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Effect of Affinity for Droplet Surfaces on the Fraction of Analyte Molecules Charged during Electrospray Droplet Fission

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The effect of uneven fissioning of mass and charge from electrospray droplets on the amount of analyte charged during the electrospray process was explored. A surface selectivity factor (S) was developed to describe the affinity of an analyte for the droplet surface, and both theoretical and experimental response curves were compared for analytes with various S values. The theoretical response curves were generated by calculating the overlap between the charge and analyte spawned from parent droplets to determine the amount of analyte charged. This overlap was then graphed as a function of analyte concentration. Differences in the amount of analyte charged during droplet fission were predicted for analytes of varying surface affinities. The issue of analyte partitioning between the surface and interior phases of the ESI droplet was also included in the discussion. This was accomplished by applying the equilibrium partitioning model to a set of offspring droplets to determine the amount of analyte on their surfaces and then calculating the overlap between fissioning analyte and excess charge. Experimental response curves resembled theoretical ones, and S values predicted from theory were in excellent agreement with those predicted on the basis of the structural characteristics of the analytes.

In the attempt to understand electrospray ionization mass spectrometry (ESI-MS), both solution-phase and gas-phase factors have been studied at length. In particular, the issue of how ESI droplets form gas-phase ions has been the subject of significant debate.¹ In this debate, the role of analyte surface activity (the analyte's affinity for the ESI droplet surface) in determining the fraction of analyte charged during evaporation has been largely ignored. On the other hand, the equilibrium partitioning model² has modeled electrospray response based solely on partitioning with the ESI droplet, while ignoring effects of droplet evaporation. Here we explore the effect of surface activity on the amount of analyte charged during droplet fission. In addition, we present a method by which theoretical ESI response curves can be generated that introduces some of the effects of droplet fissioning into the equilibrium partitioning model.

In ESI, droplets are formed from a capillary to which a high voltage is applied. These droplets have an excess of charge of the same polarity as the applied voltage. Due to energy considerations, this excess charge must necessarily reside at or near the droplet surfaces. The amount of charge per solution volume is fixed, having been determined by the experimental variables of flow rate and applied voltage.³ As the droplets begin to evaporate, the charge density on their surfaces becomes higher, and as they approach the Rayleigh limit (the point at which the Coulombic repulsion of the surface charges is equal to the surface tension of the droplet), they fission, forming offspring droplets.^{3,4} The offspring droplets produced have significantly higher surface-to-volume ratios than that of the parent droplet. After the first fissioning event (Coulombic explosion), more solvent evaporates from the parent and offspring droplets until the charge density on their surfaces becomes high enough that they either fission again or lose charge by ion desorption.³ Ultimately, gas-phase ions are formed by one of two mechanisms,⁵ evaporation from droplet surfaces during the fission process (the ion evaporation model)⁶ or formation of final droplets containing only one ion at the end of the fission process (the charged residue model).⁷

When a charged droplet undergoes a Coulombic explosion, the offspring droplets are formed from the solvent close to the droplet surface. Since charge resides at the droplet surface, these offspring droplets are enriched in charge relative to the initial droplets. This was demonstrated experimentally by Taflin and co-workers,⁴ who trapped individual microdroplets in electric fields and studied their size and charge before and after droplet fission. They determined that when a droplet undergoes a Rayleigh explosion, it loses between 10 and 18% of its charge and only between 1 and 2% of its mass.⁴ These values have been accepted throughout the literature; however, it is important to note that the experiments performed by Taflin et al. made use of organic solvents such as dodecanol and heptadecane. These solvents are significantly different from the methanol/water mixtures typically employed for ESI analysis.

Regardless of the solvent used, it can be assumed that the observations of Taflin et al. are at least qualitatively true, that mass

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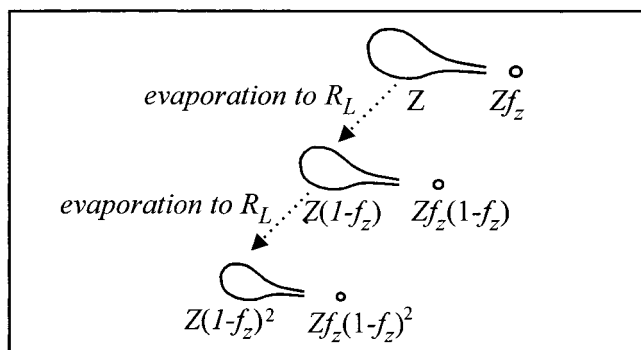


Figure 1. Fissioning of an ESI droplet with initial number of excess charges equal to Z and initial number of analyte molecules equal to T_A . The assumption is made here that a fixed percentage of the charge and a fixed percentage of the mass (or volume) is lost from the parent droplet each time it fissions. After each fissioning event, solvent evaporates from the parent droplet until it reaches the Rayleigh limit (R_L) and fissions again.

and charge spawn unevenly in the fissioning process. It has been suggested that this uneven spawning of mass and charge could have a significant effect on the amount of analyte charged in ESI.^{5,8} The key to whether the analyte becomes charged is whether it is on the surface of the droplet. Analytes with high surface affinity should follow the charge during the fissioning process, and thus, the uneven spawning of mass and charge will not significantly affect their responses. However, the uneven spawning of mass and charge could have a pronounced effect on the response of analytes with a high affinity for the droplet interior, since these analytes may be left behind in the initial droplet when the highly charged offspring droplets are formed. Thus, the effect of uneven spawning of mass and charge from electrospray droplets is expected to be most severe in cases where the analyte has low affinity for, or possibly even an aversion to, the droplet surface.

