Effects of Solvent and Counterion on Ion Pairing and Observed Charge States of Diquaternary Ammonium Salts in Electrospray Ionization Mass Spectrometry

Guangdi Wang

Department of Chemistry, Xavier University of Louisiana, New Orleans, Louisiana, USA

Richard B. Cole

Department of Chemistry, University of New Orleans, New Orleans, Louisiana, USA

Two diquaternary ammonium chloride salts have been used to examine the roles of solvent and counterion in determination of the degree of ion pairing in solution and the resultant charge state distributions in electrospray ionization mass spectrometry (ESI-MS). Three series of solvents, that is, alcohol, polar aprotic, and chlorinated solvents, have been employed to test the influence of solvent polarity and other parameters on the desorption behavior of diquaternary ammonium ions observed in ESI-MS. Solvents of higher polarity were found to yield gas-phase ions of higher charge states, in accordance with their reduced tendency toward ion pairing in solution. Counterion effects were investigated via the following approaches: (1) increase the diquaternary ammonium salt concentration; (2) increase the concentration of an external electrolyte that contained the common counterion Cl^- ; (3) replace Cl^- with trifluoroacetate (TFAC^-); (4) increase the concentration of an external electrolyte that contained TFAC^- . These experiments indicate that variation of the specific counterion employed alters the degree of influence that the counterion exerts (via ion pairing) on electrospray ionization mass spectra. Increasing amounts of trifluoroacetate ions in a variety of solvent systems invariably led to a progressive shift of the observed ESI-MS charge states of diquaternary ammonium ions toward lower values. (*J Am Soc Mass Spectrom* 1996, 7, 1050-1058)

Among the most attractive features of electrospray ionization mass spectrometry (ESI-MS) are two unique characteristics, namely, (1) multiple charging of analyte species, which enables observation of macromolecules with molecular weights up to 5 million units at relatively low mass-to-charge ratio values [1], and (2) the ease with which samples in solution are analyzed in a wide variety of commonly used solvent systems. Much interest exists in comprehension of the fundamental mechanisms that underlie electrospray processes that lead to the formation of multiply charged gas-phase ions and, in particular, that relate the observed multiple charging back to various solution conditions that were used to introduce the analytes into the mass spectrometer [2-19].

Most of the research that relates solution chemistry to electrospray ionization mass spectra has focused on proteins, peptides, oligonucleotides, and other

molecules that contain multiple basic or acidic sites. Chait and co-workers [7, 10, 11] and Smith and co-workers [4, 8, 21] have investigated the effects of protein conformational changes on the charge state distributions of protein ions observed in the gas phase. Variations in the degree of heating [9, 10], solution pH, chemical denaturant content [11], and organic solvent composition [21] have been employed to induce alterations of protein tertiary structure. The resultant shift in protein charge states in ESI mass spectra was in the same direction as predicted by solution chemistry. Indeed, based on aqueous titration data of selected proteins, Guevremont et al. [12] concluded that the shape of the ion abundance versus mass-to-charge ratio profile observed in ESI mass spectra matched the distribution of preformed ions in aqueous solution. However, this notion contradicts the findings by Loo et al. [22] and Kelly et al. [13] in which multiply charged positive ions of proteins were observed from basic solutions and multiply charged negative ions were observed from acidic solutions. Findings similar to the

Address reprint requests to Richard B. Cole, Department of Chemistry, University of New Orleans, New Orleans, LA 70148.

latter also were obtained for small peptides that underwent little conformational change over a wide range of variation of solution pH [16].

Experiments that involved nonaqueous solvent effects on analyte charge states in negative ion ESI-MS [18] investigated a different aspect of the contributions of solution chemistry to ESI mass spectra. For a neutral species (AB_2), which represents an anionic analyte A^{2-} bearing two B^+ counterions that dissociate upon solvation,

the analyte may be detected either in the doubly charged (A^{2-}) or singly charged (AB^-) form. Solvents of higher polarity will facilitate a shift of the dissociation equilibria given in eqs 1 and 2 to the right, because the product ions to the right of the equilibrium sign bear more charges than those on the left [23]. The influence of solvent polarity (as indicated by the dielectric constant) on charge state distributions in ESI mass spectra was tested using several solvent series. Charge state distributions were observed to shift consistently toward lower values in homologous series of solvents whose dielectric properties were progressively weakened [18].

