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# A Predictive Model for Matrix and Analyte Effects in Electrospray Ionization of Singly-Charged Ionic Analytes

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**In electrospray ionization (ESI), droplets with a surface excess charge are created. The rate of production of surface excess charge is a constant and is equal to the rate of ion production. The ions appearing in the mass spectrum are postulated to be those that formed the surface excess charge at the time of droplet formation (or their collision products). An equilibrium model based on competition among the ions in the solution for the limited number of excess charge sites has been developed. This model accurately predicts the response curves of singly-charged ionic analytes as a function of the concentration of electrolyte and other analytes and provides an explanation for the selective effectiveness of ESI. At low concentrations of total analyte (micromolar and less), the response curves are linear, indifferent to the presence of other low concentration analytes, and suppressed by electrolyte concentrations in excess of the minimum required. At higher analyte concentrations, the response becomes independent of analyte concentration but highly affected by the presence of other analytes.**

The introduction of electrospray ionization (ESI) methods to mass spectrometry is creating a great number of opportunities for the increased application of mass spectrometry.<sup>1–7</sup>

At the same time, the technique sometimes seems capricious in terms of the types of molecules amenable to ESI and the wide range of response factors attained. In addition, it has been difficult to predict the effects of changes in the solvent composition, the electrolyte employed, or the (generally adverse) presence of other analytes. For singly-charged ions and doubly-charged inorganic ions, it is generally the case that the relative intensities of ions in the spectrum do not reflect the relative concentrations of these ions in solution, sometimes by several orders of magnitude.<sup>8</sup> In much of the literature on this topic, it has been assumed that the basis for these effects lay in the way in which the free (unsolvated) ions were formed from the charged droplets. In this paper, I suggest first that the principal phenomenon affecting the relation-

ship between solution composition and the relative abundances of ions in the mass spectrum is the partitioning of the ionic species between the two phases that are part of the charged droplet system. These phases are the interior, solvated, ion-paired phase and the surface excess charge phase. Second, I suggest that the relative ion abundances observed are proportional to the relative concentrations in the surface excess charge phase (with a few caveats discussed below). And finally, I suggest that the relative concentrations in the surface excess charge phase can be quantitatively predicted by the equilibrium-based model presented here.

**A Brief Review of the Electrospray Process.** The most common implementation of the general ESI process is illustrated in Figure 1. A metallic, narrow-bore capillary, continuously filled with a solution containing the analyte to be ionized, is connected to the positive terminal of a high-voltage dc power supply. The negative terminal of this supply is connected to a metal plate located a few millimeters away from the capillary tip. The intense electric field at the capillary tip draws some of the cations in the exposed solution to the liquid surface. The anions that formerly neutralized the charge on these cations are driven back toward the tip of the capillary. The electrohydrodynamics of the charged liquid surface cause it to take the shape of a cone (called the Taylor cone) from which a thin filament of solution extends until it breaks up into droplets.<sup>9–12</sup>

Like the solution making up the Taylor cone, the droplets produced have a surface composition enriched in unneutralized cations (which make up the excess charge) while the droplet interiors are solutions of uncharged molecules and neutral salt. These positively charged droplets are attracted to move downfield toward the metal plate (while fanning out through mutual repulsion as they go). On their trip to the plate, much of the solvent evaporates from the droplets and, by one or more of several proposed mechanisms, some solvent-free cations are formed. When the vapor-phase cations and the remaining charged droplets hit the metal plate, electrons from the metal neutralize their charge. The net motion of cations from the capillary tip to the metal plate constitutes an electrical current passing clockwise around the circuit through the power supply, up to the capillary metal, into the solution, out into the droplets, and so on. An electrochemical oxidation process must occur where the current

