Anal. Chem. **2001,** *73,* 23−28

A Method for Determination of Ion Distribution within Electrosprayed Droplets

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A simplified method for determination of ion distribution within electrospray ionization droplets has been developed. The method is based on an electrospray ionization mass spectrometry equilibrium partitioning model recently developed by Enke (Enke, C. G. *Anal. Chem.* 1997, *69*, 4885−4893). This "simple" method required only two samples to be analyzed at each solution composition compared to the method previously reported. Furthermore, as the same m/z value is monitored in both experiments, possible effects of the mass-dependent ion transmission in the quadrupole were eliminated. Tetraalkylammonium compounds with variable hydrophobicity were used as model compounds and the effect of methanol−water composition in the electrosprayed solution was studied. It was found, as expected, that the signal optimized at a high content of methanol in the electrosprayed solution. The distribution coefficient for analyte ions between the droplet surface and bulk solution maximize, however, at a lower content of methanol in the electrosprayed solution.

Electrospray is a technique where a liquid is dispersed into small charged droplets by an electrostatic field. In the late 1960s and early 1970s, Dole et al.[1,2] made the first reports of the creation of gas-phase ions at atmospheric pressure using an electrostatic sprayer, i.e., electrospray. Polystyrene macroions were separated by the use of a retarding potential and the ion current was detected with a Faraday cage. In the 1980s, several research groups[3−6] used mass spectrometry for detection of gas-phase ions generated from charged droplets at atmospheric pressure. In 1988, the research group of Fenn[7,8] presented results which demonstrated that multiply charged ions could be generated by electrospray ionization (ESI). The analytical capabilities of electrospray ionization mass spectrometry (ESI-MS) have increased significantly during the 1990s and an overview can, for example, be found in a compilation edited by Cole.[9] The electrospray process, which is described below, have been the subject of a large number of publications (see refs 10−19). When an electric field is generated between a liquid-filled capillary tip and a counter electrode, by applying a potential difference between them, ions present in solution will migrate under the influence of the field to the opposite electrode until a charge distribution, (charge separation), is obtained that counteracts the field. This so-called electrophoretic charging mechanism[20] results in the generation of droplets with excess charge and a polarity depending on the electric field. The charged droplets will drift toward the counter electrode and reduce in size as the solvent evaporates.[21,22] Droplet shrinkage leads to higher surface charge density, and at a given droplet radius, electrostatic charge repulsion overcomes the surface tension, which results in droplet "fission". This process can continue until very small droplets are formed ($\leq$10 nm)[21,23] from which solvated gas-phase ions are generated. The actual mechanism by which gas-phase ions are generated from the charged liquid droplets has been a subject of much debate,[24,25] and the exact details in the last steps are not fully understood. Two main models have been proposed, the ion evaporation model, by Iribarne and Thomson[26,27] and the charge residue model by Dole et al.[1,2] and

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Röllgen et al.[28,29] In both theoretical models, solution chemistry plays an important role in the ionization process. The chemical composition of the electrosprayed solution will change during the spray process due to, for example, charge separation,[20] electrochemical redox reactions,[30,31] charge enrichment,[32] and solvent evaporation.[21,22] It has usually been difficult to predict the effects of changes in solvent composition as a large number of parameters influence the analytical ion signal response in electrospray mass spectrometry. For example, parameters closely related to the solvents and additives used, such as volatility, surface tension, viscosity, conductivity, ionic strength, dielectric constant, electrolyte concentration, and pH, will influence the analytical ion signal. Furthermore, properties of the analyte including pK_a, hydrophobicity, surface activity, ion solvation energies, and gas-phase basicity will also influence the analytical ion signal.