The current study extends our investigation to the positive ion ESI-MS mode by using diquaternary ammonium salts. In an analogous way, the dissociation of dicationic analytes (C^{2+}) that initially bear two D^- counterions (CD_2 neutral form) may be written as

where CD^+ and C^{2+} may be detected by the mass spectrometer. An advantage of the use of diquaternary ammonium salts over, for example, tertiary amines for studies into solvent effects on charge state distributions is that the positive charges on the ammonium cations are "permanent." Thus, complications presented by solvent-induced solution phase shifts in the equilibrium of protonation-deprotonation or charge (e.g., proton) transfer reactions in the gas phase are avoided. Moreover, loss of a neutral ion pair subsequent to anion attachment to the charge site is not possible in the gas phase or elsewhere. Compared to multiply charged metal cation species that give rise to abundant peaks that involve solvent coordination and clusters [e.g., $M^{n+}(\text{solvent})_m$ with $n \geq 2$ and $m \geq 1$], the relatively bulky diquaternary ammonium ions are much less solvated in solution and they yield rather simple ESI mass spectra. Furthermore, a related phenomenon, the degree of ion pairing in solution, which depends upon both the solvent dielectric and the nature of the counterion, also was examined by exchanging the anions that participate in the ion pair.

Experimental

A Vestec-201 mass spectrometer (PerSeptive Biosystems/Vestec Corporation, Houston, TX), equipped with an electrospray ion source, which has been previously described [24], was used to acquire all mass spectra. A Sage syringe pump (Orion Research, Boston, MA) was used to deliver solutions into the electrospray needle at a flow rate of $1.6 \mu\text{L}/\text{min}$. Each series of experiments was carried out under constant instrumental conditions, which include the needle-skimmer distance, the temperature in the vicinity of the electrospray needle ($45 \pm 1^\circ\text{C}$), source block temperature ($230 \pm 1^\circ\text{C}$), and the electric potentials at the needle (2.4–2.7 kV), the nozzle (300 V), and the skimmer (13 V). Obtained $2+ / 1+$ ratios in ESI mass spectra are biased systematically slightly toward higher values relative to the $2+ / 1+$ ratio of ions produced in the ion source due to the transmission bias of the quadrupole analyzer. This transmission bias will be constant for a given analyte, however, and thus will have no bearing on the *direction* of shift of the $2+ / 1+$ ratio in response to changing experimental conditions.

Diquaternary ammonium salts were purchased in the chloride salt form from Aldrich Chemical Co. (St. Louis, MO). Cesium chloride, cesium trifluoroacetate, and silver trifluoroacetate also were purchased from Aldrich and were used without further purification. Precipitation titration was used to prepare the diquaternary ammonium trifluoroacetate salt.

Results and Discussion

The electrospray mass spectra of the two diquaternary ammonium salts in 1 : 1 aqueous methanol solution are given in Figure 1a and b. Notably, the doubly charged diquaternary ammonium ions appear as the base peaks in each case. The corresponding singly charged ions are characterized by the attachment of one chloride anion to the doubly charged ion. Several unidentified background ions, which may arise from impurities in the samples, are also present in each spectrum. Because the analytes carry two permanent positive charges, the only factor that can cause the charge states to shift to lower values is increased ion pairing between the diquaternary cations and the anions present in solution. We will start with an investigation of the solvent effect on the degree of ion pairing and the resultant charge states of gas-phase ions.

Solvent Effect on Ion Pairing and Analyte Charge State Distributions

Shown in Figure 2 are ESI mass spectra obtained for diquat 1 dissolved in four different alcohol solvents to form solutions of identical concentration. It is obvious that in moving downward from methanol to ethanol, 1-propanol, and 1-butanol (order of decreasing polarity as measured, for example, by the dielectric constant),

Figure 1. Electrospray ionization mass spectra of (a) 10^{-5} -M diquatary ammonium salt 1 (diquat 1) and (b) 10^{-5} -M diquatary ammonium salt 2 (diquat 2), each dissolved in 1:1 (v:v) methanol: water.

the ratio of the abundance of the doubly charged ion (m/z 328) to that of the singly charged ion (m/z 691) diminishes progressively (from about 17:1 to less than 1:1). This reflects the fact that increasing fractions of Cl^- counterions are attached to the diquatary ammonium ions, which results in the detection of a higher proportion of singly charged analyte ions as one moves from Figure 2a to d. Similar results were obtained for another diquatary ammonium salt (diquat 2) that carries the same counterion Cl^- as shown graphically in Figure 3a, where the $2+ / 1+$ abundance ratios are plotted versus alcohol solvent (displayed in order of decreasing dielectric constant) for both diquat 1 and diquat 2. Again, the $2+ / 1+$ ratio descends as the solvent is varied from methanol (highest polarity, left) to butanol (lowest polarity, right), which indicates that solvents of lower polarity favor production of gas-phase ions of lower charge states.