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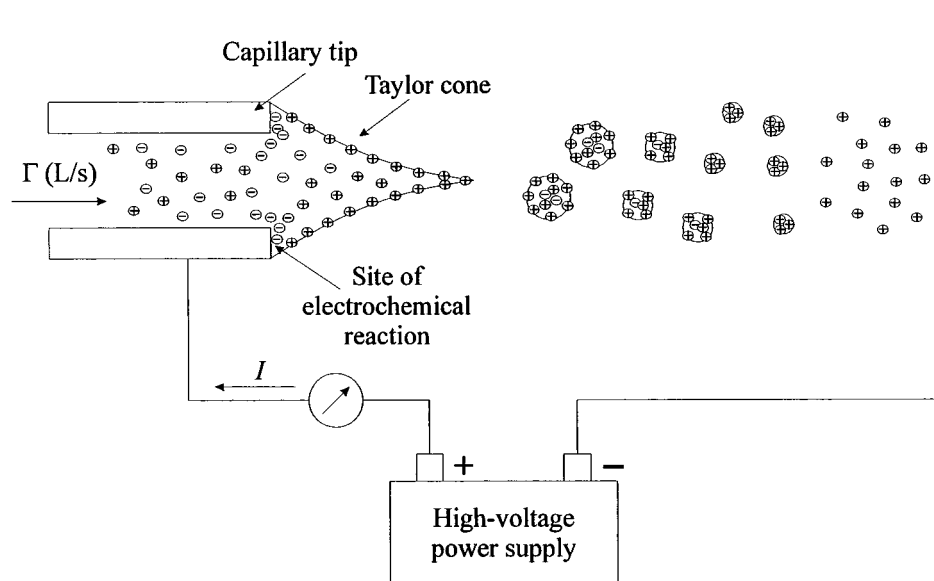


Figure 1. Cartoon illustrating several aspects of the electrospray ionization process.

passes into the solution.<sup>11–13</sup> Since this is a series circuit, the value of the current is the same everywhere in the circuit.

In practical mass spectrometry employing ESI, the metal plate is part of an assembly that separates the atmospheric pressure electrospray area from the hard vacuum of the mass spectrometer. An orifice exists in this metal plate through which some of the vapor-phase ions pass into the mass analyzer. Current designs generally involve two or more stages of pressure reduction between the atmospheric pressure ESI region and the vacuum of the mass analyzer.<sup>14,15</sup> The description given above is for the production of positive ions. Simply reversing the polarity of the power supply can generate negative ions instead. In this case, the positive counterions are driven back away from the tip of the Taylor cone and the electrochemical process is reduction.

**Existing Models.** Two principal mechanisms have been proposed for the desolvation of the observed ions. One is *Coulomb fission*, in which the repulsive effect of the increasing surface charge density eventually completely offsets the droplet surface tension so that the droplet divides, temporarily increasing the overall surface-to-volume ratio. The fission process may result in product droplets of roughly equal size, or more often, it may spawn the ejection of a series of much smaller droplets<sup>16</sup> in a process now called “droplet jet fission.”<sup>21</sup> Dole<sup>17</sup> imagined the fission process continuing until the particles remaining contained only a single charged species. The second proposed mechanism is *ion evaporation*<sup>18</sup> in which the increase in surface charge density as a result of solvent evaporation produces a Coulombic repulsion that exceeds the charged species’ adhesion to the drop surface and thus some ions are expelled from the surface. This process also continues as the droplet decreases in surface area due to solvent evaporation. Ion evaporation is assumed by some to be

the principal mechanism for droplets 10 nm or less in radius.<sup>19</sup> Of major concern to the mass spectrometrists, of course, is which of the ions will be expressed in the mass spectrum.

In 1991, Tang and Kebarle proposed a model based on the hypothesis that the ion evaporation rate from the droplets is proportional to the ion concentration in the droplet.<sup>20</sup> The basis for this model was the assumption that the Iribarne ion evaporation model was the principal mechanism for the ion transfer to the gas phase. The fraction of the ion current due to the analyte species is represented as its fraction of the total ion formation rate,  $I$ . The resulting equation is

$$I(A^+, ms) = Pf \frac{k_A[A^+]}{k_E[E^+] + k_A[A^+]} \quad (1)$$

where  $ms$  refers to the ion current converted to source ions,  $f$  is the fraction of the droplet charge that is converted into gas-phase ions,  $P$  is the sampling efficiency of the system,  $k$ ’s are the rate constants, and the bracketed ions are the analytical concentrations of the analyte and electrolyte species, respectively. Inherent in this model is the realization that the total circuit current  $I$  will be apportioned among the various species according to their proclivity for undergoing the process. Ion evaporation was selected as the principal process affecting the ability of an ionic species to form gas-phase ions, though in a later work,<sup>21</sup> the  $k$ ’s were referred to simply as “sensitivity coefficients” without indication of the mechanism of selectivity.

