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Feasibility of Formation of Hot Ions in Electrospray

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Internal energy changes during the electrospray process have been studied. Our results suggest that, contrary to conventional wisdom, ions that are quite hot are formed in electrospray, even without acceleration in the cone-skimmer region. The main role of the curtain gas seems to be not to break up existing clusters but, rather, to cool down the nascent ions and to prevent cluster formation by shielding them from solvent vapors.

In the past decade,^{1–3} electrospray has become one of the most frequently used soft ionization methods. This technique is extremely useful, since it accomplishes the transfer of ions from solution to the gas phase, regardless of their molecular weight.^{4,5} It is applicable for a wide variety of ions and molecules, from singly charged atomic ions to multiply charged proteins or nucleic acids. Two principal mechanisms have been put forward to explain the formation of gas-phase ions from charged droplets. One pathway follows a series of Coulomb-fission of the droplets formed in electrospray, leading to a final droplet that contains only one ion. Subsequent evaporation of the last few solvent molecules yields a free gas-phase ion in this scenario. This mechanism was proposed by Dole in 1968, and it is referred to as the charged residue model (CRM).⁶ The other mechanism, proposed by Iribarne and Thomson,^{7,8} deals with direct transfer of ions from nanodroplets to the gas phase. As in the case of CRM, the size of the droplets decreases by sequential Coulomb-fissions and solvent evaporation. Below a certain size threshold ($r < 10$ nm), the electric field on the surface of a charged droplet becomes so high that evaporation of ions may occur. This scenario is termed the ion evaporation mechanism (IEM). After debates in the mid 1990s, a solid consensus emerged⁹ that ionized macromolecules, such as proteins or polymers, are formed by CRM, but that ionization

of “small” molecules follows IEM. The borderline between the two mechanisms was set at ~ 3300 Da by Fernandez de la Mora.⁹

Although the principal mechanism of gas-phase ion formation in electrospray has become well-established,^{10,11} there are still some aspects not fully understood. In the present communication, we focus our attention on the internal energy content of ions formed in the ES process. Conventionally, cold ions are supposed to be formed in ES.^{4,11,12} This idea is mainly based on the known fact that solvent evaporation and gas expansion into vacuum requires a lot of energy, which cools the molecules significantly. It is indeed observed, so ES ion sources are often heated to overcome this cooling effect and to help solvent evaporation. Another argument supporting the formation of cold ions in ES is that large molecules (e.g., proteins) rarely fragment in ES. This latter feature is, however, predominantly due to degrees of freedom (DOF), not internal energy effects.

In the present communication, we summarize a number of experiments which suggest an altogether different picture and indicate that quite hot ions can be formed in ES. We focus our attention on relatively small molecules, which are thought to be formed by the ion evaporation mechanism,^{4,9} whereas large molecules formed by the charge residue model may behave differently. In our present studies, we have used a Sciex API-2000-type mass spectrometer, but previous studies using Micromass instruments showed similar characteristics.^{13–15}

Experimental conditions in the present study were the following: A 4.0 kV spray voltage was applied, and pressures of nebulizer and curtain gases were set to 200 and 100 kPa, respectively. *p*-Cl- and *p*-methoxy-benzylpyridinium salt solutions in methanol–water 1:1 mixture were used at a concentration of 0.01 M and at a flow rate of 20 $\mu\text{L min}^{-1}$. Calculation of the characteristic temperature (T_{char}) was performed as described previously using the benzylpyridinium salts mentioned above.¹⁴ This and modeling the cooling effects were performed by MassKinetics Scientific (version 1.2) software, which is an upgraded version of the freely available