Another series of experiments was performed by using chlorinated solvents (i.e., CH_2Cl_2 , $CHCl_3$, and CCl_4). In this series, due to solubility considerations, methanol was used as a "carrying solvent"; to 9 parts of each of the chlorinated solvents was added 1 part of

Figure 2. Electrospray ionization mass spectra of 5×10^{-5} -M diquat 1 dissolved in (a) methanol (MeOH), (b) ethanol (EtOH), (c) 1-propanol (PrOH), and (d) 1-butanol (BuOH).

Figure 3. (a) Electro spray 2+ / 1+ charge state ratio observed for diquat 1 (filled squares) and diquat 2 (empty circles) versus solvent employed. (b) Sum of the signal intensities of singly and doubly charged ions of diquat 1 (filled squares) and diquat 2 (empty circles) dissolved in methanol, ethanol, 1-propanol, and 1-butanol, listed in order of decreasing polarity. The data shown in parts a and b correspond to one series of experiments.

methanol that contained 2×10^{-4} M of the diquaternary ammonium salt. As shown in Figure 4a, the 2+ / 1+ ratio decreases steadily as the solvent is changed from CH_2Cl_2 to CCl_4 for both diquat 1 and diquat 2. The observed shift in charge state distributions toward lower values with decreasing dielectric constant in the series of chlorinated solvents echoes the shift obtained in alcohol solvents.

It should be borne in mind that although solution phase separation of ion pairs may be attributed largely to solvent polarity considerations, the factors that influence analyte charge states observed in ESI mass spectra are more complicated. Other solvent properties, such as volatility, surface tension, viscosity, and conductivity, may affect ESI mass spectra in a variety of ways, and the solvent influence on the degree of ion pairing observed in the gas phase does not necessarily mirror solvent effects in solution. Although it is virtually impossible to change a single solvent parameter while the others are held constant, evidence was provided [18] that some solvent characteristics (e.g., con-

Figure 4. (a) Electro spray 2+ / 1+ charge state ratio observed for diquat 1 (filled squares) and diquat 2 (empty circles) versus solvent employed. (b) Sum of the signal intensities of singly and doubly charged ions of diquat 1 (filled squares) and diquat 2 (empty circles) dissolved in 10% methanol and 90% of the following solvents: methanol, dichloromethane (CH_2Cl_2), chloroform (CHCl_3), and carbon tetrachloride (CCl_4), listed in order of decreasing polarity. The data in parts a and b correspond to a single series of experiments.

ductivity, surface tension, and viscosity) exhibit no obvious influence upon the distribution of analyte charge states observed for certain compounds that bear fixed anionic sites in the negative ion mode.

For both the alcohol series and the chlorinated solvent series, volatility decreases monotonically with diminishing solvent polarity as shown in Table 1. Thus, with increasing chain length (from methanol to butanol) or with increasing Cl content (from CH_2Cl_2 to CCl_4), solvent polarity and solvent volatility each are decreasing progressively. This makes it difficult, if not impossible, to differentiate the contributions of these two solvent parameters to lower the observed charge states. Solvent volatility clearly affects the rate of droplet evaporation in the electrospray process. Fenn has proposed [6] that the observed distribution of charge states of an analyte in ESI-MS is influenced by the evaporation rate of the charged droplet. Moreover, the specific surface-charge density of the droplet at

Table 1. Solvent dielectric constants, vapor pressures, and boiling points [20, 27]