THEORY

The overlap between the charge and analyte in offspring droplets can be described mathematically. If the initial droplet has a charge of Z , and the fraction of charge lost in the first fission event is f_z , the resulting parent droplet will have a charge of $Z(1 - f_z)$ and the offspring droplet a charge of Zf_z . The amount of charge on the offspring droplet produced in the second fission event is $Zf_z(1 - f_z)$, while that produced in the third has a charge of $Zf_z(1 - f_z)^2$, and so on (Figure 1). Note that Figure 1 and the calculations above and below show one offspring droplet being produced each time the parent droplet fissions. The actual number of offspring droplets produced can vary depending on the experimental conditions.^{3,9,10}

Equation 1 expresses the amount of charge on the offspring droplets (z_n) produced in the n th fissioning event. The distribution

$$z_n = Z(f_z)(1 - f_z)^{(n-1)} \quad (1)$$

of analyte among offspring droplets can be determined in much the same way. However, an additional complication is added by

considering how droplet surface affinity affects the fraction of analyte spawned. If an analyte has extremely high surface affinity and, therefore, like the charge, resides completely on the droplet surface, the fraction of analyte lost during droplet fission (f_a) will be equal to the fraction of charge lost (f_z). If, however, the analyte is equally distributed throughout the droplet, the fraction spawned will be equal to the fraction of the volume spawned, f_v . Finally, a situation where the analyte has a high affinity for the droplet interior can also be imagined. In this case, when the outer layer of the droplet forms offspring droplets, the analyte in the droplet interior will be left behind. Thus, a fraction lower than f_v (as low as 0) of the analyte could be spawned into offspring droplets.

Equation 2 expresses the range of possible values for f_a by introducing S , which we will call the surface selectivity factor. For this equation to have physical significance, S can range from 0 ($f_a = 0$) to 1 ($f_a = f_z$). Equation 1 can now be rewritten for the analyte

$$f_a = S(f_z) \quad (0 \leq S \leq 1) \quad (2)$$

(eq 3), where a_n represents the amount of analyte in the offspring

$$a_n = T_A(f_a)(1 - f_a)^{(n-1)} \quad (3)$$

droplet after n fissioning events, T_A is the total number of analyte molecules in the initial droplet, and f_a depends on S , the surface selectivity.

METHODS

All samples were analyzed on a Finnigan TSQ 7000, triple quadrupole mass spectrometer (Thermoquest, San Jose, CA) with modified¹¹ Finnigan ESI source. Data were collected by scanning the mass range of Q1 from 0 to 1000 at a scan rate of 1 s/scan. A total of 80 scans were averaged for each spectrum, and the mass spectral response at each analyte concentration was determined by averaging the response of the analyte of interest from three such spectra.

A $5 \times 10^{10} \Omega$ resistor was inserted in series with the ESI power supply, causing stabilization of the spray current.¹² A 50- μ m-i.d. fused-silica capillary positioned 4 mm from the entrance to the mass spectrometer was used as the ESI spray capillary. Analyte solution was pumped to the spray capillary through a 250- μ m-i.d. fused-silica transfer line from a Harvard Apparatus syringe pump (South Natick, MA). A voltage of 4.5 kV was applied through direct metal contact to a union 3 mm from the capillary tip. The resulting spray current was monitored through a digital ammeter, which was placed in series with the ESI capillary. The temperature of the heated capillary interface was set at 200 °C.

Samples were diluted in 50% methanol/50% water solution/0.5% acetic acid solution. Stock solutions of trimethyloctadecylammonium chloride and cesium hydroxide (Sigma, Saint Louis, MO) were made to a concentration of 5×10^{-3} M. Less concentrated solutions were made through serial dilutions of these stock solutions.

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RESULTS AND DISCUSSION

Generation of Theoretical Response Curves by Assuming Ion Evaporation. Gomez and Tang suggested that ESI parent droplets fission 32 times before they reach the field desorption limit and begin to produce ions directly.³ This result was based on the assumption that offspring droplets carry a constant fraction of the parent droplet volume and charge when they fission. In actual application of ESI, it is questionable whether there is enough volume and enough time for the parent droplet to undergo 32 Coulombic explosions. Under typical ESI operating conditions, the parent droplets generated are between 1 and 5 μm in diameter.^{13–15} These tiny droplets lose volume very quickly throughout the fissioning process since they must evaporate down to the Rayleigh limit after each Coulombic explosion. In addition, the amount of time available for the fissioning process is finite; the fissioning process will end when the droplets either become neutralized upon impact with metal surfaces or freeze upon entering the vacuum. The exact amount of time during which fissioning can proceed has yet to be determined and will certainly vary depending on experimental parameters such as the solvent composition, the distance between the spray capillary and the counter electrode, and the configuration of the ESI interface.