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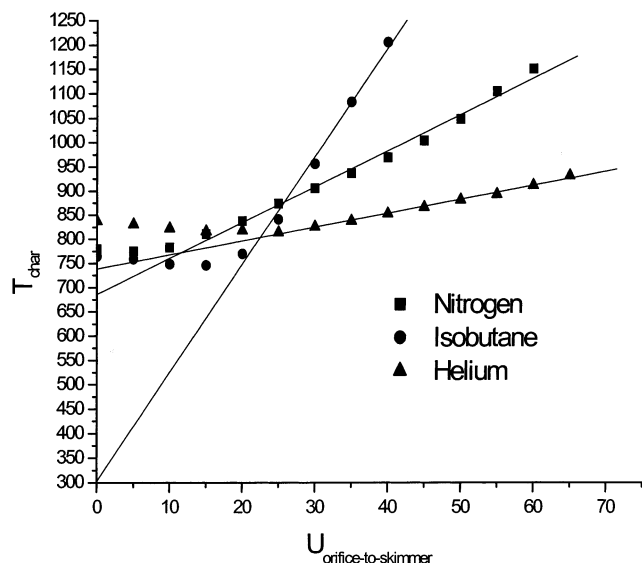


Figure 1. T_{char} vs U_{or} plot using nitrogen, isobutane, and helium as curtain gases at 100 kPa pressure.

MassKinetics 1.2 Light.¹⁶ The program is based on the algorithm presented previously.¹⁷ Calculations shown here can also be downloaded from this site, allowing further calculations or tests to be performed by the interested reader. Vibrational frequencies necessary for RRKM (used in MassKinetics) were calculated at the B3-LYP/6-31G level.^{18–20} Frequencies were scaled by 1.0013, as suggested for low-frequency modes,²¹ which is relevant for RRKM calculations.

The first set of experiments to be discussed is related to the determination of ion internal energy distributions using the ion survival yield (SY) method.^{13–15,22} This technique is based on the measurement of the molecular ion survival yield for a series of model compounds, usually substituted benzylpyridinium salts. Knowing the energetics of their fragmentation, appearance energies were determined by MassKinetics calculations.¹⁴ Internal energy distributions were determined by plotting the derivative of the molecular ion survival yield values for a series of benzylpyridinium salts vs appearance energies. It was found that the internal energy distribution of electrosprayed ions is close to a thermal distribution.¹⁴ For this reason, the internal energy distributions were characterized by a temperature-like parameter, called “characteristic temperature” (T_{char}). This is analogous to the use of “transition state temperature” with respect to the finite heat bath theory developed by Klotz²³ and used subsequently by Futrell at all.²⁴ The characteristic temperature was found to increase linearly with the orifice voltage (U_{or}), so extrapolating the results to zero orifice voltage (T_{char}^0), the ion formation process can be characterized. Such plots using various curtain gases are shown in Figure 1. In a control experiment, an ion population in real

Table 1. Zero-Orifice Extrapolated Characteristic Temperatures Using Various Curtain Gases

curtain gas	mass	T_{char}^0	DOF ^a
He	4	740	3
CH ₄	16	570	15
N ₂	28	680	6
Ar	40	730	3
C ₄ H ₁₀	56	300	42

^a Including transitional and rotational degrees of freedom.

thermodynamic equilibrium was evaluated in this way in an FT-ICR experiment.¹⁴ It was determined that the resulting T_{char}^0 value was, within experimental error, identical to the real temperature of the ions. This gives strong support to the validity of internal energies determined using the SY technique.

The results indicated that T_{char} values were typically in the range of 600–800 K for various instruments, experimental conditions, and test compounds.¹⁴ These suggested that ions formed in the ES process, contrary to expectations, must be quite hot. In the last 2 years, we have devised various experiments to determine where this energy originates from and whether it indeed relates to the ES ion formation process or to secondary excitation. It was found that the zero-extrapolated characteristic temperature depends on neither the spray voltage nor the voltage put on the curtain plate (under normal operating conditions it is ~1 kV). This suggests that T_{char} is independent of accelerating potentials in the atmospheric pressure region. In our previous paper,¹⁴ we tentatively suggested that the ring electrode (located between the skimmer and the orifice) may have an effect, but our new experiments excluded this possibility: The ring electrode is normally at 20 V, but decreasing it to zero did not influence significantly the curves shown in Figure 1. In these experiments, no heating was used, so all parts of the instrument had a temperature below 350 K. These experiments suggest, therefore, that the high internal energy of ions entering into the orifice-skimmer region is not a consequence of instrument design or of static electric fields, but rather is an inherent feature of the ion formation mechanism.

