Internal Energy Distributions in Desorption Electrospray Ionization (DESI)

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The internal energy distributions of typical ions generated by desorption electrospray ionization (DESI) were measured using the “survival yield” method, and compared with corresponding data for electrospray ionization (ESI) and electrosonic spray ionization (ESSI). The results show that the three ionization methods produce populations of ions having internal energy distributions of similar shapes and mean values (1.7–1.9 eV) suggesting similar phenomena, at least in the later stages of the process leading from solvated droplets to gas-phase ions. These data on energetics are consistent with the view that DESI involves “droplet pick-up” (liquid-liquid extraction) followed by ESI-like desolvation and gas-phase ion formation. The effects of various experimental parameters on the degree of fragmentation of p-methoxy-benzylpyridinium ions were compared between DESI and ESSI. The results show similar trends in the survival yields as a function of the nebulizing gas pressure, solvent flow rate, and distance from the sprayer tip to the MS inlet. These observations are consistent with the mechanism noted above and they also enable the user to exercise control over the energetics of the DESI ionization process, through manipulation of external and internal ion source parameters. (J Am Soc Mass Spectrom 2008, 19, 420–427) © 2008 American Society for Mass Spectrometry

Desorption electrospray ionization (DESI) is an ambient ionization technique in which solid samples are analyzed with minimal or no sample preparation by spraying fast-moving charged solvent droplets onto the sample surface. Analytes present at the surface are extracted and carried by the offspring droplets into the atmospheric pressure interface of the mass spectrometer (MS). Since its introduction in 2004 [1], DESI has found numerous areas of application, including forensics [2–8], imaging [2, 3, 9–11], metabolomics [12–15], pharmaceuticals [8, 14, 16–25], characterization of natural products [1, 8, 19], bacteria [26], polymers [27, 28], proteins [1, 24, 29–32], and explosives detection [4, 8, 33–37]. Investigations into the fundamentals of the droplet dynamics and ionization mechanism have also been conducted through systematic measurements of the droplet size and velocity [38], computer simulations [39], surface charging effects [40], or more simply, by evaluating the mass spectra of various types of analytes in conjunction with variations in the experimental parameters [41]. These investigations suggested that the primary ionization mechanism in DESI is “droplet pick-up”, i.e., extraction of the analyte into the droplet, followed by electrospray ionization (ESI)-like mechanism in which the progeny droplets are desolvated yielding ionized molecules. One of the arguments supporting this conclusion, the

similarity between the ESI and DESI mass spectra, also implies that the two ion sources should generate populations of ions with similar internal energy distributions, $P(E)$. It should be noted though, that the initial events in the two experiments are quite different, and recent simulations show that the process of formation of progeny droplets from the initial $\sim 4\ \mu\text{m}$ droplets in DESI is not dependent on charge build-up as it is in ESI. These dissimilarities need not result in differences in the final internal energy (E) since the later processes occurring in the atmospheric pressure interface are probably the same for these two processes and, as we will show later, dominate the energetics of the process.

Here we use the “survival yield” (SY) method [42] applied to a set of thermometer ions (benzylpyridinium cations) to judge internal energy deposition associated with soft ionization techniques [43] to measure and compare the internal energy distributions of the ions generated by DESI and two related methods which produce ions directly from solution, namely ESI and electrosonic spray ionization (ESSI) [44]. We also examine the effect of various operating parameters in the DESI and ESSI sources on the extent of fragmentation of the thermometer ions. The ESSI technique is closely related to ESI but one which operates at high nebulizing gas flow rates. The conditions in ESSI are more similar to those used in DESI making this method more appropriate than ESI for comparison to DESI with regards to the ion internal energy.

The internal energies of the molecular ions govern their propensity to fragment. The full scan mass spec-

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Scheme 1. Structure and fragmentation pathway of the benzylpyridinium cations.