The electrospray current is generated by redox reactions taking place at the metal–liquid interface in the spray capillary.[30,31] Due to the effect of charge repulsion, it can be assumed that the excess charge in a droplet will be at or near the liquid surface. Recently, a simplistic equilibrium partitioning model for singly charged ionic analytes was developed by Enke.[33] This model is based on competition among the charged species in the solution for the limited number of excess charge sites at the droplet surface. It is assumed that the relative abundances of ions in the mass spectrum are proportional to the relative concentration of the different charged species at the droplet surface. In other words, partitioning of the ionic species between the two phases, the interior, solvated, ion-paired phase (i) and the surface excess charge phase (s), is important for the relative mass spectrometric response. Enke[33] assumed that the partitioning process is sufficiently rapid and a displacement reaction for a two-component system, one analyte ion (A^+) and one electrolyte ion (E^+), was written.

$$(A^+X^-)_i + (E^+)_s \leftrightarrow (E^+X^-)_i + (A^+)_s \qquad (1)$$

The counterion is represented by X^-. The equilibrium constant for the displacement reaction is

$$\frac{K_A}{K_E} = \frac{[E^+X^-]_i[A^+]_s}{[A^+X^-]_i[E^+]_s} \qquad (2)$$

Further, by assuming that the fraction of the total analyte or electrolyte concentration that is on the surface is very small, an equation similar to Kebarle's equation[11] was developed.

$$R_A = Pf\frac{C_AK_A}{C_AK_A + C_EK_E}[Q] \qquad (3)$$

In this equation, R_A is the mass spectrometric ion response, and the Ks represent ion equilibrium constants for the phase distribution. [Q] represents the excess charge concentration, which is the difference in the concentration of cations and anions in the electrosprayed solution. The proportionality constant, f, represents the fraction of excess droplet charge that is converted into free gas-phase ions, i.e., ion desolvation efficiency. P is the proportionality constant that represents the ion sampling, transfer, and detection efficiency of the system from the ion source to the detector. The excess charge concentration can be calculated by dividing the total electrospray current, I, with the liquid flow rate, L, and Faradays constant, F.

$$[Q] = I/LF \qquad (4)$$

The equilibrium constant for the displacement reaction (eq 2) was rewritten by Enke,[33] by introducing two mass balance equations, one for the analyte

$$C_A = [A^+]_s + [A^+X^-]_i \qquad (5)$$

and one for the electrolyte

$$C_E = [E^+]_s + [E^+X^-]_i \qquad (6)$$

and an equation for the surface excess charge, Q.

$$[Q] = [A^+]_s + [E^+]_i \qquad (7)$$

The resulting equation is

$$\frac{K_A}{K_E} = \frac{[A^+]_s(C_E - [E^+]_s)}{(C_A - [A^+]_s)[E^+]_s} = \frac{[A^+]_s(C_E - [Q] + [A^+]_s)}{(C_A - [A^+]_s)([Q] - [A^+]_s)} \qquad (8)$$

This equation was further rewritten to obtain a response function, $[A^+]_s$ vs C_A. The equilibrium partitioning constant will be influenced by a number of properties of the ion and solvent including solvation energy, ion-pairing energy, charge density, and hydrophobicity.

When only electrolyte, and no analyte, is present in the electrosprayed solution, it can be assumed that the surface excess charge, Q, is carried only by the electrolyte, $[Q]_{C_A=0} = [E^+]_{s,C_A=0}$, and an instrumental response factor, Pf_E, can be calculated by the use of eq 9.

$$Pf_E = R_{E,C_A=0}/[Q]_{C_A=0} \qquad (9)$$

When analyte ions also are present in the electrosprayed solution, eq 9 together with the assumption that Pf_E, is unchanged when

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analyte is present can be used to derive eq 10, which express the surface concentration of the electrolyte ions.

$$[E^+]_s = \frac{R_E}{Pf_E} = \frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0} \qquad (10)$$

Furthermore, by combining eqs 7 and 10, an equation that expresses the surface concentration of the analyte ions is obtained.

$$[A^+]_s = [Q] - \frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0} \qquad (11)$$

By substitution of $[E^+]_s$ and $[A^+]_s$ in eq 8 with eqs 10 and 11, the following equation is obtained for the equilibrium partitioning constant.

$$\frac{K_A}{K_E} = \frac{[Q] - \frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0}}{\frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0}} \, \frac{C_E - \frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0}}{C_A + \frac{R_E}{R_{E,C_A=0}}[Q]_{C_A=0} - [Q]} \qquad (12)$$

This equation now contains only two responses for the electrolyte, one when no analyte is present ($R_{E,C_A=0}$) and one when analyte is present (R_E), the analytical concentration of the analyte (C_A) and electrolyte (C_E). The equation further contains two surface excess charge concentrations, one when no analyte is present ($[Q]_{C_A=0}$) and one when analyte is present ($[Q]$), and finally the distribution coefficients for analyte and electrolyte ions between the droplet surface and bulk solution (K_A/K_E).