In this study, we make the assumption that as long as enough excess charge ends up in the offspring droplets, all of the analyte molecules that fission into offspring droplets will be charged. Furthermore, we will assume that the offspring droplets produced in the ESI process lose all of their charge by desorption of ions rather than by further fissioning. This assumption was also made by Gomez and Tang,³ but may be an oversimplification, since it is entirely possible that offspring droplets could reach the Rayleigh limit and fission during the evaporation process. Whether or not this occurs has not yet been experimentally verified due to the minute size of the offspring droplets produced. Finally, we make the same assumption about offspring droplet sizes made by Gomez and Tang,³ that the offspring droplets produced in each fissioning event carry a constant fraction of the volume (0.02) and charge (0.15) of the parent droplet (Figure 2).

Consistent with our assumption that offspring droplets do not fission, is the assumption that the parent droplet fissions only until it reaches the size of the first offspring droplet. Since the parent droplet loses 15% of its charge each time it fissions, it should evaporate after each fissioning event until it reaches a size such that its surface area is 85% of what it was prior to fissioning. If this is indeed the case, the parent droplet reaches the size of the first offspring droplet after 16 Coulombic explosions (Table 1). The question then arises as to the fate of any analyte that remains in the parent droplet after 16 fissions. If indeed the droplet were to continue fissioning, as Gomez and Tang³ suggested, the majority of this analyte would end up in droplets without enough charge left to charge it. This is demonstrated by Figure 2, which was generated by graphing the distribution of charge (from eq 1) and the distribution of analyte (from eq 3) after 32 fissioning events for an analyte that is equally distributed throughout the ESI droplet. In this figure, the shaded area represents the amount of analyte that overlaps with the charge and thus becomes

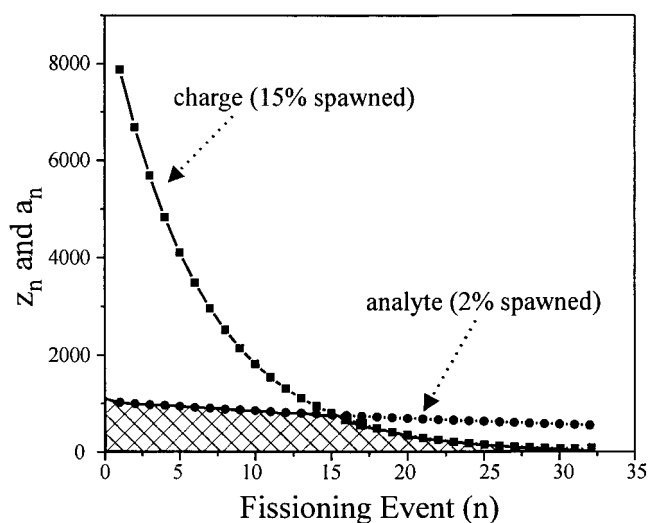


Figure 2. Number of analyte molecules and charges in offspring droplets generated in the n th fissioning event. Parameters: $T_A = Z = 52\,500$, $S = 0.13$ (analyte equally distributed within the droplet), $f_z = 0.15$, $f_v = f_a = 0.02$, and $n = 32$. The outlined area represents the amount of analyte charged. Note that most of the charge fissions into the offspring droplets generated early in the fissioning process.

Table 1. Information on Evaporating Droplets^a

n	V parent (μm^3)	surface area parent (μm^2)	V offspring (μm^3)	T_E in offspring
0	8.18	19.63	1.64×10^{-1}	1.97×10^5
1	6.28	16.47	1.26×10^{-1}	1.93×10^5
2	4.82	13.81	9.65×10^{-2}	1.89×10^5
3	3.70	11.58	7.41×10^{-2}	1.85×10^5
4	2.84	9.71	5.69×10^{-2}	1.82×10^5
5	2.18	8.14	4.37×10^{-2}	1.78×10^5
6	1.67	6.83	3.36×10^{-2}	1.75×10^5
7	1.28	5.73	2.58×10^{-2}	1.71×10^5
8	0.99	4.80	1.98×10^{-2}	1.68×10^5
9	0.76	4.03	1.52×10^{-2}	1.64×10^5
10	0.58	3.38	1.17×10^{-2}	1.61×10^5
11	0.44	2.83	8.97×10^{-3}	1.58×10^5
12	0.34	2.38	6.89×10^{-3}	1.55×10^5
13	0.26	1.99	5.29×10^{-3}	1.52×10^5
14	0.20	1.67	4.06×10^{-3}	1.49×10^5
15	0.16	1.40	3.12×10^{-3}	1.46×10^5
16	0.12	1.18	2.40×10^{-3}	1.43×10^5

^a Calculations assume that, for the initial droplet, $r = 1.25$ mm and that when this droplet fissions, it spawns offspring droplet(s) carrying 2% of its mass and 15% of its charge. The initial droplet then evaporates until its surface area is 85% of the original value and the fissioning process repeats.

charged. From Figure 2, it is apparent that most of the charge fissions into droplets produced early in the fissioning process. The shape of the analyte curve will vary depending on the value of S . For an S value of 1, the analyte and charge curves would be identical.