trum reflects the internal energies deposited during the ionization process. These ion internal energies are difficult to measure, although estimates can be made based on the degree of dissociation, i.e., using a process which is the inverse of that used to calculate mass spectra [45]. This has given rise to the deconvolution method [46] and the thermometer ion method [42] of estimating $P(E)$. The latter was originally developed two decades ago for activated ions [42] and is applicable to species, i.e., thermometer ions, assumed to have similar distributions $P(E)$ and to fragment by a sequence of reactions of known thermochemistry. The fundamental assumption when using this method is that all ions with internal energy below the critical energy, E_0 , do not dissociate and are observed as intact precursor ions, while all ions with an internal energy above the critical energy do dissociate and are observed as fragment ions. “Survival yield” (SY) is the name applied to the modified version of the thermometer ion method, which has been developed for the investigation of soft ionization techniques [47, 48]. The survival yield is the fraction of ions with internal energy below the critical energy; it can be calculated as shown in eq 1, where $I(\text{parent ion})$ and $I(\text{fragment ion})$ represent the peak abundances corresponding to the precursor and the fragment ions, respectively. The $P(E)$ is obtained by taking the derivative of the breakdown curve; in cases where each molecule shows a single fragmentation channel and all are considered to have the same kinetic parameters, this is given simply as the plot of SY versus E_0 .

$$SY = \frac{I(\text{parent ion})}{I(\text{parent ion}) + \sum I(\text{fragment ion})} \quad (1)$$

Since its initial development, the SY method has been applied for measuring the internal energy distributions of ions produced in such ionization methods as ESI [49], matrix assisted laser desorption ionization (MALDI) [50], secondary ion mass spectrometry (SIMS) [51], and fast atom bombardment (FAB) [48]. Studies involving the effect of various instrumental parameters on the energetics of the ESI source have also been conducted, and they aimed at gaining a better understanding of the

ionization mechanism and of the possibility of efficiently tuning the internal energy of the ions generated [49, 52–54]. It has been demonstrated that in ESI the shape of the $P(E)$ curve corresponds approximately to a thermal energy distribution [53]. Characteristic temperatures have been calculated for a “cold” ion source (i.e., zero cone voltage) and for conditions corresponding to varying degrees of in-source ion activation. These studies revealed that at the time of formation in the ESI process, the ions are “hot”, and supplemental energy can be added by appropriate manipulation of the voltages applied to the lenses in various parts of the atmospheric pressure interface and by increasing the temperature of the heated transfer capillary [54]. It is therefore necessary that the energetics of the in-source collision induced dissociation (CID) process be maintained constant when different atmospheric pressure ionization sources are compared. It has been recognized [54] that using the SY method with no correction for the kinetic shift provides the shape of the internal energy distribution but can only be used to approximate the mean value of E . This level of approximation is adequate for comparing the internal energy distributions of ions generated in different but related ion sources such as DESI, ESI, and ESSI, successively installed on the same instrument. The benzylpyridinium cations used in this study have the general structure and fragmentation behavior presented in Scheme 1. The various substituents (R) in these cations and the calculated values for the critical dissociation energies [43] are listed in Table 1.

Experimental

Experiments were carried out using a commercial Thermo Electron LTQ (San Jose, CA) linear ion trap mass spectrometer with a mass/charge range up to 2000 units equipped with a heated transfer capillary. The $P(E)$ measurements were conducted for each ion source, DESI (home-built [41] but similar to that of Prosolia, Inc., Indianapolis, IN), ESI (Thermo Electron LTQ, San Jose, CA), and ESSI (home-built [55]) under identical operating conditions of the atmospheric pressure interface. The external source parameters were those routinely employed for each ion source. Figure 1 shows a diagram of the DESI source indicating the most important geometrical parameters. The operating conditions chosen for the $P(E)$ measurements were: α : 55°, β : 0–15°, h : 1 mm, s : 1 mm, and b : 1 mm. The ESSI

Table 1. Critical dissociation energies of the benzylpyridinium ions

R	$E_0(\text{V})^a$
OCH ₃	1.51
CH ₃	1.77
Cl	1.90
CN	2.10
NO ₂	2.35

^aTaken from reference [43].

Figure 1. Diagram of the DESI source. The geometric parameters are: incident angle (α), height of the spray tip (h), spray tip-to-sample distance (l), collection angle (β), spray impact point-to-substrate edge distance (s), substrate-to-MS inlet distance (b), and spray impact point-to-MS inlet distance (L).

measurements were conducted by directing a sprayer, identical to that of the DESI source, towards the MS inlet at a distance of 2 mm. The pressure of the nebulizing gas (nitrogen) as measured at the gas tank was 150 psi while the temperature of the sample was room temperature as no heating was involved in DESI. The capillary temperature was maintained at 150 °C, while the capillary and the tube lens voltages were optimized for this study as explained later in the text. The solvent flow rate and the spray voltage were 5 μ L/min and 5 kV, respectively, for all three sources. Each data point was obtained by averaging at least three experimental values.