The knowledge of the distribution coefficients for analyte and electrolyte ions could, for example, aid the analyst in selecting a suitable buffer−solvent system in order to minimize matrix and suppression effects and also predicting the concentration-dependent linear range in the mass spectrometric response. In the method developed by Enke, K_A/K_E values are obtained by curve fitting to the measured data points. To make this fitting, a minimum of three data points is necessary but in reality more are often needed to get an acceptable result. The aim of the present study was to develop a simplified method to determine equilibrium partitioning constants, K_A/K_E, for singly charged analytes. Only a maximum of two solutions needs to be analyzed to get a K_A/K_E value in the proposed method, thus making it a more rapid and better suited method for the determination of K_A/K_E values for large series of analytes or solvent systems. Furthermore, the effect of organic content in the electrosprayed solution on analytes with different hydrophobicities was evaluated. For this purpose, a series of tetraalkylammonium (TAA) compounds with increasing number of carbons in the alkyl chains were used as model compounds and analyzed at different methanol−water solution compositions.

EXPERIMENTAL SECTION

Sample and Solution Preparation. The model compounds used in this study were tetramethylammonium bromide (TMeA), tetraethylammonium bromide (TEtA), tetrapropylammonium bromide (TPrA), tetrabutylammonium bromide (TBuA), tetrapentylammonium bromide (TPeA), and tetraoctylammonium bromide (TOcA), all from Fluka Chemie AG (Buchs, Switzerland). All chemicals were of at least 99% purity and used without further purification. Several stock solutions, at 10 mM concentrations, of the six different tetraalkylammonium bromide salts were prepared in methanol (LC gradient grade), Merck (Darmstadt, Germany). Sample solutions were prepared by diluting the stock solutions with different methanol−water mixtures (ranging from 100 to 10% methanol, v/v) to a total tetraalkylammonium concentration of ∼0.2 mM. Water was obtained from a Milli-Q plus purification system (Millipore Corp., Bedford, MA).

Sample Introduction. Flow injection analyses were performed using a six-port injection valve, C6W (Valco Instruments, Houston, TX) equipped with a large sample loop (40 μL) in order to reach a steady sample concentration in the central part of the sample plug. A Harvard Apparatus model 22 syringe pump (South Natick, MA) was employed together with a 250-μL gastight syringe (Hamilton, Reno, NE) for the delivey of sample solution, from the injection loop to the spray, via a fused-silica capillary (Polymicro Technologies, Phoenix, AZ) of 50 μm i.d. and 187 μm o.d. at a constant flow of 5 μL/min. The same solvent as used to dilute the different stock solution of the quaternary ammonium salt was used as mobile phase in the flow injection experiments. Prior to use, the mobile phase was degassed using ultrasonication. Two types of samples were analyzed for each mobile-phase composition, one containing only a reference compound (electrolyte) at 0.2 mM concentration, giving Pf_E and one sample containing 0.1 mM electrolyte and 0.1 mM analyte to determine the K_A/K_E coefficient.