## DISCUSSION

The excess charge in a droplet is necessarily carried on, or very near, its surface. This is required by the effect of mutual charge repulsion. By contrast, the interior of the droplet is neutral, containing solvent, other molecules, and salts. Though much attention has been paid to which of the desolvation mechanisms

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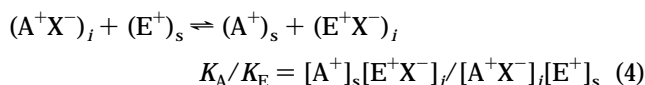
is at work under a given set of conditions, from the standpoint of which ions get expressed, that may not be the most critical question. This is because, in all proposed mechanisms, the ions that are freed from the liquid phase are most likely those that comprise the surface excess charge, i.e., those that prefer the charged state at the droplet surface over the completely solvated, ion-paired state in the droplet interior. Those cations that prefer to be paired with anions in the interior of the droplet will increasingly tend to do so as evaporation proceeds and their concentration in the solution increases. The desolvation process does not generally result in charge separation beyond that which makes up the excess charge (of which there was a fixed amount at the time of droplet formation). If these arguments are valid, an investigation of the process that determines the relative abundance of the species making up the surface excess charge is warranted.

The model proposed here is based on the consideration of the surface excess charge state as a *phase separate* from that of the droplet interior. Ions are then free to partition between the surface and interior phases. If the partitioning process is sufficiently rapid, we could assume that the relative concentrations come to an equilibrium state. For the analyte ions  $A^+$  and the necessary electrolyte ions  $E^+$ , these equilibrium partition reactions and their partition coefficients will be



These reactions have been written with the associated anion included to remind us that the analyte ions in the droplet interior are all neutralized by an equivalent amount of countercharge indicated here by  $X^-$ .

The equilibrium can also be expressed as a displacement reaction since the two components  $A^+$  and  $E^+$  are both competing to supply a fixed number of surface charges. This reaction and its equilibrium constant are



The goal of the next sections is to develop an equation for the surface concentration of analyte on the droplet in terms of the analytical concentration of analyte in the original solution and other knowable concentrations and constants. In this development, I have chosen to use the partition form of the equilibrium (eqs 2 and 3) since the equilibrium constant for the analyte will be more useful if it is independent of the electrolyte species present.

**The Equivalent Concentration of the Excess Charge.** The difference in the concentrations of the cations and anions in the electrosprayed solution can be called the concentration of excess charge. The rate of excess charge production in equivalents per second is equal to the circuit current,  $I$ , divided by the Faraday constant,  $F$ . When this rate is divided by the flow rate  $\Gamma$ , in liters per second, the result is the concentration of excess charge  $[Q]$  in equivalents per liter that exists at the instant of droplet formation (before significant solvent evaporation).

$$[Q] = I/F\Gamma \quad (5)$$

For typical conditions of  $I = 10^{-7}$  A and  $\Gamma = 6 \mu\text{L}/\text{min}$ , the value of  $[Q]$  is  $\sim 10^{-5}$  equiv/L. Over the normal range of operating conditions,  $[Q]$  does not deviate greatly from this value. The significance of  $[Q]$  is that it is the sum of the concentrations of all the charged species on the surface. For the case of a single analyte  $A^+$  and electrolyte  $E^+$ ,

$$[Q] = [A^+]_s + [E^+]_s \quad (6)$$

From this equation we can see that when the total cation concentration in the drop exceeds  $[Q]$ , there will be a competition among the cations for which ones will be part of the surface excess charge (and thus available to become vapor-phase ions) and which will remain in the droplet interior paired with the counterions (and thus unavailable for mass spectrometric detection). It is also interesting to note that the total cation concentration must be at least  $[Q]$  to provide the charges for the current  $I$ .

If we assume that the fraction of the total analyte or electrolyte concentration that is on the surface is very small, the partition equilibrium constants of eqs 2 and 3 can be written

$$K_A = [A^+]_s[X^-]_i/C_A \quad \text{and} \quad K_E = [E^+]_s[X^-]_i/C_E \quad (7)$$

where  $C_A$  and  $C_E$  are the analytical concentrations of the analyte and electrolyte.