If the parent droplet does not continue to fission after 16 fissioning events, what happens to any analyte remaining in the parent droplet? Because it has undergone so much evaporation, the parent droplet has become highly concentrated in electrolyte by the time it has fissioned 16 times, and it may form a solid salt crystal. Alternately, it may enter the vacuum region without completely desolvating, at which point it will freeze and all the analyte it contains will be lost. As mentioned earlier, Gomez and Tang calculated that the parent droplet did not reach the field

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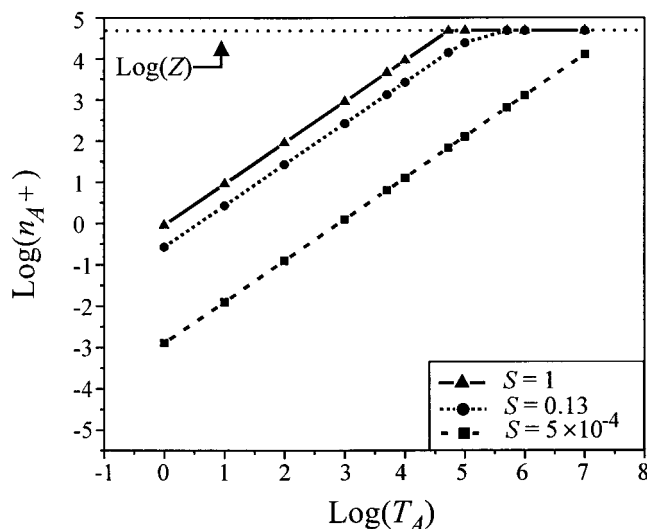


Figure 3. Theoretical response curves generated by calculating the overlap between the analyte and excess charge in offspring droplets at various values of T_A (total number of analyte molecules in initial droplet). For $S = 1$, the analyte is completely on the droplet surface; for $S = 0.13$, the analyte is equally distributed throughout the droplet; and for $S = 5 \times 10^{-4}$, the analyte has a high affinity for the droplet interior. Parameters: $Z = 52\,500$, $f_z = 0.15$, $f_v = 0.02$, and $n = 16$.

desorption limit until it fissioned 32 times.³ On the basis of this calculation, it is unlikely that the parent droplet produces ions by ion evaporation after 16 fissioning events. However, Gomez and Tang based their calculations on solvents atypical to ESI analysis (heptane doped with an antistatic fuel additive) and therefore it is possible that these calculations are not directly applicable to methanol/water mixtures.

Although the possibility that the parent droplet may produce ions after 16 fissioning events cannot be ruled out, we will assume in this work that, after 16 fissioning events, any analyte that remains in the parent droplet is lost by one of the aforementioned mechanisms. This assumption is in agreement with the results of theoretical studies of charge density and time scale for evaporating droplets by Tang and Smith,⁹ who concluded that the offspring droplets are the major source of ions observed in ESI-MS.

Response curves generated using the calculated (with Excel) overlap between eqs 1 and 3 are shown in Figure 3. In applying these equations, the number of electron charges in the initial droplets (Z) was set at 52 500. This value was chosen to allow for comparison with our experimental results, in which a spray current of 86.0 nA was measured for droplets on the order of $2.5\ \mu\text{m}$ in diameter (see eq 4 of the next section for calculation of Z). This value for the number of charges on methanol and water droplets $2.5\ \mu\text{m}$ in diameter is in good agreement with the value of 44 000 reported by Fenn.¹⁴

Figure 3 compares the effect of surface selectivity on theoretical ESI response curves. When the analyte is completely on the droplet surface ($S = 1$), the number of analyte molecules charged (n_{A^+}) increases linearly with concentration and then levels off sharply when the total number of analyte molecules in the initial droplet (T_A) reaches the number of excess charges available (Z). The value of $S = 0.13$ was chosen to represent a case in which the analyte has no particular affinity for the droplet surface and is equally distributed throughout it (by eq 3, when $S = 0.13$, $f_a =$

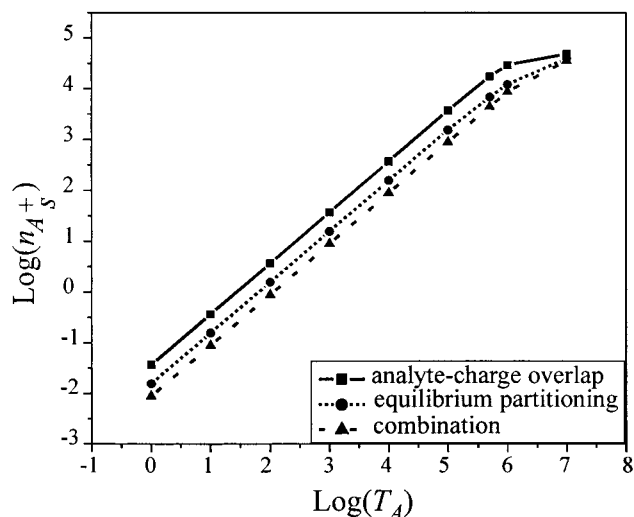


Figure 4. Comparison of response curves generated with three different methods, the analyte charge overlap method, the equilibrium partitioning model, and a combination of both. The combination of the two models predicts a slight decrease in the amount of analyte charged and an extension in the linear dynamic range as compared to either model alone. In general, however, the three models predict very similar behavior. Parameters: $S = 0.016$ ($K_A/K_E = 3$), $Z = 52\,500$, $f_z = 0.15$, $f_v = 0.02$, $n = 16$, $r = 1.25\ \mu\text{m}$, and $T_E = 9.85 \times 10^6$.