	MeOH	EtOH	PrOH	BuOH	CH ₂ Cl ₂	CHCl ₃	CCl ₄	ACN	DMF
Dielectric constant	32.70 (25 °C)	24.30 (25 °C)	20.33 (25 °C)	17.51 (25 °C)	8.93 (25 °C)	4.81 (20 °C)	2.24 (20 °C)	37.5 (20 °C)	37.78 (25 °C)
Vapor pressure (torr)	125 (25 °C)	40.0 (19 °C)	14.5 (20 °C)	5.0 (20 °C)	436 (25 °C)	158.4 (20 °C)	89.55 (20 °C)	88.8 (20 °C)	2.7 (25 °C)
Boiling pt. (°C)	64.7	78.3	97.2	117.7	39.8	61.2	76.8	81.6	153.0

the moment of ion evaporation will exert an important influence upon the charge state of an ion desorbed at a certain instant in time. Thus, ions desorbed when the surface-charge densities are low will carry fewer charges than those desorbed when the surface charge density has increased. This theory has been supported by experiments [6] wherein charge state distributions were shifted to higher values when the droplet evaporation rate was increased by augmented flow rate or by raised temperature of the ESI counterflow bath gas. Other factors being equal, lower volatility solvents should thus increase the length of time that a low surface-charge density regime is maintained on the droplet surface, which leads to an increased proportion of lower charge state ions in the total population of desorbed ions as compared to higher volatility solvents.

As a means to probe the relative importance of solvent polarity and volatility, two aprotic solvents, namely, acetonitrile (ACN) and dimethylformamide (DMF), were employed, along with methanol (MeOH) as a reference solvent. The three solvents have similar dielectric constants [18] ($\epsilon_{\text{DMF}} = 36.7$ at 25 °C, $\epsilon_{\text{ACN}} = 33.5$ at 45 °C, and $\epsilon_{\text{MeOH}} = 28.9$ at 45 °C), yet rather different volatilities (DMF: 2.7 torr at 20 °C, ACN: 88.8 torr at 25 °C, and MeOH: 125 torr at 25 °C). The observed 2+ /1+ ratios of diquat 1 and diquat 2 are 26 and 57 in DMF, 16 and 28 in ACN, and 17 and 32 in MeOH, respectively. It is noted that DMF, which has the highest dielectric constant and the greatest cation solvation power yet the lowest volatility, yielded the highest 2+ /1+ ratio for both diquat 1 and diquat 2. The results obtained with this limited selection of solvents suggest that solvent polarity can exert a more important influence than solvent volatility in determination of the distribution of charge states in ESI-MS.

In a previous study of small peptides [16], two factors were proposed to contribute to the increased yield of gas-phase ions of lower charge states in DMF compared to other solvents tested. These factors were the relatively low volatility of DMF and the increased propensity for proton attachment to DMF. Evidence for the latter was given by the highly intense peaks that arise from protonated solvent monomers and noncovalently bound dimers of DMF in the ESI mass spectra of peptides initially introduced in the hydrochloride salt form. In the current study, protonated DMF peaks in ESI mass spectra are virtually absent, which is indicative of a limited source of protons in the DMF solution of diquatery ammonium salts. These observed dif-

ferences in the ESI mass spectra support the notion that the high propensity to form protonated solvent molecules was indeed largely responsible for lowered relative abundances of more highly charged (multiply protonated) peptide ions [16]. The charge states of diquatery ammonium cations appear to be influenced heavily by the degree of ion pairing in solution, which, among the three solvents employed, is expected to be the lowest for DMF.

A further analysis of the solvent influence on desorption in ESI-MS was performed by quantification of the total analyte signal intensities obtained by using the data plotted in Figures 3a and 4a. The total ESI signal intensity was calculated by summation of the intensities of the singly and doubly charged forms. Results are plotted as a function of solvent employed in Figures 3b and 4b. For alcohol solvents, the total signal intensity of both diquat 1 and diquat 2 decreased steadily as the solvent polarity was diminished with increasing alkyl chain length from methanol to butanol (Figure 3b). A similar trend of reduced signal intensity was observed for the chlorinated solvent series as the polarity decreased (Figure 4b), with one exception where the signal intensity of diquat 2 in chloroform was higher than that in methylene chloride. The general trend of a weakening signal in progressively lower polarity solvents may be rationalized by consideration of the dissociation equilibria given in eqs 3 and 4. In the lowest polarity solvents, dissociation of ion paired analyte can be minimal. As the solvent polarity is raised and the solvation energy increases, each of these dissociation equilibria will be shifted to the right. Thereby, the concentrations of both doubly and singly charged diquatery ammonium ions can be increased in solution. As an apparent consequence, the total ESI signal intensity (sum of the singly and doubly charged ion abundances) has increased with solvent polarity in these homologous series of solvents. Despite this general conclusion, other solvent factors clearly play a role in determination of total signal intensities in ESI-MS, as demonstrated by the one aberration from a perfectly monotonic descent in signal intensity with solvent polarity displayed in Figure 4b.

