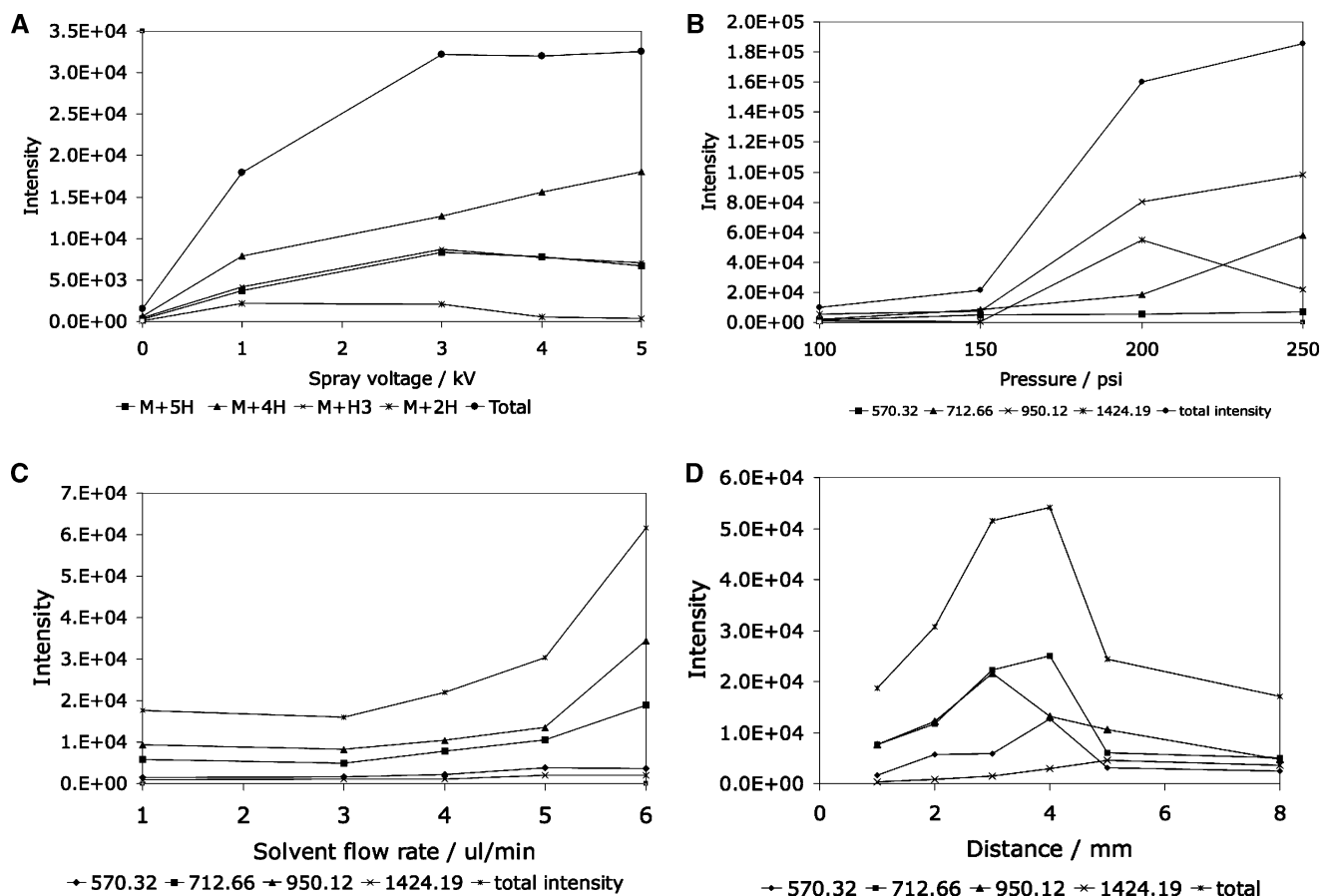
With an increase in supply pressure (controlled at the gas regulator) the mean velocity of the droplets increased up to a maximum value controlled by the restriction imposed on the gas flow by the narrow capillary at the sprayer tip. For the sprayer used in the Rhodamine experiments this maximum was reached at a velocity of 190 m/s at 1820 kPa (250 psi). The sprayer used for the mellitin experiments had a longer section of outer capillary, and a maximum was reached at 120 m/s at 1480 kPa (200 psi). A further increase in applied pressure no longer accelerated the droplets for measurements made 1 mm above the surface with the sprayer a total of 2 mm away from the surface. In both cases the maximum ion intensity was observed when the droplets had average velocities between 100 and 120 m/s. Due to the particular sprayer construction used in the mellitin analysis, faster velocities could not be obtained and a plateau in intensity was observed at an applied pressure higher than 200 psi. For the Rhodamine experiment, faster velocities decreased the observed intensities. There are two possibilities to account for this observation. On one hand it could be due to geometrical factors where faster moving droplets have a different takeoff trajectory; alternatively, it could be an effect of droplet residence time on the surface. These effects require further investigation.