f_z). When the analyte is equally distributed throughout the droplet, the number of analyte molecules charged at each value of T_A decreases and an extension in the linear region of the response curve is observed. The extension of the linear range is a result of the fact that when only a fraction of the number of analyte molecules present in the initial droplet becomes charged, a greater value of T_A is required to reach a value of n_{A^+} equal to Z . Finally, a value of S equal to 5×10^{-4} was chosen to demonstrate the effect of a high affinity for the droplet interior on the number of analyte molecules charged. The result is a decrease by several orders of magnitude in the amount of analyte charged at a given T_A as well as an extension in the linear dynamic range of the response curve well beyond Z .

Extension of the Equilibrium Partitioning Model To Account for Droplet Fission. The previous discussion assumed that all of the analyte that overlaps with charge in offspring droplets is charged. An additional consideration is the issue of partitioning of analyte species in the offspring droplets. According to the equilibrium partitioning model,² two phases exist in an ESI droplet, a neutral interior phase and a surface phase where the excess charge resides (see Figure 4). Only analytes that are part of the surface phase become charged in the evaporation process, while those that exist in the interior are lost as neutrals. The extent of nonpolar character of a given analyte helps to determine its affinity for the droplet surface and, therefore, is an important factor governing its ESI response.^{16,17}

In view of this model, it is of interest to consider partitioning within offspring droplets and its effect on the amount of analyte charged. Thus far, the equilibrium partitioning model has only considered the partitioning of analytes within the initial droplets formed from the Taylor cone.² Here we extend this model to

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consider partitioning within the offspring droplets that fission from the initial droplet. In so doing, we assume that only a single droplet is produced each time a droplet fissions. Previously, the equations of the equilibrium partitioning model have related concentration of analyte on the droplet surface to analytical concentration of analyte.^{2,11} Since the volumes of parent and offspring droplets differ and are dependent on evaporation, it is convenient in considering partitioning within parent and offspring droplets to derive equations in terms of number of analyte molecules rather than analyte concentration and therefore eliminate the variable of droplet volume. The derivation of that relationship, which is analogous to that for concentration reported earlier,^{2,11} is presented in the next few equations.

The number of excess charges on the droplet surface, Z , is defined by eq 4, where i is the current measured in the needle

$$Z = (i/\Gamma F) V_{\text{dr}} N_A \quad (4)$$

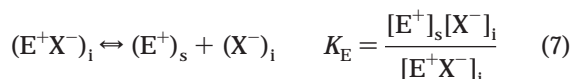
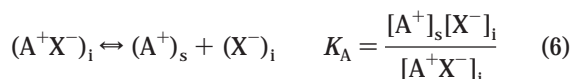
circuit (in A), F is Faraday's constant (96 485 C/equiv), Γ is the solution flow rate (in L/s), V_{dr} is the volume of the electrospray droplet (in L), and N_A is Avogadro's number (6.022×10^{23} charges/mol).

For a system containing a single analyte (A) and a single electrolyte (E), which compete with each other for the excess charge, the excess charge will be carried either by charged analyte (A^+) or charged electrolyte (E^+), as shown by eq 5. This equation

$$Z = n_{A^+} + n_{E^+} \quad (5)$$

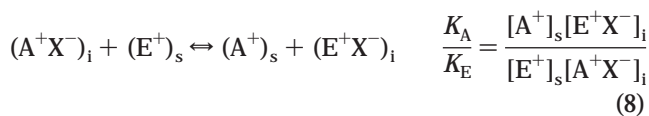
applies to any single analyte system, since some type of electrolyte is necessary to increase the conductivity of the solvent thus and facilitate the ESI process.¹⁸ This electrolyte can be in the form of background impurities in the solvent¹⁸ but is more often added to the solution in the form of an acid such as acetic acid (for positive ion analysis) or a base such as ammonium acetate (for analysis in the negative ion mode). In the case of positive ion analysis in methanol/water solutions acidified with acetic acid, the electrolyte is protonated methanol.^{16,17}

The equilibrium reactions and associated equilibrium constants for the analyte and electrolyte partitioning between the surface (s) and interior (i) phases of the ESI droplet are demonstrated in eqs 6 and 7.² The overall competition between the electrolyte and



analyte can be written in terms of a displacement reaction (eq 8), whose equilibrium constant is the ratio of the equilibrium constants from eqs 6 and 7.