Mass Spectrometric Conditions. A PE-Sciex API III$^+$ triple quadrupole mass spectrometer (Concord, ON, Canada) was used in the present study. To be able to handle a large range of methanol−water solution compositions, IonSpray, pneumatically assisted electrospray, were employed as the spray technique. The fused-silica spray capillary, 50 μm i.d. and 187 μm o.d., was centered in a stainless steel capillary auxiliary assembly delivering 1.0 L/min air (zero grade, generated in-house) for pneumatically assisted ESI-MS. The flow rate of dry nitrogen countercurrent curtain gas (99.999% purity, 5.0 from AGA, Stockholm, Sweden) which was heated to 65 °C, was 1.2 L/min over the sampling orifice. The following mass spectrometer parameters were given set values: ion spray voltage (ISV) 4000 V, interface plate voltage (IN) 650 V, orifice lens (OR) 50 V unless otherwise indicated, and ac entrance rod (R0) 30 V. Data were acquired using a Macintosh IIfx computer with PE-Sciex Tune software version 2.5-FPU. Mass spectral data were recorded at unit mass resolution either by selected ion monitoring (SIM) or by scanning the approximate 10−700 u region in multichannel acquisition mode (MCA). A dwell time of 100 ms was used in SIM mode. The articulated IonSpray assembly tip was positioned 20 mm from the interface plate, ∼6 mm off-axis, and aimed ∼14 mm at the opposite side of the orifice (spray angel ∼45°), to artificially decrease the sensitivity. Furthermore, to avoid saturation of the channel electron multiplier detector in SIM mode, the isotopic peak (m/z 75) for the TMeA ion rather than the monoisotopic peak was monitored. Mass scale calibration was performed using a poly(propylene glycol) solution (PPG) supplied by the instrument manufacturer.

Spray Current Measurements. The current, *I*, leaving the spray capillary is a measure of the rate at which excess positive electrolyte ions leave the capillary. The spray current was measured using a test point on the high-voltage power supply board (ISV board). The voltage output from the test point on the ISV board was calibrated against the spray current measured with an off-line test rig.[34]

Safety Considerations. All work was done with commercial instrumentation, which provides sufficient protection against electrical shock; however, caution should be exercised when high voltages are used. Furthermore, all chemicals used should be handled with care.

RESULTS AND DISCUSSION

As the aim of this study was to investigate how the distribution of analyte ions with different hydrophobicities is affected by changes in organic content of the electrosprayed solution, a series of tetraalkylammonium compounds with increasing number of carbons in the alkyl chains were chosen as test compounds. The quaternary ammonium ions are already present in solution, and the ions are not affected by pH changes. Furthermore, the quaternary ammonium ions cannot be generated or lost due to gas-phase ion/molecule reaction such as proton transfer.[13] Therefore, quaternary ammonium salts can be considered as a good starting point for exploring some aspects of electrospray ionization mechanisms. These types of compounds have also been utilized extensively before by others[4,13−17,23,33,35−42] when different aspects of electrospray mass spectrometry have been explored.

In Figure 1, the total ion response (filled circles) for TMeA is shown, which represents the general trend for the total ion response for the test analytes used in this study when the percentage of methanol in the electrosprayed solution was varied between 10 and 100%. It can be seen, as expected, that the total response maximized at a high percentage of methanol (100%). It was further observed that the signal level generally decreased with increasing number of carbons in the test analyte (data not shown), which is in contradiction to what was reported previously by Fenn et al.[24] It is well known that the quadrupole mass analyzer have a mass-dependent ion transmission. [23,43] However, other factors, such as ion sampling from atmospheric pressure, might contribute to ion discrimination. In this study, we were spraying at a large off-axis position in order to artificially decrease the sensitivity, which might induce mass-dependent ion discrimination. Different aspects that influence ion discrimination and the determination of the droplet surface partitioning coefficient are being currently investigated in our laboratory and the results will be published in a separate report.

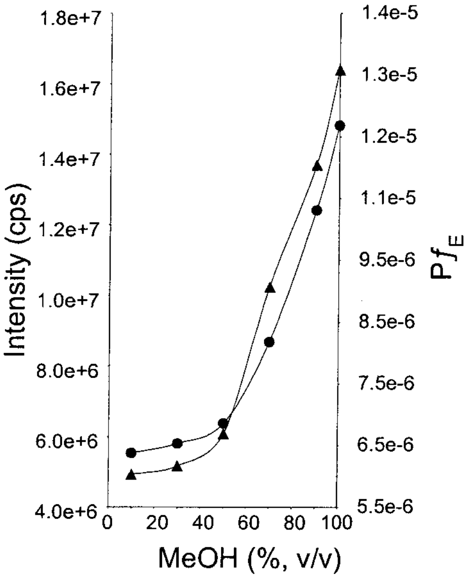

Figure 1. Total ion response (●) and the instrumental response factor, Pf_E (▲), for 0.2 mM tetramethylammonium bromide in methanol−water mixtures. For further details, see text.