p-Chloro-benzylpyridinium chloride (p-Cl), p-methylbenzylpyridinium bromide (p-CH₃), p-methoxybenzylpyridinium tetrafluoroborate (p-OCH₃), and p-nitro-benzylpyridinium bromide (p-NO₂) were purchased from the Florida Center for Heterocyclic Compounds, University of Florida-Gainesville, while p-cyano-benzylpyridinium chloride (p-CN) was synthesized in-house as described elsewhere [56]. Methanol/water (1:1) solutions of the mixed salts were prepared at concentrations of 10^{−4} M each for the DESI experiments and 10^{−6} M each for the ESI and ESSI experiments. Samples were prepared for DESI by depositing 0.2 μ L of the sample solution on etched glass. Etched glass substrate was chosen for its ability to provide longer lasting signals. For the experiments where the influence of various experimental parameters on the fragmentation extent of the ions was studied, a 10^{−4} M solution of p-OCH₃ was used.

Calculations

The method used to calculate the internal energy distribution is based on that previously reported by Gabelica and De Pauw [43]. The SY values for each thermometer ion were calculated using eq 1 and plotted as a function of the dissociation energies, E_o . Two additional SY values 0 at 0 eV and 1 at 3.5 eV were assumed and included in the curve. Equation 2 shows the 3-parameter sigmoid used to fit the breakdown curve thus generated. (The breakdown curve of an ion defines its fragmentation and is the plot of normalized fragment ion abundances against internal energy.) E is the internal energy and a , b , and c are the coefficients of the 2D sigmoidal equation which were calculated using a web-based curve fitting program (found at <http://zunzun.com/>). The $P(E)$ curve was calculated by taking the derivative of the breakdown curve.

$$SY = \frac{a}{1 + \exp\left(\frac{b - E}{c}\right)} \quad (2)$$

Results and Discussion

Effects of the Capillary and the Tube Lens Voltages

The instrument was operated using inlet capillary and tube lens voltages such that the internal energies of the thermometer ions led to a large spread of the survival yield values between 0 and 1 (Figure 2). The fact that the chosen conditions led to the correct ranking of the critical energies [54] justifies their selection. (Note that the same operational parameters are routinely varied/adjusted for maximizing signal and that the same conditions were used for DESI and for the comparison methods of ESI and ESSI. We therefore limit the present study to the softest in-source energetics that still allows the survival yield method to be used).

The survival yields were measured for the five

Figure 2. Variation of the survival yield as a function of the capillary voltage, for ESSI-MS at 100 V tube lens voltage.

Figure 3. Calculated breakdown (a) and $P(E)$ (b) curves for DESI, ESSI, and ESI sources using the survival yield method.

thermometer ions at various tube lens voltages while varying the capillary voltage. More than one combination of the two parameters was found optimal for $P(E)$ measurements for each ionization method. For example, in the case of ESSI, when holding the tube lens at 100 V (see Figure 2), any voltage between 70 and 100 V applied to the capillary produced a well-spread series of survival yields. The measurements were repeated for each source and results similar to those presented in Figure 2 were obtained for the DESI and ESI sources. This allowed us to easily find a unique set of operational conditions (70 V capillary voltage and 100 V tube lens voltage) that could be applied for all three ion sources.

$P(E)$ Measurements

Internal energy distributions were measured for the DESI, ESSI, and ESI sources at 70 V capillary voltage and 100 V tube lens voltage. The operating parameters are listed in the experimental section. The breakdown and $P(E)$ curves were calculated as described earlier in this paper and plotted in Figure 3a and b.

Internal energy distributions similar in both shape and mean value (between 1.7 and 1.9 eV) were obtained

for the three types of ion sources. This outcome suggests that (1) the deposition of the internal energy, at least in the final desolvation stages of the ionization event, is similar between the three ionization methods and (2) the differences in the mechanisms of droplet formation in ESI, ESSI, and DESI appear to have limited effects on the overall distribution of internal energy of the ions. The mechanisms of droplet formation for ESI, ESSI, and DESI are electrospray, nebulization assisted by high velocity nitrogen, and secondary droplet formation through collision of a primary solvent droplet with the surface liquid film, respectively. The final desolvation stages of the ionization event refer to the ion evaporation [57–59] or the charge residue mechanisms [60] believed to lead to the gas-phase ions from a flux of charged solvent droplets entering the mass spectrometer.