Effect of Counterions

The foregoing results demonstrate that the solvent medium exerts pronounced effects on the signal intensities and on the extent of ion pairing and the resultant

charge states of diquatery ammonium salts observed in ESI mass spectra. Because singly charged diquatery ammonium ions arise solely from the attachment of one anion without complicating secondary equilibria, these compounds are excellent candidates for studies of the role of the counterion in determination of the distribution of charge states observed in ESI mass spectra.

A first investigation probed the effects of an increasing total diquatery ammonium salt concentration on the observed yield of doubly versus singly charged species, that is, the concentration of the indigenous anion Cl^- increases simultaneously with that of the diquat 1 cation. Second, an increasing amount of CsCl was added to a solution that contained a fixed concentration of diquat 1. Results are shown in Figure 5a and b, respectively, where the $2+/1+$ ion abundance ratios observed in ESI mass spectra are plotted versus diquat 1 concentration (Figure 5a) or CsCl concentration (Figure 5b). It is interesting to note that when Cl^- is increased by increasing the total diquatery ammonium salt concentration, the abundance of doubly charged diquatery ammonium ion relative to the singly charged ion decreased substantially (Figure 5a). In contrast, if Cl^- is introduced in increasing amount as the anion of external electrolyte (e.g., CsCl), the $2+/1+$ ratio is rather constant over the whole range of CsCl concentration (Figure 5b).

Both of these results conform to findings from previous studies in our laboratory [17, 19]. We have proposed [19] that with increasing analyte concentration, a decreasing ratio of N/N_0 (where N is the total number of excess charges in all droplets and N_0 is the total number of analyte molecules in all droplets) is chiefly responsible for yielding gas-phase ions of lowered charge states. The ratio N/N_0 is an indicator of available excess charges per analyte molecule, and a decreasing value of this ratio at progressively elevated analyte concentrations implies that the number of ion pairs of multiply charged analyte species and counterions in the droplets is increasing. The results presented here for diquat 1 that show increasing attachment of counterions (and thus a higher proportion of singly charged ions) with increasing analyte concentration, provide additional evidence to support this argument.

On the other hand, the near constancy of ESI-MS charge states of diquat 1 with increasing CsCl concentration is consistent with previous observations on the effect of solution ionic strength on charge state distributions of proteins in positive ion ESI-MS and for other small analytes in negative ion ESI-MS [17]. In that study, in spite of a severe reduction of overall signal intensities, the charge state distributions of analytes were found to remain virtually constant after the progressive addition of increasing amounts of CsCl or quaternary butylammonium chloride. These added electrolytes were termed "spectator" electrolytes because they did not participate in charge attachment-removal from analyte ions and their presence did not

Figure 5. Semilogarithmic plot of the ESI-MS abundance ratio of doubly charged to singly charged diquat 1 versus (a) the concentration of diquat 1 dissolved in 1:1 methanol-water solution and (b) the concentration of external electrolyte CsCl at a fixed diquat 1 concentration of 10^{-5} M.

alter the distribution of charge states of desorbed analyte species. The same study showed that acetate ions, introduced in the form of ammonium acetate, could shift the distribution of analyte charge states. These acetate anions, which were thus capable of removal of protons from analyte species, could not be considered to be "spectators." In a follow-up study [19], the charge state distributions of smaller molecules (i.e., gramicidin S and 4,4'-bipiperidine) were observed to shift significantly toward lower charge states as a result of the addition of high concentrations of CsCl to analyte solutions. It was hypothesized that the difference in the desorption rate constant between Cs^+ and analyte cations may be related to the magnitude of the shift in analyte charge states [19]. In cases where analyte cations desorbed much more efficiently than Cs^+ , the effect of an increasing CsCl concentration on the ESI-MS charge state distributions was negligible.

In the current study, ESI mass spectra acquired for equimolar mixtures of diquat 1 and cesium salt exhibited much higher peak intensities for diquat 1 than for Cs^+ , which indicates that the desorption rate constants for diquatery ammonium cations are considerably larger than the desorption rate constant for Cs^+ . The results presented in Figure 5b, where the distribution of charge states of diquat 1 is rather constant with increasing CsCl concentration, appear to add to the plausibility of this proposition with regard to the importance of relative desorption rates of detected ions that arise from the added salt and the analyte.