A number of factors contribute to the total ion response in electrospray mass spectrometry, for example, the release of sample ions from the charged liquid droplets into the gas phase and the ion sampling efficiency of the system. The release of sample ions into the gas phase can be affected by controlling the size and charge density of the droplets, and the ion solvation, which includes both interactions with counterions and solvent molecules.In this study, we were interested in separating the effects of chemical and instrumental parameters, according to the equilibrium model developed by Enke,[33] into two components, i.e., the droplet surface partitioning coefficient (K_A/K_E) and the instrumental response factor (*Pf*).

The simplified method, developed in this work, for the determination of analyte and electrolyte ion distribution coefficients, is based on eq 12, and the assumption that the surface excess charge, Q, is carried only by the electrolyte, $[Q]_{C_A=0} = [E^+]_{s,C_A=0}$, when only electrolyte, and no analyte, is present in the electrosprayed solution. To simplify the measurements and data evaluation, it is preferred that a single peak from the electrolyte dominates the mass spectrum when only electrolyte is added to the electrosprayed solution. In this work, tetramethylammonium bromide was, for this reason, chosen as electrolyte. In Figure 2a, it can be seen that the peak from the TMeA ion (*m*/*z* 74) in 100% methanol dominates the mass spectrum and it can be concluded that the assumption is valid, saying that excess charge is carried by the electrolyte. However, upon closer examination of the spectrum, some peaks with low abundance can be seen. The contribution from impurities such as sodium was calculated to be ∼3% of the TMeA ion abundance. In this calculation, the total abundance of TMeA was calculated using the isotopic peak at *m*/*z* 75, as the monoisotopic peak saturates the detector. Furthermore, ∼2% of the total abundance of TMeA was due to fragment and dimer ions. The orifice voltage had to be adjusted carefully, as it will influence the degree of up-front fragmentation.[44] For example,

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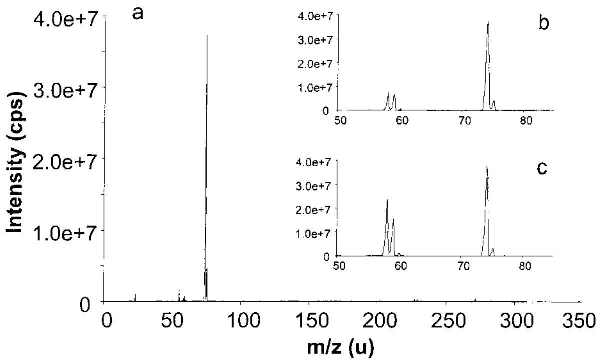

Figure 2. Mass spectrum of 0.1 mM tetramethylammonium bromide in 100% methanol at orifice voltage of (a) 50, (b) 60, and (c) 70 V.

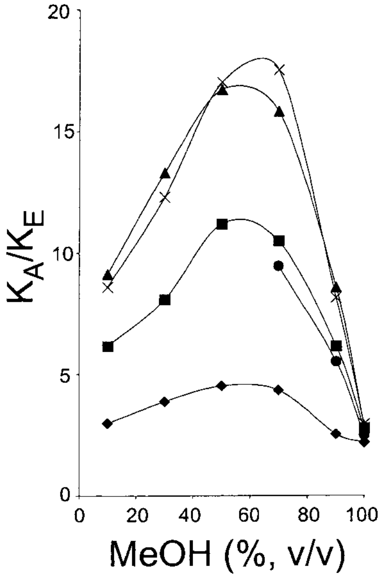

Figure 3. Droplet surface partitioning coefficient (K_A/K_E) for different tetraalkylammonium compounds as a function of methanol content in the electrosprayed solution. The compounds are marked with the following symbols: ◆, TEtA$^+$; ■, TPrA$^+$; ▲, TBuA$^+$; × TPeA$^+$; and ●, TOcA$^+$.

when the orifice voltage was increased from 50 to 60 V, the degree of fragmentation increased from 1.3 to ~16%, and by further increasing the orifice potential to 70 V, the degree of fragmentation increased to ~55%. In the above calculations, no corrections to the ion abundances were made for the mass-dependent ion transmission of quadrupoles.[23,43] The degree of fragmentation, as long as it is constant, will not influence the determination of the surface partitioning coefficient (K_A/K_E) according to eq 12 as the ratio of electrolyte responses is used. The absolute value of the instrumental response factor (Pf) will, however, be affected. The contribution from detected impurities increased slightly when the amount of water was increased in the electrosprayed solution; for example, the detected impurities increased from 3 to 7.5% in the solution containing 90% water.