The second set of experiments examines the role of the curtain gas. It is well-known that its main purpose is to prevent cluster formation.⁴ We have tested the influence of several curtain gases (nitrogen, helium, argon, methane, and isobutane); the results (in terms of T_{char}^0) are given in Table 1, and three respective T_{char} vs U_{or} curves are shown in Figure 1. The conventional and simplest assumption is that the curtain gas is likely to increase the internal energy of ions in a CID-type process as the ions are accelerated by the electric fields of the instrument. The degree of excitation in such a scenario should be proportional to the mass of the curtain/collision gas. (The higher the mass of the collision gas, the higher the center of mass collision energy and, therefore, the higher the amount of kinetic to internal energy conversion. Simply stated, the higher the mass of the collision gas, the higher should be the resulting ion temperature.) The results clearly indicate that this is not the case. T_{char}^0 shows no correlation with the mass of the collision gas (Table 1). On the other hand, T_{char} correlates fairly well with the degrees of freedom of the curtain (collision) gas (Table 1). This suggests that energy exchange occurs through

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a long-lived collision complex in which the heat capacity and not the mass of the collision gas is the determining factor. The heat capacity correlates well with the DOF of the collision gas. Because the collision gas is at room temperature and T_{char}^0 decreases with increasing DOF (Table 1), this clearly indicates collisional cooling. In simple terms, isobutane is a much better heat sink than noble gases or nitrogen, so isobutane manages to cool the ions close to room temperature, whereas with noble gases, T_{char}^0 is in the 700–800 K range. In a more quantitative way, calculations using the long-lived collision complex model have been carried out utilizing the MassKinetics software.^{16,17} The results confirm the feasibility of collisional cooling, and the calculations show the same dependence of T_{char}^0 on the collision gas, as observed experimentally.

Note that the slope of the T_{char} vs U_{or} curves at high orifice voltage (Figure 1) does depend on the collision gas, and in this case, the mass of the collision gas is the determining factor. In this region, as expected, collisional excitation occurs. The degree of excitation, indicated by the slope of the T_{char} vs U_{or} curves, is determined by the center-of-mass collision energy.

The third set of experiments performed is also connected to a curtain gas effect, in this case, formation and destruction of solvent clusters were studied. As mentioned before, the curtain gas is used to prevent cluster ions from dominating ES spectra.⁴ It is not well-established if formation of the clusters is prevented or if existing clusters are destroyed in a CID process. We suggest that the former mechanism is occurring. In our experiments, we added a small amount solvent vapor (water, methanol, acetonitrile) to the nitrogen collision gas. This resulted in extensive cluster formation, but always with the solvent added to the curtain gas, and not with the solvent used to spray the ions. The most likely explanation is that when ions are formed in ES, they lose all attached solvent molecules. The ions cool in a collision cascade, and in subsequent collisions, ion–molecule complexes may be formed. If there is no collision (curtain) gas, collisions with the remaining solvent molecules re-form cluster ions. If the usual nitrogen curtain gas is used, the primary ions are surrounded not by solvent molecules but by nitrogen in the gas phase. Because the affinity of most ions to N_2 is very small, cluster formation is not observed. If some polar or highly polarizable molecules are present, cluster formation will take place, not with the original solvent used to spray the ions, but with a compound added to the curtain gas.