The anion Cl^- , which is present in two equivalents in the commercial form of diquat 1, was now replaced with trifluoroacetate (TFAc^-) by introduction of $\text{Ag}(\text{TFAc})$ salt to remove Cl^- via precipitation of AgCl . The ESI mass spectrum of the diquat 1 trifluoroacetate salt prepared in this way (Figure 6c) is compared with that of the diquat 1 chloride salt (Figure 6a), as well as that of an equimolar mixture of diquat 1 chloride salt and $\text{Cs}(\text{TFAc})$ (Figure 6b). When an equimolar quantity of external electrolyte (CsTFAc) is present, the singly charged diquat 1 is represented predominantly by $[\text{M} - 2\text{Cl} + \text{TFAc}]^+$ at m/z 770, although the peak due to $[\text{M} - \text{Cl}]^+$ at m/z 691 is still discernible in Figure 6b. Note that there are actually two equivalents of Cl^- ions present for each equivalent of TFAc^- when the diquat 1 chloride salt and $\text{Cs}(\text{TFAc})$ are present in equimolar concentrations. Clearly, TFAc^- attaches more efficiently to the doubly charged diquat 1 ion than Cl^- when the two are in direct competition (Figure 6b). A comparison of the situation where only the chloride anion is present (Figure 6a) with the case where only TFAc^- is present (Figure 6c) indicates a more pronounced anion attachment that yields a significantly higher relative abundance of singly charged ions for TFAc^- . The absolute abundance of singly charged species also is raised when the original counterion Cl^- has been removed and TFAc^- is present as the counterion of the diquatery ammonium cation (Figure 6c); all other parameters are unchanged (e.g., solvent, diquat 1 concentration, and instrumental parameters).

In neutral solutions, different anions are known to exhibit different affinities toward a given cation [25]. Mirza and Chait [26] reported that by introduction of different anions into protein and peptide solutions, the analyte charge states observed in ESI mass spectra were shifted significantly. Their results suggest that anions with higher electroselectivity toward anion exchange chromatography columns correlate to larger shifts toward lower charge states for cationic analytes in ESI-MS. The results presented in Figure 6 support this notion that anion effects in positive ion ESI-MS depend upon the nature of the counterion. Previous results that revealed a near constancy of protein charge state distributions when certain external electrolytes termed "spectator" electrolytes (e.g., CsCl) were added in increasing concentrations [17] coincide with results

presented by Mirza and Chait [26] for anions that exhibit the *lowest* electroselectivity toward anion exchange columns, that is, those with the lowest propensity toward charge neutralization.

To compare the behavior of anions that exhibit higher electroselectivity toward anion exchange columns, ESI mass spectra of $2 \times 10^{-5}\text{-M}$ diquat 1 were acquired in the presence of progressively increasing concentrations of cesium trifluoroacetate (up to 10^{-3} M). For this anion, the $2+ / 1+$ ratio decreased dramatically from 106, for the solution with no cesium trifluoroacetate, to 9.5, for the solution that contained 10^{-3}-M cesium trifluoroacetate (Figure 7). This result contrasts with the nearly constant $2+ / 1+$ ratio observed in Figure 5b for the same analyte in the presence of increasing quantities of cesium chloride. Because the anion TFAc^- has a stronger tendency toward ion pairing with the diquatery ammonium cation than Cl^- , the possibility of formation of ion pairs with cations in solution prior to or during the electrospray process is favored for TFAc^- . Increasing the concentration of TFAc^- thus results in the promotion of ion pairing to form a higher proportion of singly charged diquat ions in ESI mass spectra.

Conclusion

Diquatery ammonium salts with fixed positive charges offer advantages to study the effects of solvent and counterion on the degree of ion pairing and the resultant charge state distributions observed in ESI mass spectra. For these compounds, solution phase protonation equilibria and gas-phase proton transfer reactions (i.e., neutral loss of a proton-anion pair) are excluded. Solvents of higher polarity, as measured for example by the dielectric constant, that exhibit stronger solvating power toward cations, were found to yield gas-phase diquatery ammonium ions of higher charge states. This observation was attributed to the decreasing extent of ion pairing in solvents with higher dielectric constants, which offer an improved ability to stabilize ions of higher charge states. Other solvent properties (including volatility), which are known to influence ESI mass spectra in a variety of ways, exhibited no obvious trend to shift charge states of diquatery ammonium ions. Investigation of the effect of counterions revealed that the charge states of diquatery ammonium ions in ESI-MS shifted toward lower values when the total analyte concentration (chloride salt form) was raised. In contrast, when the analyte concentration was held constant while CsCl was added, the charge states in ESI-MS were not affected. These results conform to our previous observations and the rationales therein [17, 19]. For another anion characterized by a higher tendency of charge neutralization toward cations (TFAc^-), the charge states of diquatery ammonium ions were lowered whenever TFAc^- was present in solution, regardless of the form in which it was introduced. We concluded that the