Droplet diameters were minimally affected by changes in applied pressure except at low pressures (<100 psi) where large droplets were produced due to inefficient nebulization. A minimum in droplet sizes was also measured at 790 kPa (100 psi), which may have a beneficial influence on ionization efficiency (Figure 9B). For the Rhodamine experiments, diameter measurements made at the pressure setting corresponding to the maximum in ion intensity were 14% smaller than the average (average excluding the large droplets at inefficient nebulizing gas pressures), while correspondingly for mellitin, droplets were 6.6% smaller. These

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**Figure 8.** Melittin ion intensities by DESI-MS as a function of operating conditions: (A) applied voltage; (B) regulator-controlled pressure of nebulizing gas; (C) flow rate of methanol/water delivered by syringe pump; (D) distance of the sample sprayer from the surface. Measurements were made 1 mm above the sample surface.

measurements appear to indicate that smaller droplets are beneficial for increasing ion intensities.

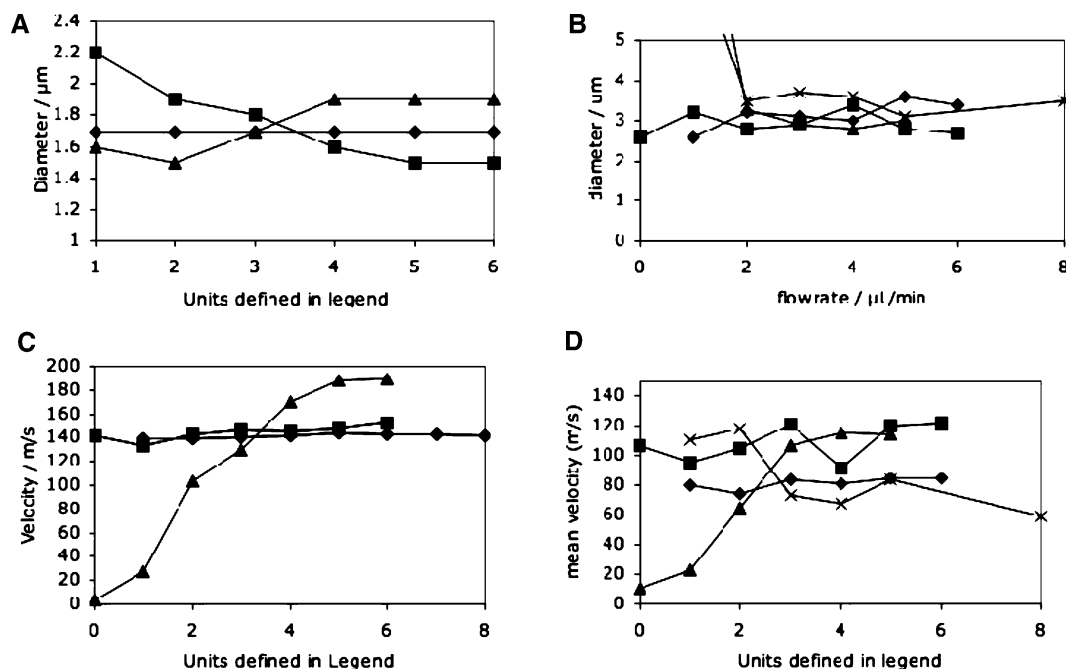
The mean velocity of the droplets decreased with distance from the spray tip, an observation ascribed above to aerodynamic drag forces. Very close to the spray tip velocities of up to 200 m/s have been measured, and velocities at or near the speed of sound in air were predicted from early calculations.<sup>27</sup> Due to interference on the droplet measurements by light scattered from the sprayer and the difficulty of measuring very dense sprays close to the sprayer, measurements were always made at least 1 mm away from the sprayer. At this position the maximum mean velocity observed was 180 m/s, while 140 m/s is typical. Droplets also have to travel 1 mm from the measurement position before impacting with the surface. Thus, the droplets reach the surface at velocities of 120 m/s or less. The mean velocity decreases to 60 m/s at 8 mm away from the spray tip. Diameters of droplets were not observed to change much by moving the sprayer further away from the surface. Evaporation times of methanol droplets were calculated to be 2.6 ms, and 50/50 methanol–water droplets will take even longer to evaporate. The residence time for a droplet in the distance window studied here is typically a few microseconds, allowing for a loss of less than 8% of the droplet volume (for methanol, even less for methanol–water mixtures) during the flight time on the way to the surface. For melittin, MS intensities are seen to increase for distances up to 4 mm. This is in contrast to the results previously reported<sup>16</sup> for melittin where a maximum was observed at 1 mm. This difference in observation