Equation 8 demonstrates that analyte or electrolyte from the droplet interior can give up its counterion and become part of the surface phase, while analyte or electrolyte on the droplet



surface can accept a counterion and become part of the neutral interior phase. The ratio K_A/K_E determines the affinity of the analyte for the surface charge phase relative to that of the electrolyte. It is therefore related to electrospray response, such that analytes with higher values of K_A/K_E will have a higher electrospray response than analytes with lower values of K_A/K_E .^{2,16,17}

The equilibrium expression in eq 8 is written in terms of concentrations, but since it is a ratio, the volume terms drop out, and it can be expressed in an equivalent manner in terms of numbers of analyte and electrolyte molecules (eq 9). The purpose

$$\frac{K_A}{K_E} = \frac{[A^+]_s [E^+X^-]_i}{[E^+]_s [A^+X^-]_i} = \frac{(n_{A^+}_s)(V_s)(n_{E^+X^-}_i)(V_i)}{(n_{E^+}_s)(V_s)(n_{A^+X^-}_i)(V_i)} = \frac{(n_{A^+}_s)(n_{E^+X^-}_i)}{(n_{E^+}_s)(n_{A^+X^-}_i)} \quad (9)$$

of this derivation is to express the relationship between total number of analyte molecules and number of analyte molecules in the surface phase of the electrospray droplet. In so doing, it is necessary to introduce the mass balance equations for A and E. According to the mass balance equation for analyte (eq 10), analyte

$$T_A = n_{A^+}_s + n_{A^+X^-}_i \quad (10)$$

$$T_E = n_{E^+}_s + n_{E^+X^-}_i \quad (11)$$

will exist either as charged analyte on the droplet surface (A^+) or analyte paired with counterions on the droplet interior (A^+X^-). An analogous mass balance equation can be written for the electrolyte (eq 11). In these equations, T_A represents the total number of molecules of A and T_E represents the total number of molecules of E.

Equations 10 and 11 can be rearranged and substituted into eq 9 to eliminate the terms $(n_{E^+X^-}_i)$ and $(n_{A^+X^-}_i)$. The $n_{E^+}_s$ terms can then be eliminated by using eq 5. The resulting equation (eq 12) relates K_A/K_E to $n_{A^+}_s$. Rearranging Eq 12 gives an equation

$$\frac{K_A}{K_E} = \frac{(n_{A^+}_s)(n_{E^+X^-}_i)}{(n_{E^+}_s)(n_{A^+X^-}_i)} = \frac{(n_{A^+}_s)(T_E - n_{E^+}_s)}{(n_{E^+}_s)(T_A - n_{A^+}_s)} = \frac{(n_{A^+}_s)(T_E - (Z - n_{A^+}_s))}{(Z - n_{A^+}_s)(T_A - n_{A^+}_s)} \quad (12)$$

that is quadratic in $n_{A^+}_s$ (eq 13). Finally, eq 13 can be solved for

$$(n_{A^+}_s)^2 \left(\frac{K_A}{K_E} - 1 \right) - n_{A^+}_s \left(Z \left(\frac{K_A}{K_E} - 1 \right) + T_A \frac{K_A}{K_E} + N_E \right) + (T_A)(Z) \left(\frac{K_A}{K_E} \right) = 0 \quad (13)$$

$n_{A^+}_s$ using the quadratic equation to yield eq 14. This equation is significant because it shows how the number of analyte molecules

(18) Tang, L.; Kiebarle, P. *Anal. Chem.* **1993**, *65*, 3654–3668.

$$n_{A^+}_s = \left[\frac{\left(Z \left(\frac{K_A}{K_E} - 1 \right) + T_A \frac{K_A}{K_E} + T_E \right) - \sqrt{\left(Z \left(\frac{K_A}{K_E} - 1 \right) + T_A \frac{K_A}{K_E} + T_E \right)^2 - 4(T_A)(Z) \left(\frac{K_A}{K_E} \right) \left(\frac{K_A}{K_E} - 1 \right)}}{2 \left(\frac{K_A}{K_E} - 1 \right)} \right] \quad (14)$$

in the excess charge phase (and therefore the electrospray response) is related to the ratio of the partitioning coefficients of the analyte and the electrolyte (K_A/K_E) and to the initial number of analyte molecules (T_A). Since K_A/K_E is a measure of the analyte's affinity for the droplet surface, it should be related to the surface selectivity factor S . It is necessary to develop a relationship between S and K_A/K_E in order to incorporate the previous discussion of charge and mass loss during the droplet fission process into the equilibrium partitioning model. Equation 2 relates S to the fraction of analyte spawned during droplet fission (f_a). Therefore, the relationship between these variables can be derived if the way in which K_A/K_E depends on f_a can be determined.