If these two equations are solved for  $[X^-]_i$  and equated to eliminate  $[X^-]_i$

$$[A^+]_s = C_A K_A / C_E K_E [E^+]_s \quad (8)$$

Then, if eq 6 is solved for  $[E^+]_s$  and substituted in eq 8

$$[A^+]_s = \frac{C_A K_A}{C_A K_A + C_E K_E} [Q] \quad (9)$$

If we assume that the ion abundance in the spectrum is proportional to the concentration of that ion in the surface excess charge phase of the droplet, the instrumental response for that ion will be

$$R_A = Pf \frac{C_A K_A}{C_A K_A + C_E K_E} [Q] \quad (10)$$

where  $P$  is the efficiency of vapor-phase ion transfer into the mass spectrometer and  $f$  is the efficiency of converting the ions forming the surface excess charge into vapor-phase ions. The selection of  $P$  and  $f$  for these constants follows the convention of Kebarle.<sup>20</sup>

When this equation is compared with the Kebarle equation (eq 1) it will be seen that they have exactly the same form. In other words, the fraction of  $[Q]$  that is  $[A^+]_s$  in eq 10 is the same as the fraction of  $I$  that is carried by  $A^+$  in eq 1 except that, in eq 1, the  $k$ 's are ion evaporation rate constants and, in eq 10, the  $K$ 's are equilibrium constants for the phase distribution reaction. The similarity of eqs 1 and 10 is interesting since even though they were arrived at from very different principles, the response functions they predict are exactly the same. However, the assumption made in the equilibrium approach, namely, that the

surface concentration of analyte is small compared to the analytical concentration, bears closer scrutiny.

**The Introduction of Mass Balance Equations.** Since the ions having the polarity of the excess charge may exist in either the interior or surface charge phases, a mass balance equation can be written for each of the species involved in the partition. For the single analyte  $A^+$  and electrolyte  $E^+$ , the mass balance equations are

$$C_A = [A^+]_s + [A^+X^-]_i \quad (11)$$

and

$$C_E = [E^+]_s + [E^+X^-]_i \quad (12)$$

In this case we will begin with eq 5 since the  $[X^-]$  term is already eliminated. Then the interior concentrations will be eliminated by the substitution of eqs 11 and 12 and  $[E^+]_s$  will be eliminated by substitution of eq 6.

$$\frac{K_A}{K_E} = \frac{[A^+]_s[E^+X^-]_i}{[A^+X^-]_i[E^+]_s} = \frac{[A^+]_s(C_E - [E^+]_s)}{(C_A - [A^+]_s)[E^+]_s} = \frac{[A^+]_s(C_E - [Q] - [A^+]_s)}{(C_A - [A^+]_s)([Q] - [A^+]_s)} \quad (13)$$

This is now an equation containing only the surface concentration of  $A^+$ , the analytical concentrations of  $A^+$  and  $E^+$ , the distribution coefficients of analyte and electrolyte, and the equivalent concentration of surface excess charge  $[Q]$ —all physical constants or knowable parameters. Equation 13 is quadratic in  $[A^+]_s$  as seen in the rearrangement shown in

$$[A^+]_s^2 \left( \frac{K_A}{K_E} - 1 \right) - [A^+]_s \left( [Q] \left( \frac{K_A}{K_E} - 1 \right) + C_A \frac{K_A}{K_E} + C_E \right) + C_A [Q] \frac{K_A}{K_E} = 0 \quad (14)$$

The quadratic formula can be used to solve this equation to obtain the response function ( $[A^+]_s$  vs  $C_A$ ). It is a curve that asymptotes to straight lines at high values of  $C_A$  and at very low values of  $C_A$ . At high values of  $C_A$ , where only terms including  $C_A$  are considered,  $[A^+]_s = [Q]$ . In this region, all the surface excess charge is composed of  $A^+$  and the response (proportional to  $[A^+]_s$ ) is not a function of  $C_A$ . In other words, this region is not useful for quantitation. At values of  $C_A$  lower than  $[Q]$ , eq 14 reduces to

$$[A^+]_s = C_A \left( \frac{K_A/K_E}{K_A/K_E - 1 + C_E/[Q]} \right) \quad C_A \ll [Q] \quad (15)$$

In this region, the response is proportional to  $C_A$ , the response factor being the portion of eq 15 in parentheses. Since this is the region in which quantitation may be accomplished, it is worthwhile to look at the factors that make up this response factor more closely.