underscores the importance of the geometry in DESI analysis. In previous work the geometries were re-optimized and signal strength maximized for each new distance setting. Changing the distance of the inbound spray changes the optimum impact-to-MS inlet distance and/or the collection angle.

Predictably, changes in the flow rate of the spray solvent did not affect the mean velocity of droplets. A small upward trend was observed for the average sizes of droplets with an increase in flow rate. A modest increase in signal with an increase in flow rate is observed for both Rhodamine and melittin analysis. This is likely the consequence of an increase in droplet flux since the small increase in droplet diameter does not account for changes in mass flow. An increase in the flux of droplets has a positive effect on the ion intensities. This is different from previously reported results<sup>16</sup> where an increase in signal was observed up to 3 µL/min, after which it started to decrease slightly.

## CONCLUSIONS

The effect of operating conditions on droplet sizes and velocities and the influence of these on signal intensities has been studied. It was found that the nebulizing supply pressure and distance between sprayer and surface have a large effect on the velocities of the droplets and on the signal intensities. It appears that the optimal velocities of droplets used for analysis by DESI is between 100 and 120 m/s. Scattered droplets had higher velocities when scattered close to the plane of the surface, and these are the droplets which are assumed most likely to be



**Figure 9.** PDPA measurements of the sizes and velocities of electrosprayed droplets made immediately (A and C) after the measurements in Figure 7 and (B and C) after the measurements in Figure 8 without changing the position of the sprayer or sample surface: (◆) flow rate in  $\mu\text{L}/\text{min}$ ; (■) spray voltage in kV; (▲) Gas supply pressure in psi/50; (×) distance between sprayer and surface in mm.

effective in analyte transfer in the droplet pickup DESI mechanism. Droplet sizes were mostly unaffected by changes in operating conditions. Modest effects on the droplet sizes were observed for changes in the electrical potential on the spray tip and by the nebulizing gas flow rate.

Contributions from complementary processes have been proposed to account for the ionization of different kinds of analytes by DESI. Ionization of proteins and peptides is generally accounted for by the droplet pick-up mechanism. Lower mass compounds with acidic or basic functionality may be ionized by the vapor-phase ionic process of chemical sputtering, droplet pick up, or a combination of these. In rare cases radical anions and cations may be produced through gas-phase ionization processes, although this study has adduced no new evidence for this. The merits of these ionization mechanisms are now considered in terms of the results reported above.

Sputtering by inelastic nuclear collisions or electronic processes or even conventional momentum transfer is impossible due to the inherently small cluster energy per nucleon, which is calculated to be less than 0.6 meV/water molecule for typical droplets impacting on a surface at less than 200 m/s. Ionization by shockwave formation too is not expected to contribute to ionization during a DESI experiment. In order for a shockwave to occur the velocity of an impacting projectile needs to exceed the speed of sound of the surface or projectile material. In DESI, droplets impact on the surface with velocities well below 200 m/s, which is much slower than the speed of sound in typical solid materials, liquids, or gaseous mixtures. Studies of droplets by PDPA do not provide enlightenment on the chemical sputtering ionization mechanism as this involves charge transfer from species too small to be observed with this system. The observations of ions produced by chemical sputtering will be further studied by atmospheric pressure ion gun experiments and other methods of chemical analysis.

The evidence acquired in this study supports the droplet pick-up model as the major process leading to ionization of the analytes considered under the conditions studied here. Condensed phase analytes are solvated by the impinging droplets during interaction with the surface followed by typical electrospray ionization processes. Further evidence supporting this mechanism is found in the observation of droplets surviving surface collisions and production of multiple charged ions of peptides and proteins. Further experiments studying the effects of surface properties including surface roughness, hardness, and dielectric constant on charging of the surface and the emission of secondary droplets and ions are being conducted. Experiments where a neutral spray impinges on the sample surface and picks up analyte before a voltage is applied to the analyte-carrying droplets are also being investigated.

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## SUPPORTING INFORMATION AVAILABLE

Video clip showing droplets impinging on a surface; micro-photograph of spray colliding with a smooth glass surface. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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