The fraction of analyte on the droplet surface is equal to $n_{A^+}_s/T_A$ (the ratio of the number of analyte ions on the surface to the total number of analyte molecules). It is assumed that 15% of the charge, which already resides on the droplet surface, fissions from the parent droplet with each Coulombic explosion. If this is true, it is also reasonable that 15% of the analyte on the droplet surface be transferred to the offspring droplet in a fissioning event (assuming that the analyte is equally distributed on the droplet surface). This is shown in eq 15. When eqs 15 and 2 are set equal

$$f_a = f_z(n_{A^+}_s/T_A) \quad (15)$$

to each other, the term f_z drops out yielding eq 7, and since $n_{A^+}_s$ depends on K_A/K_E (eq 14), this equation relates S to K_A/K_E . By eq 16, it is apparent that S is equal to the fraction of charged

$$S = n_{A^+}_s/T_A \quad (16)$$

analyte on the surface of the droplet. Using the equations presented here, it is possible to apply the equilibrium partitioning model to *each offspring droplet* in order to determine what fraction of the analyte spawned into that droplet resides on the droplet surface. One slight complication is the fact that T_E is required in the application of eq 14. It is therefore necessary to calculate how many electrolyte ions end up in each offspring droplet. Provided that the electrolyte (in this case protonated methanol) is equally distributed throughout the parent droplet, the number of electrolyte ions that end up in the first offspring droplet can be calculated from the volume of the offspring droplet and the initial concentration of electrolyte (3×10^{-3} M). The number of electrolyte molecules in successive droplets can then be calculated in a similar way by once again applying the assumption that the initial droplet evaporates down to a surface area 85% of its initial value before fissioning again. The data used in calculating T_E and the resulting values of T_E in each offspring droplet are shown in Table 1.

Figure 4 shows the result of applying the equilibrium partitioning model to each individual offspring droplet and then calculating

the overlap of analyte and charge (dashed line). The two other curves in the figure are shown for the sake of comparison. One considers only the overlap of excess charge and analyte during the droplet fissioning process (solid line). The other (dotted line) was generated using the equilibrium partitioning model without considering droplet fission, i.e., considering only partitioning within the initial droplet. Each model alone (the equilibrium partitioning and charge overlap) overestimates the amount of analyte that can be charged. This is to be expected. The equilibrium partitioning model considers only the competition for the excess charge phase between analyte and solvent and ignores effects of uneven fissioning of mass and charge. Conversely, the analyte charge overlap method considers only uneven fission of mass and charge while ignoring competition for the excess charge phase. Combining the two models takes both factors into account, and as a result, the predicted amount of analyte charged is lower.

The ESI response curve predicted from the equilibrium partitioning model alone is qualitatively identical to that generated when the model is modified to account for droplet fissioning. The response curves in both cases are linear until the number of analyte molecules becomes a significant fraction of Z , at which point they level off due to charge limitation. Thus, former assertions that the equilibrium partitioning model can be used to predict ESI response without considering factors such as droplet fissioning and ion evaporation^{2,17} may be at least qualitatively true. The form of the equations² used to predict ESI response is still correct, as would be expected since they have been successfully applied to fit experimental data.¹⁷ However, the significance of the previous values calculated for K_A/K_E may not be just the distribution coefficient as previously suggested.¹⁷ In situations where droplet fissioning occurs to a significant extent, this ratio is affected not only by the competition of the analyte with the solvent for the excess charge but also by the fraction of analyte molecules charged during the fissioning process.

Comparison of Theoretical and Experimental Response Curves. Experimentally generated response curves for two different analytes are shown in Figure 5. One of the analytes is trimethyloctadecylammonium (TMOA⁺), a tertiary amine with surfactant properties. Its 18-carbon hydrophobic chain gives it a high affinity for the ESI droplet surface, and thus its S value should be close to 1. The other analyte is Cs⁺, which prefers to be solvated and therefore will likely be evenly distributed throughout the ESI droplet or even have some affinity for the droplet interior. We assume therefore that the S value for Cs⁺ is somewhere between 0 and 0.13. The response curves were generated for the two analytes run individually, so they should compete for excess charge with the protonated solvent only and should compare well to the theoretically generated response curves.

The data in Figure 5 are compared to theoretical response curves generated using the combination of the two models (charge overlap and equilibrium partitioning). To allow for this comparison,

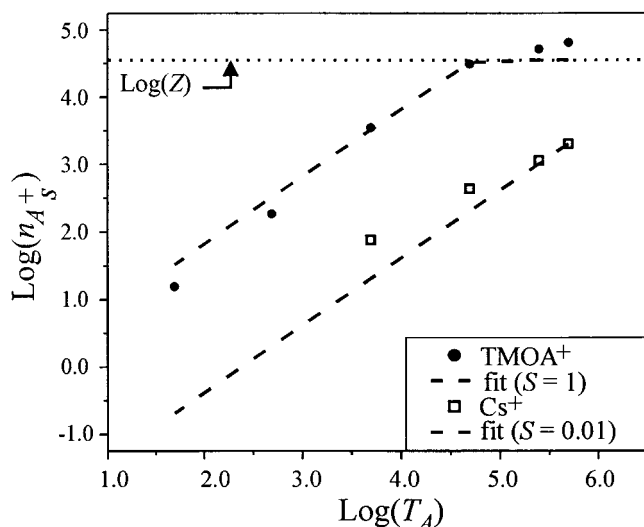


Figure 5. Experimental response curves for two analytes with different droplet surface affinities. The response curves were generated separately but under the same conditions, with a spray current of 86.0 nA (± 1.0 nA) and a flow rate of 5 μ L/min. The parameters assumed in calculating from mass spectral response and T_A from analytical concentration were as follows: response units/analyte ion = 54.4 and $d = 1.25$ μ m. Parameters used in generating the data from the combination of the models were identical to those used in Figure 3.