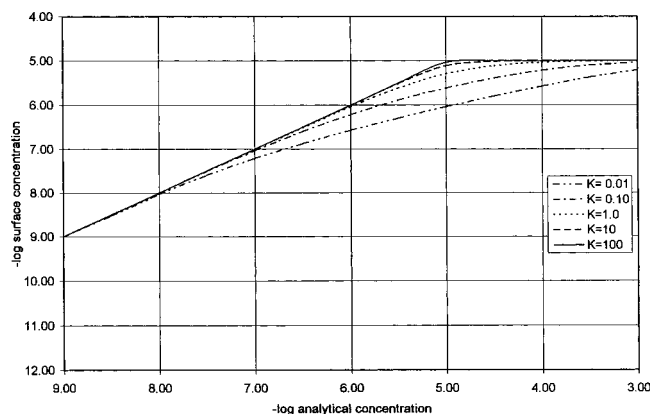


Figure 2. Response function for a singly-charged analyte with a singly-charged electrolyte.  $[Q] = 10^{-5}$  M,  $C_E = 10^{-5}$  M, and  $K_A/K_E = 0.01-100$ .

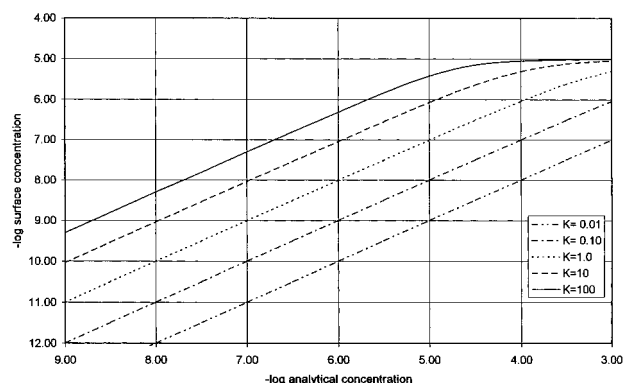


Figure 3. Response function for a singly-charged analyte with a singly-charged electrolyte.  $[Q] = 10^{-5}$  M,  $C_E = 10^{-3}$  M, and  $K_A/K_E = 0.01-100$ .

For operation at vanishingly small values of analyte concentration, virtually all the excess charge must come from the electrolyte. Thus, the minimum electrolyte concentration is  $[Q]$  and the fraction  $C_E/[Q]$  must be at least 1. If  $C_E$  were equal to  $[Q]$ , the response factor is unity. In this case, *essentially all the analyte is part of the surface excess charge*, and this is true regardless of the value of  $K_A/K_E$ ! The complete response curve over the entire concentration range for this condition is shown in Figure 2. The effect of various values of  $K_A/K_E$  is seen to be the degree of rounding in the response curve between the low-concentration region and the "saturation" region at higher concentrations. For values of  $K_A/K_E$  greater than unity, the surface concentration of analyte is essentially equal to the analytical concentration until the analytical concentration approaches  $[Q]$ . Obviously, this is a very favorable operating condition for the response factor is not only as high as possible but there is little difference in the response factors among different analytes as long as their  $K_A/K_E$  values are greater than unity.

The effect of increasing the concentration of electrolyte above the minimum value of  $[Q]$  is shown in Figure 3. Here eq 14 has been solved for various values of  $K_A/K_E$  when  $C_E$  is  $100[Q]$ . In this case, the response factor is less than unity even for a value of  $K_A/K_E$  of 100. For lower values of  $K_A/K_E$ , the response factor is even lower, for example, 0.01 for a  $K_A/K_E$  of 1. With higher values of  $C_E$ , the range of concentrations over which quantitation can be achieved extends to values much higher than  $[Q]$ , but at great cost in terms of the sensitivity. In addition, the response

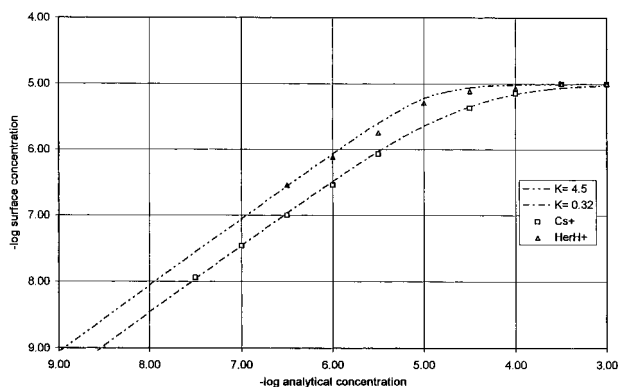


Figure 4. Fit of eq 14 to experimental data from Kebarle and Tang.<sup>11</sup>

factor will vary greatly among the analytes, depending on their  $K_A/K_E$  value.