Figure 6. Electrospray ionization mass spectra of 10^{-4} -M diquat 1 in 1:1 methanol:water solutions with (a) Cl^- as the original counterion, (b) Cl^- as the original counterion but containing 10^{-4} -M CsTFAc, and (c) with TFAc^- as the original counterion.

Figure 7. Effect of increasing concentration of external electrolyte CsTFAc on the charge state distribution of diquat 1 chloride salt form (10^{-5} M in 1:1 methanol:water) observed in ESI-MS.

stronger affinity of TFAc^- toward the cation is chiefly responsible for a greater degree of ion pairing in solution and, hence, the observed shifts in charge states of diquatery ammonium ions toward lower values in ESI mass spectra.

References

- Nohmi, T.; Fenn, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 3241-3246.
- Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675-679.
- Meng, C. K.; Mann, M.; Fenn, J. B. *Z. Phys. D* **1988**, *10*, 361-368.
- Loo, J. A.; Udseth, H. R.; Smith, R. D. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 411-414.
- Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. K. *Mass Spectrom. Rev.* **1990**, *9*, 37-70.
- Fenn, J. B. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 524-535.
- Chowdhury, S. K.; Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 9012-9013.
- Loo, J. A.; Edmonds, C. G.; Udseth, H. R.; Smith, R. D. *Anal. Chem.* **1990**, *62*, 693-698.
- La Blanc, J. C. Y.; Beuchemin, D.; Siu, K. W. M.; Guevremont, R.; Berman, S. S. *Org. Mass. Spectrom.* **1991**, *26*, 831-839.
- Mirza, U. A.; Cohen, S. L.; Chait, B. T. *Anal. Chem.* **1993**, *65*, 1-6.
- Vorm, O.; Chait, B. T.; Roepstorff, P. *Proceedings of the 41st ASMS Conference on Mass Spec. and Allied Topics*; San Francisco, CA, 1993; pp 621a-621b.
- Guevremont, R.; Siu, K. W. M.; Le Blanc, J. C. Y.; Berman, S. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 216-224.
- Kelly, M. A.; Vestling, M. M.; Fenselau, C. C. *Org. Mass Spectrom.* **1992**, *27*, 1143-1147.
- Ashton, D. S.; Beddel, C. R.; Cooper, D. J.; Green, B. N.; Oliver, R. W. A. *Org. Mass Spectrom.* **1993**, *28*, 721-728.
- Gatlin, C. L.; Turecek, F. *Anal. Chem.* **1994**, *66*, 712-718.
- Wang, G.; Cole, R. B. *Org. Mass Spectrom.* **1994**, *29*, 419-427.
- Wang, G.; Cole, R. B. *Anal. Chem.* **1994**, *66*, 3702-3708.
- Cole, R. B.; Harrata, A. K. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 546-556.
- Wang, G.; Cole, R. B. *Anal. Chem.* **1995**, *67*, 2892-2900.
- Maryott, A. A.; Smith, E. R. *Table of Dielectric Constants of Pure Liquids*; National Bureau of Standards Circular 514; U.S. Department of Commerce, U.S. Government Printing Office: Washington, DC, 1951.
- Loo, J. A.; Ogorzalek Loo, R. R.; Udseth, H. R.; Edmonds, C. G.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 101-105.
- Loo, J. A.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 207-210.
- Bates, R. G. In *Solute-Solvent Interactions*; Coetzee, J. F.; Ritchie, C. D., Eds.; Dekker: New York, 1969; pp 46-93.
- Allen, M. A.; Vestal, M. L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 18-26.
- Garst, J. F. In *Solute-Solvent Interactions*; Coetzee, J. F.; Ritchie, C. D., Eds.; Dekker: New York, 1969; pp 539-601.
- Mirza, U. A.; Chait, B. T. *Anal. Chem.* **1994**, *66*, 2898-2904.
- Lide, D. R., Ed. *Handbook of Chemistry and Physics*, 71st ed.; CRC Press: Boca Raton, FL, 1990.