A good example illustrating those above is a lithium salt (LiF) sprayed from aqueous solution. Using dry nitrogen curtain gas, the spectrum was of low abundance. When the curtain gas was switched off, $\text{Li}(\text{H}_2\text{O})_n^+$ clusters dominated the spectrum (Figure 2a). Note that some $\text{NH}_4(\text{H}_2\text{O})_n^+$ clusters that were formed from trace amounts of ammonium ions present were also observed. When a small amount of acetonitrile vapor was added to the curtain gas, the spectrum changed completely: all water-containing clusters disappeared, and abundant acetonitrile clusters appeared in the spectrum (Figure 2b).

The last question we want to address is, where does all the energy that is necessary to heat up the ion by a few eV (to 600–

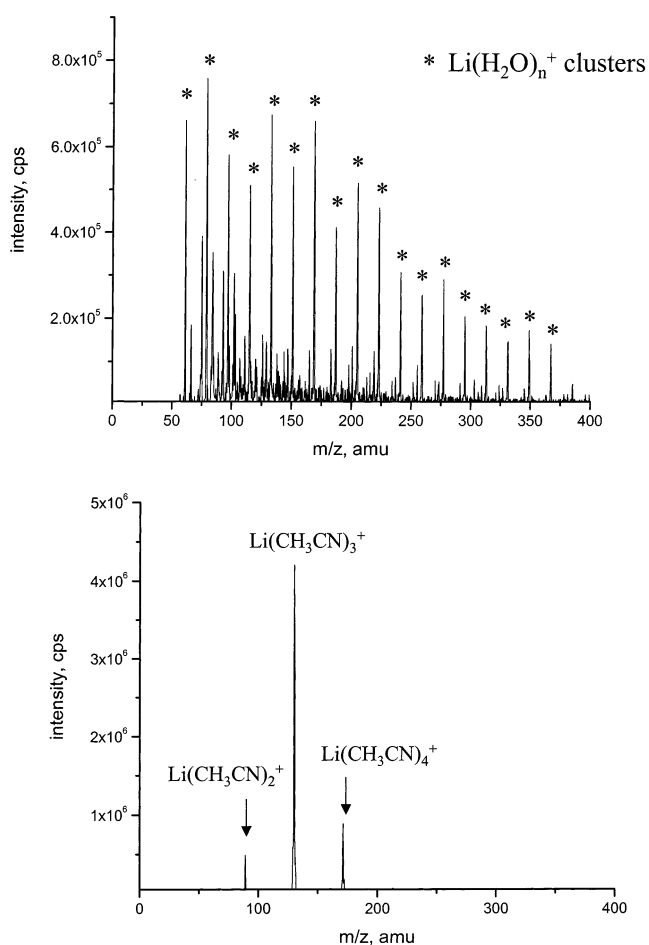


Figure 2. Electrospray spectrum of 0.01M aqueous LiF solution using no curtain gas (a) and nitrogen curtain gas containing 1% acetonitrile vapor (b).

1000 K) come from? We suggest that most of it may originate from Coulomb repulsion between a highly charged droplet and the “ejected” ion (ion-evaporation model). The potential energy of a singly charged ion in the field of a ~ 20 -nm-diameter droplet at the Rayleigh limit is ~ 20 – 30 eV. This is converted into kinetic energy as the ion moves away from the charged droplet. In a collision cascade, 5–10–15% of this energy^{25,26} may be converted into internal energy. Roughly this amount would explain the observed T_{char}^0 values. At this point, we cannot claim that Coulomb repulsion (which is inherent in the ion evaporation model) completely explains excitation in ES, but only that this is a reasonable hypothesis.

In conclusion, we have briefly described three sets of experiments, all indicating that small- and medium-sized molecules are produced with a relatively high internal temperature in electrospray. For benzylpyridinium salts, it is in the range of 600–800 K; for leucine enkephalin, it is 530 K¹⁴ under typical operating conditions. This is supported by the measured T_{char}^0 values, by the influence of the type of curtain gas on T_{char}^0 , and by cluster formation when polar molecules are added to the collision gas.

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