Fitting eq 14 to experimental response curves for a single analyte is a test of its validity and the conclusions derived from it. The instrumental response factor (the fraction of the excess charge that appears at the mass spectrometer detector) can be obtained by dividing the limiting intensity observed at high concentrations for a single analyte by  $[Q]$ . This will give the number of detector response units per mole of analyte. The best determination of  $[Q]$  is to use measured values for  $I$  and  $\Gamma$  from eq 1. Data from an experiment reported by Kebarle and Tang<sup>11</sup> are plotted in Figure 4. In this experiment, the response intensities of heroin hydrochloride ( $\text{HerH}^+$ ) and  $\text{Cs}^+$  were separately measured as a function of analyte concentration. The electrolyte present was principally the  $\text{NH}_4\text{CH}_3\text{O}$  impurity present in the methanol solvent, estimated by the authors to be  $10^{-5}$  M. The fit parameters were  $K_A/K_E$  and  $C_E$ . The values that fit were  $C_E = 1.6 \times 10^{-5}$  M and  $K_A/K_E$  for  $\text{Cs}^+$  and  $\text{HerH}^+$ , 0.32 and 4.5. These results demonstrate the ability of eq 14 to fit experimental data in the low-concentration ( $<10^{-5}$  M) range where eq 1 did not work. Not unexpectedly, the values of  $k$  obtained by Kebarle and Tang from fitting eq 1 to the data do not exactly match the values of  $K_A/K_E$  obtained by fitting eq 14. They do agree in the general trend of the  $k$  values but differ by a factor of 2.5 in the ratio of the  $K$ 's for  $\text{Cs}^+$  and  $\text{HerH}^+$ .

At this point, it is interesting to consider the special case of the recently developed techniques that perform electrospray ionization at much reduced flow rates. From eq 5 we see that the value of  $[Q]$  is inversely proportional to the flow rate. Thus, if the flow rate were reduced to one-hundredth the typical value of  $6 \mu\text{L}/\text{min}$  used in the above discussion and the needle current remained essentially the same (as it typically does), the value of  $[Q]$  would become  $10^{-3}$  equiv/L. The effect of this change on the predicted response curves is quite significant. The quantitative concentration range is extended upward by 2 orders of magnitude as is the required concentration of electrolyte. The greater tolerance for electrolytes in the analytical solution may account for some of the observed increases in sensitivity (according to eq 15) when low flow rate ESI techniques are used. Additional benefits almost certainly come from a more complete desolvation of the ions forming the surface excess charge (See a later section.)

**ESI of Multiple Analytes.** The presence of additional analytes provides more competition for the limited charge. The mathematical treatment employed below parallels that for the single analyte. First the charge balance equation (eq 6) is modified to include the surface concentration of both analyte ions

$\text{A}^+$  and  $\text{B}^+$  and the electrolyte  $\text{E}^+$ .

$$[\text{A}^+]_s + [\text{B}^+]_s + [\text{E}^+]_s = [Q] \quad (16)$$

The mass balance equation for the analyte  $\text{B}^+$  is  $C_B = [\text{B}^+]_s + [\text{B}^+\text{X}^-]_i$ . An additional equilibrium equation (comparable to eq 4) is also involved.

$$\frac{K_B}{K_E} = \frac{[\text{B}^+]_s[\text{E}^+\text{X}^-]_i}{[\text{B}^+\text{X}^-]_i[\text{E}^+]_s} = \frac{[\text{B}^+]_s(C_E - [\text{E}^+]_s)}{(C_B - [\text{B}^+]_s)[\text{E}^+]_s} \quad (17)$$

A third equilibrium equation can also be derived, though it is not independent of the other two.

$$\frac{K_A/K_E}{K_B/K_E} = \frac{K_A}{K_B} = \frac{[\text{A}^+]_s(C_B - [\text{B}^+]_s)}{(C_A - [\text{A}^+]_s)[\text{B}^+]_s} \quad (18)$$