Effects of DESI and ESSI Source Parameters on the Survival Yield of *p*-Methoxy-Benzylpyridinium Ions

The offspring droplets obtained in DESI after the impact with the sample surface are smaller and slower than those directly sprayed [38]. However, as shown above, the internal energy distributions of the final ions are similar for DESI and ESSI under the experimental conditions employed in the measurements. It has been reported [38] that the offspring droplets collected at about 10° above the surface have diameters of 2 μ m or less and velocities below 10 m/s while the directly sprayed droplets are 2 to 4 μ m in diameter and have velocities of 100 to 140 m/s. There are likely differences in internal energy of the progeny ions associated with these differences in droplet size and velocity; however, given the complexity of the phenomena occurring upon droplet and ion transport through the atmospheric pressure interface, these effects are not straightforward. We have conducted a series of experiments to evaluate the effects of various attributes of the charged droplet flux (mainly the velocity acquired outside the MS as that varies the most after the surface impact) on the internal energy of the thermometer ions as measured by their extent of fragmentation. Thus, we examined in parallel for DESI and ESSI, the effects of several source parameters on the extent of fragmentation of *p*-methoxy-benzylpyridinium ions. The comparison was aimed at those parameters of the ion source that are known to affect the droplet velocity, are common between the two ionization techniques, and/or are routinely optimized in applications: nebulizing gas pressure, distance from the MS inlet to the sprayer tip (in ESSI) or to the sample (in DESI), and solvent flow rate. The relative contribution of the operational parameters of the outer source and those at the atmospheric pressure interface (e.g., capillary temperature and voltage, tube lens voltage) was also evaluated.

The internal energy of ions generated through spray ionization techniques originates in the thermal energy

of the droplets from which they emerge [53], and in the conversion of kinetic energy into internal energy in the course of collisions with the gas molecules in the atmospheric pressure interface. Earlier studies on the thermal energy distribution in ESI [53] revealed that the characteristic temperature of ions at the time of formation, assumed to be the temperature of the droplet, was 500–600 K, much higher than the temperature of the ion source (300–350 K). Such high temperatures may be acquired through friction of the fast-moving droplets with gas molecules in the interface, which overcompensates for the effect of evaporative cooling. The droplet temperature increases with increasing the droplet velocity, surrounding gas pressure, and duration of the frictional process, but may also depend on the type of solvent [49] and the size of the droplets. Solvents with high vapor pressure for which the heat at the droplet surface cannot be easily dissipated through evaporation [49], as well as those with low thermal conductivity, will generate hotter ions. The size of the droplet affects its temperature through the evaporative cooling rate which is inversely proportional to the droplet radius [61] and the heat dissipation efficiency, which decreases for smaller surface area to volume ratios. In other words, smaller droplets warm up and cool down faster than the larger droplets and the relative contribution of the two opposite effects is not straightforward.

The kinetic energy of the ions is acquired through acceleration in the electric field of the skimmer and during the postcapillary expansion [62], in the course of ion ejection from the charged droplets and due to the flow dynamics in the spray. Ionic collision with gas molecules in the skimmer region converts a fraction of the kinetic energy into internal energy. If sufficient time is available, the ions dissociate (i.e., they undergo in-source CID). The kinetic energy of the ions emitted during ion evaporation is governed by the strength of the electric field at the droplet surface and therefore by the charge density and droplet size. In addition, to the kinetic energy at the moment of collision, the pressure in the atmospheric pressure interface, the type of gas, and the degree of ion solvation also affect the internal energy build up. Higher pressures in the skimmer region, easily achieved by increasing the solvent intake, lead to multiple short-range collisions, which enhance desolvation while preventing extensive ion activation [54]. The systems most affected by pressure are those which fragment most easily, such as noncovalent complexes.

Increasing the nebulizing gas pressure from 75 to 400 psi caused the extent of fragmentation of the $p\text{-OCH}_3$ cation to increase slowly and then stabilize above 250 psi in both the DESI and ESSI experiments (Figure 4a). The spray parameter mainly affected by the nebulizing gas pressure is the droplet velocity [38], which for the directly sprayed droplets increases abruptly from nominal zero to about 180 m/s (measured at 2 mm from the sprayer) with increase in the pressure up to about 200 psi. At higher gas pressures the restriction imposed on

Figure 4. Variation of the survival yield of $p\text{-OCH}_3$ with (a) the nebulizing gas (nitrogen) pressure and (b) the distance to the MS inlet for DESI and ESSI. Error bars correspond to one standard deviation.

the gas flow by the sprayer capillary prevents further acceleration of the droplets. Apparently the droplet velocity acquired outside the MS makes only a small contribution to the kinetic energy of the gas-phase ion under the experimental conditions applied here and ultimately to the extent of fragmentation. Generalizing, the difference in the droplet velocities between ESSI and DESI should make only a modest contribution to the total internal energy of the ions.