The ionic strength of the electrosprayed solution was found to influence Pf, and therefore, when the "simplified" method were used, the ionic strength was kept constant in both types of samples that were analyzed, one that contained only TMeA as electrolyte and one sample containing both electrolyte and analyte. The effect of the ionic strength observed in this study is in agreement with a recent report by Constantopoulos et al.[41] They observed that the analyte response increased with increasing electrolyte concentration below 1 mM, which they attributed to be due to either the ionic strength affecting the thickness of the electrical double layer on the charged droplet surface or a decrease in droplet size due to increased conductivity of the solution.

A series of experiments were performed to investigate how the instrumental response factor for TMeA, Pf_E, is influenced by changes in solution composition. The results are shown together with the total ion signal in Figure 1, where separate solutions containing only 0.2 mM of TMeA were analyzed. The instrumental response factor, Pf_E, was calculated by the use of eq 9. Generally, as the water content increases, the instrumental response factor decreases, which is the same trend that was observed for the total ion response. Furthermore, the electrospray current shows a similar behavior. For 100% methanol, the electrospray current was 190 nA, which dropped to ~145 nA for 10% methanol.

The results from the experiments, with the electrosprayed solution containing both electrolyte (TMeA) and analyte at different solution compositions, are shown in Figure 3. In contradiction to the instrumental response factor, which optimized at a high concentration of methanol, the droplet surface partitioning coefficient (K_A/K_E) optimized at a lower percentage of methanol, about 70−50%. Furthermore, K_A/K_E increases with the number of carbons in the alkyl chains, as expected, up to five but decreased slightly for the tetraoctylammonium ion. The reason for the general increase in K_A/K_E when the methanol content is decreased from 100 to 50% could be that, as the solution becomes more polar, i.e., more water, the hydrophobic tetraalkylammonium ions tend to avoid the polar bulk solution and the content at the surface increases. From the above reason, it could be expected that K_A/K_E should show a continuous increase when the bulk solution becomes more polar. However, when the methanol content is further decreased below 50%, the droplet surface partitioning coefficient starts to decrease again. The relative decrease in K_A/K_E for the larger, more hydrophobic, ions is larger than the decrease in K_A/K_E for the TEtA ions. For example, the K_A/K_E for TPeA decreased to ~49% of its maximum value compared to TEtA, which decreased to ~66% of its maximum value. This observation might be due to a solubility problem of the different hydrophobic TAA ions as it could be expected that the larger, more hydrophobic, TAA ions are more difficult to solvate when the water content is increased, i.e., when the solution becomes more polar. The mass spectrum was inspected to see whether IF there were any differences in the tendency to form multimers when the methanol content was decreased; however, no differences could be observed. Limitation in solubility for tetraoctylammonium bromide was visually observed when the water content increase above 30% and no K_A/K_E values for TOcA were obtained for higher water content. Further experiments are currently in progress in our laboratory with different solvents to evaluate the effect of solvent polarity.

The reproducibility in the K_A/K_E coefficient determination was evaluated in a series of experiments for TEtA and TPeA at three different methanol content levels. The results are shown in Table 1. Generally the relative standard deviation was in the 3−7% range.

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Table 1. Reproducibility in the K_A/K_E Coefficient Determination (±95% Confidence Interval)

	TEtA			TPeA		
MeOH (%, v/v)	$K_{A/KE}$	±	n	$K_{A/KE}$	±	n
100	3.0	0.25	5	5.3	0.34	5
50	3.8	0.16	5	17.6	0.90	5
10	2.8	0.14	4	9.9	0.97	4