From eq 18, the following expression can be obtained for  $[\text{B}^+]_s$ .

$$[\text{B}^+]_s = \frac{[\text{A}^+]_s C_B}{(K_A/K_B)C_A - (K_A/K_B)[\text{A}^+]_s + [\text{A}^+]_s} \quad (19)$$

Equation 19 is now substituted into eq 16 for  $[\text{B}^+]_s$ . Then this equation is solved for  $[\text{E}^+]_s$ .

$$[\text{E}^+]_s = [Q] - [\text{A}^+]_s - \frac{[\text{A}^+]_s C_B}{(K_A/K_B)C_A - (K_A/K_B)[\text{A}^+]_s + [\text{A}^+]_s} \quad (20)$$

This equation is then used for  $[\text{E}^+]_s$  wherever it appears in the middle expression in eq 13. A lot of algebra then ensues, but what results is the cubic equation

$$[\text{A}^+]_s^3 [K_B - K_A + K_E(1 - K_B/K_A)] + [\text{A}^+]_s^2 [C_A(2K_A - K_B - K_E) + C_B(K_A - K_E) + C_E K_E(1 - K_B/K_A) + [Q](K_A - K_B - K_E(1 - K_B/K_A))] - [\text{A}^+]_s C_A [[Q](2K_A - K_B - K_E) + C_B K_A + C_A K_A + C_E K_E] + C_A^2 [Q] K_A = 0 \quad (21)$$

This equation has been solved for a number of operating conditions. The first is for the case where  $C_A$  and  $C_B$  are equal. In the series of response curves shown in Figure 5,  $K_A/K_E$  is held constant at 1.0 while  $K_B/K_E$  varies from 0.1 to 100. The electrolyte concentration,  $C_E$ , is equal to  $[Q]$  at  $1 \times 10^{-5}$  M. In the low-concentration region (below  $10^{-6}$  M), the response curves for both analytes are linear with a response factor of nearly unity. A comparison of these curves with those of Figure 2 for a single analyte indicates that the presence of the second analyte has essentially no effect on the response function. In this region, where the surface concentrations of analytes are much less than the surface concentration of electrolyte, the analytes are independently competing with the electrolyte for the surface positions. At higher analyte concentrations, where the sum of the surface concentrations of analytes begins to be significant with respect to  $[Q]$ , the presence of the second analyte affects the response of