it was necessary to convert analyte concentration into number of analyte molecules (T_A), and mass spectral response into number of analyte ions on the droplet surface (n_{A^+}). To accomplish this conversion, the analyte concentration was multiplied by the volume of the initial droplets (assuming a droplet radius of 1.25 μ m). The analyte response was converted to n_{A^+} s by dividing by 54.4. The value of 54.4 response units/analyte ion was determined by assuming that TMOA⁺ is completely on the surface of the droplet, and therefore that at the point that the TMOA⁺ response reaches saturation, a number of TMOA⁺ ions equal to Z (52 500) are being charged. The value of $Z = 52$ 500 was calculated from eq 4 using the experimentally measured 86.0 nA for i .

It is obvious upon inspection of Figure 5 that the predicted enhanced response of the surface-active species relative to that of the non-surface-active one is indeed observed. Although there is significant scatter in the data for the Cs⁺, the increased range of linearity for this less-surface-active species predicted by the theoretical response curves seems to occur; the response for TMOA⁺ levels off when n_{A^+} reaches Z , while the slope of the line for Cs⁺ does not change. Theoretical response curves generated using an S value of 1 for TMOA⁺ and an S value of 0.01 for Cs⁺ best fit the experimental data. This is in agreement with the S values predicted on the basis of characteristics of the analytes proposed at the beginning of this section.

The theoretical response curve generated using a value of 1 for S fits the TMOA⁺ data very well in the low-concentration range and reasonably well in the high-concentration range. The slight deviation of the experimental data from the predicted curve at high concentrations could be a result of the fact that the desolvation process in our experiments is different from that proposed in theory. The TSQ-7000 mass spectrometer uses a heated capillary interface to aid in desolvation of analytes as they pass from atmospheric pressure into the high vacuum of the mass

spectrometer. This could cause deviations of the experimentally measured data from the ideal, since the process is clearly accelerated within the heated capillary and the fissioning process may not occur. Another possibility is that the TMOA⁺ is not completely on the surface of the droplet. The fact that the response increases slightly beyond the value of T_A equal to Z suggests that this may indeed be the case. Finally, it has yet to be proved experimentally that the roll-off in ESI calibration curves at high concentrations is a result of the limited amount of charge available. Kostianen and Bruins^{19,20} suggested that a limited amount of space rather than charge on droplet surfaces may be the cause of saturation. It is also possible that, in experiments using the TSQ-7000, saturation in response at high concentrations is a result of an upper limit in ion transmission through the heated capillary interface.

Theory predicts that the response curve for Cs⁺ in Figure 5 should have a slope of 1. In actuality, the slope is somewhat less than 1, or alternately, the two data points at the lowest concentrations deviate from the ideal line. The nonlinearity in the data for Cs⁺ may be a result of the fact that it is difficult to obtain reproducible data for low-mass analytes such as Cs⁺. This is due to the significant level of background noise in the form of solvent clusters observed in the low-mass range of the ESI mass spectrum, as evidenced by the poor detection limit for Cs⁺ as compared to that of TMOA⁺.

CONCLUSIONS

A number of assumptions were necessary in developing the theory to predict overlap between analyte and charge during droplet fissioning. It was assumed that the offspring droplets produced during fissioning had a fixed percentage of the parent droplet mass and charge. Furthermore, these droplets were assumed not to fission further, but to produce ions almost immediately due to their small size and high charge density. Finally, we assumed that any analyte left in parent droplets after 16 fissions did not become charged. In light of all these assumptions, it is difficult to make quantitative predictions based on the theoretical response curves generated. As more information regarding the droplet fissioning process is obtained, these assumptions can be modified and new theoretical response curves can be generated. The process presented here for generating these response curves can serve as a model for doing so.

Ultimately, this study demonstrates that analyte surface affinity may be significant in determining what fraction of analyte is charged during droplet fission. It shows that if an analyte has a very high affinity for the droplet interior, only a very small fraction of the analyte molecules may be charged during the ESI process, even if an abundance of excess charge is available in the parent droplets. These results are of interest to the ESI user in that they demonstrate that analytes that will be most successfully analyzable with ESI are those with a high affinity for the droplet surface. It has been shown previously that an analyte's affinity for the droplet surface can have a significant effect on ESI response, but this effect was rationalized based solely on partitioning within the ESI droplet.^{16,17} These results show that the effect of uneven spawning

(19) Bruins, A. P. In *Electrospray Ionization Mass Spectrometry*; Cole, R. B., Ed.; John Wiley and Sons: New York, 1997; Vol. 1, pp 107–135.

(20) Kostianen, R.; Bruins, A. P. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 549–558.

of mass and charge can compound the effect of partitioning within an ESI droplet and make the issue of droplet surface affinity even more important in determining ESI response.

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