The distance between the MS inlet and the surface impact site in DESI (or the sprayer tip in ESSI) has been investigated as this parameter also affects droplet velocity [38] due to aerodynamic drag forces. Differences in excess of 100 m/s, similar to those observed upon increasing the nebulizing gas pressure have been measured [38] when retracting the ESSI sprayer tip about 8 cm away from the MS inlet. An increase in the SY is therefore expected, the opposite effect of raising the pressure, but of similar absolute magnitude. Indeed, as seen in Figure 4b, the value of SY increases for both the

directly sprayed and the DESI droplets. In the case of ESSI, it is interesting to note a steady increase in fragmentation extent of the electrosprayed droplets over the first 2 cm from the MS inlet, although the velocity was shown to drop abruptly [38]. It is therefore likely that what we witness is a more complex process and not just the result of a simple decrease in droplet velocity. For example, the solvent intake and therefore the pressure in the skimmer region decreases as the spray plume is sampled further from the sprayer tip where the droplet density is significantly reduced. This effect is similar to that obtained when varying the solvent flow rate (see below). The ions will reach the skimmer less solvated in the absence of solvent vapor and will be more easily activated through collisions. This effect is less prominent in DESI as the flux of the offspring droplets is smaller than that of the electrosprayed droplets at the same distance from the MS inlet.

The solvent flow rate has a negligible effect on the droplet velocity and size [38]; however, the droplet production increases to accommodate the higher flow rates. The degree of fragmentation decreases (SY increases) with increasing flow rate for both DESI and ESSI (Figure 5). A similar effect has previously been reported for ESI [63] and was attributed to the incomplete desolvation of the ions in the atmospheric pressure interface of the MS.

The results presented in this section provide additional evidence for the similarity between the internal energy distributions for the two types of ion sources. We interpret this as indicating a commonality in the ionization mechanisms in the latter stages of the ionization process (early differences will tend to be obscured in a system that approaches thermal equilibrium). Moreover, the data show that the characteristics of the droplets at formation (e.g., size, velocity) affect, to a limited extent, the final internal energy of the gas-phase ions under the experimental conditions employed, i.e., fairly energetic, as explained earlier in this text. It is

Figure 5. Variation of the survival yield of p-OCH₃ with the solvent/solution flow rate for DESI and ESSI. Error bars correspond to one standard deviation.

quite possible that a “colder” atmospheric pressure interface (where the kinetic energy acquired by ions in the electric field in the skimmer region is kept at the minimum), together with a series of thermometer ions with lower dissociation energies, would allow a more sensitive investigation of the external source parameters. Nevertheless, this study is adequate both for fundamental and practical purposes, as the demonstrated trends of the survival yields as a function of the operational parameters would maintain in the low-energy region. Moreover, it enables the user to exercise some degree of control over the energetics of the ionization process.

Conclusions

We have shown that the internal energy distributions of the ions produced in ESI, ESSI, and DESI have similar shapes and mean values. Although no corrections have been made for the kinetic shift, it is reasonable to believe that the three related ionization techniques have similar time scales when utilized on the same mass spectrometer. These energetics results are consistent with—although they do not prove—the proposal that the main ionization mechanism in DESI is a “droplet pick-up” (liquid-liquid extraction) mechanism followed by an ESI-like ionization process. The results add to the earlier evidence for the droplet pickup mechanism derived from the agreement between experimental data on postcollision secondary droplet velocities and angles [38] with data obtained by modeling atmospheric transport and droplet-thin film (surface) collisions using finite element computational fluid dynamics [39]. Recent experiments on electrochemical effects in DESI [40] also support this mechanism.

The effects of outer source parameters of DESI and ESSI, such as nebulizing gas pressure, solvent flow rate, and distance from the sprayer tip or surface impact spot to the MS inlet, on the degree of fragmentation of the p-methoxy-benzylpyridinium ions have also been investigated. The data show that the characteristics of the droplet flux have a smaller contribution to the final internal energy of the ions compared with the effects caused by the choice of electrical potentials at the interface and the temperature of the inlet capillary. Any differences in the initial stages of droplet formation among the three ionization techniques are therefore reduced. However, for each type of source, the internal energy of the ions produced can be tuned by manipulating one or more of the outer source parameters.

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