Initially, a series of experiments were performed with increasing concentrations ranging from 10 nM to 1 mM of some tetraalkylammonium compounds in methanol−water solutions, and eq 8 was fitted to the experimental data points in order to obtain a K_A/K_E value. Using this method, memory effects between the different solution compositions were a serious problem, and in order to reduce the amount of adsorbed tetraalkylammonium compound, the injection valve, sample loop, and injection syringe required extensive cleaning using ultrasonication. However, it was of interest to compare the results from both methods, and a series of experiments were performed with increasing tetrapentylammonium bromide concentration in 50/50 methanol−water and tetramethylammonium bromide as electrolyte at a concentration of 0.10 mM. The concentration of analyte ions at the surface, $[A^+]_s$, was obtained using a instrumental response factor from the high-concentration (saturation) region by taking the total ion response divided by the surface excess charge concentration according to the work by Enke.[33] Furthermore, as the conductivity of the solution increases with analyte concentration, the electrospray current increases, and separate surface excess charge concentration was calculated depending on the magnitude of the electrospray current. In Figure 4, both the surface concentration of tetrapentylammonium ion (filled circles) and the fitted data points (triangles) using eq 8 are plotted as a function of the analytical concentration of TPeABr giving a K_A/K_E value of 2.4. The K_A/K_E value obtained in this experiment is significantly different from the value obtained using the “simplified” method (K_A/K_E value, 17). When eq 8 was fitted to the experimental data points generated with increasing concentration of a tetrapentylammonium bromide, it is assumed that the instrumental response factor, *Pf*, is constant over the whole concentration range. A series of experiments were performed in order to determine the instrumental response factor for TPeA at each ionic strength level, and it was found that Pf_A increases with increasing ionic strength up to 1.1 mM. When the surface concentration of TPeA ion was calculated using the corresponding *Pf* value for respective ionic strength, a K_A/K_E value of 9.5 was obtained when eq 8 was fitted to the experimental data points. This value is still different from the value obtained with the simplified method, and more detailed experiments are currently in progress in our laboratory in order to further explore this behavior. One possible explanation could be that K_A/K_E is also influenced by the ionic strength.

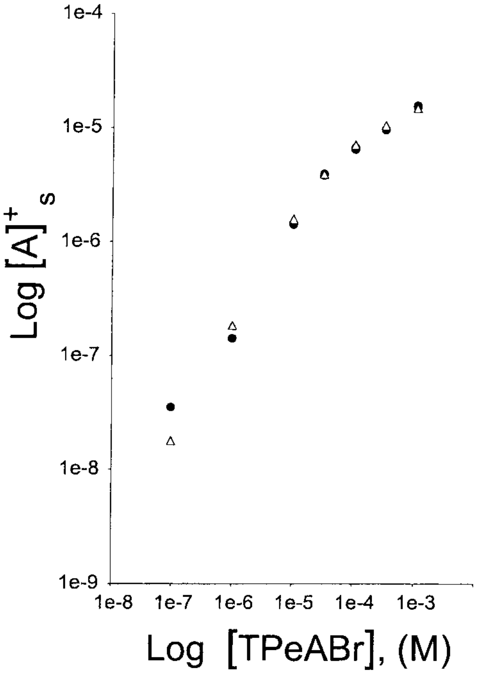

Figure 4. Surface concentration of tetrapentylammonium ion (●) and the fitted data points (Δ) using eq 8 plotted as a function of the analytical concentration of TpeABr in 50/50 methanol−water solution also containing tetramethylammonium bromide as electrolyte at a concentration of 0.10 mM.

CONCLUSIONS

In this study, a simplified method for determination of the droplet surface partitioning coefficient was developed. The method has several advantages, including reduced memory effect between the different experiments and reduced number of samples that have to be analyzed compared to the method reported by Enke.[33]

The results presented in this report show that the instrumental response factor is influenced by the ionic strength and that it optimizes at high methanol content in the electrosprayed solution and generally follows the total ion response. Furthermore, the surface partitioning coefficient maximizes at a lower content of methanol in the electrosprayed solution, and the results obtained in this study also indicate that the surface partitioning coefficient is not constant over a large analytical ion concentration range.

ACKNOWLEDGMENT

Financial support from the Swedish Natural Research Council, project K-1439-326, and from the Swedish Foundation for Strategic Research are gratefully acknowledged.

Received for review July 3, 2000. Accepted October 19, 2000.

AC000759T