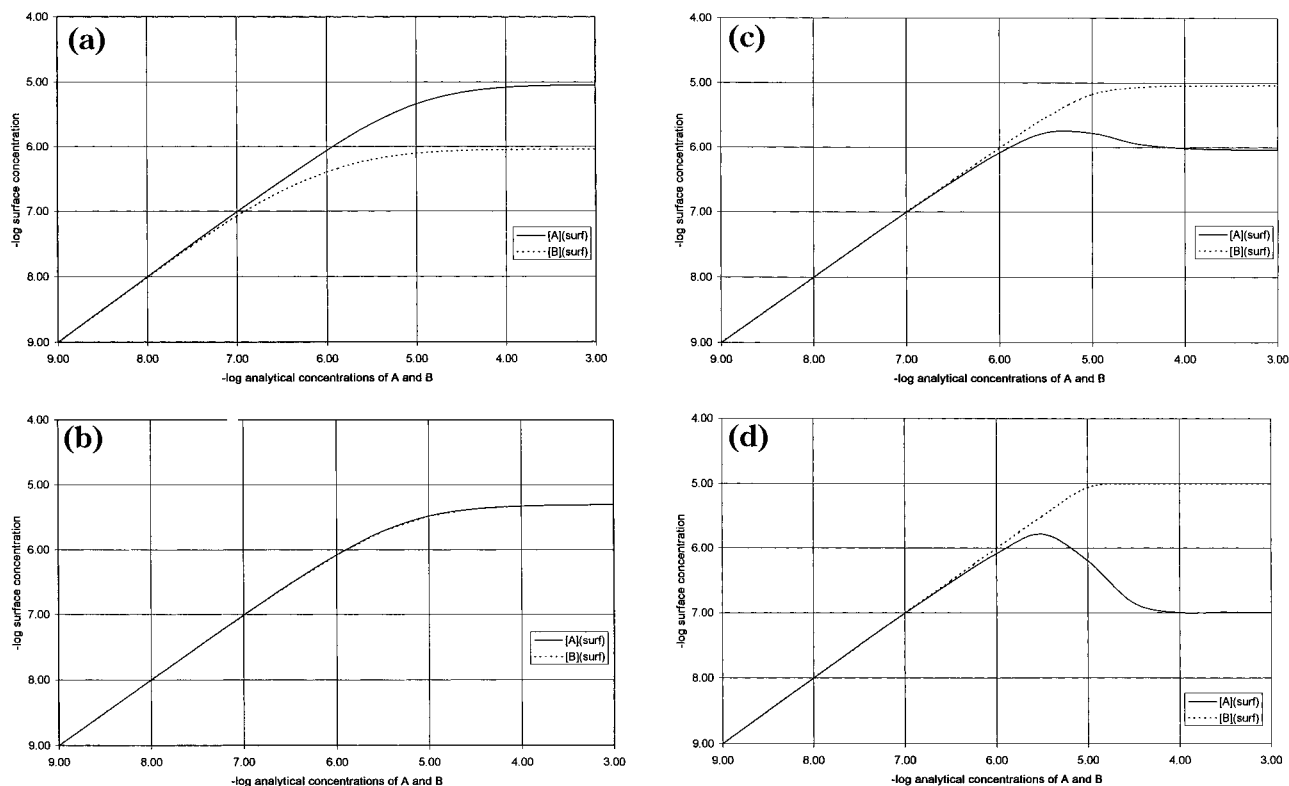


Figure 5. Response function for two singly-charged analytes.  $C_E = [Q] = 10^{-5}$  M and  $K_A/K_E = 1$ . (a)  $K_B/K_E = 0.10$ . (b)  $K_B/K_E = 1.0$ . (c)  $K_B/K_E = 10$ . (d)  $K_B/K_E = 100$ .

the first. The analyte with the higher value of  $K$  begins to have a higher surface concentration than the other. At still higher concentrations, where the analytes have displaced essentially all of the electrolyte from the surface, the sum of the analyte surface concentrations is  $[Q]$ .

In Figure 5a, both analytes have a relatively low value of  $K$  so that the break between the linear response part of the curve and the saturation area is quite gradual. Both analyte response curves are identical in Figure 5b, where they have the same value of  $K$ . In this curve, the saturation value for each is  $[Q]/2$  since they are equally sharing the surface sites. An interesting hump develops in the response curve for the less favored analyte at higher values of  $K$  as seen in Figure 5c and d. This hump occurs in the region where both analytes and the electrolyte have a significant fraction of the surface excess charge. At low concentrations, the analyte with the lower value of  $K$  can compete better with the remaining electrolyte than it can at higher concentrations, where it must displace the more favored analyte for the surface positions. The final ratio of the analyte surface concentrations, in the saturation region, is equal to the ratio of their  $K$ 's.

This same effect was observed by Kebarle and Tang.<sup>11</sup> Data from their paper have been fit to eq 21 in Figure 6. The fitting parameters were chosen in the following way. First it was observed that the two analytes (tetraalkylammonium salts) had only slightly different response curves at low concentrations even though they had clearly different  $K$  values. This meant that the electrolyte concentration was only a little larger than  $[Q]$ . The value of  $[Q]$  was obtained by recognizing that, in the linear region at low concentrations, the response function is nearly unity. In this region, the sensitivity of the system (response counts per liter of analyte) can easily be determined. From this calculation, the saturation surface concentrations for each analyte can be determined. The sum of these two concentrations is necessarily

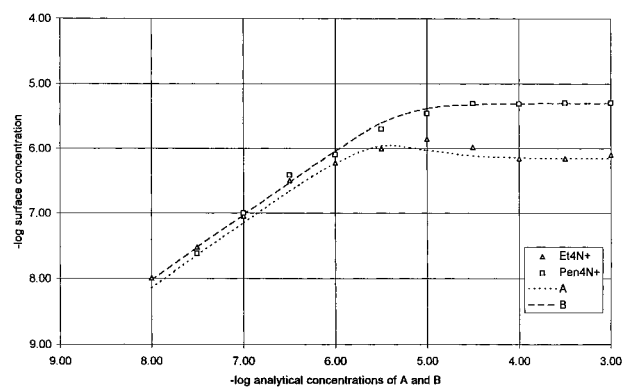


Figure 6. Fit of eq 21 to experimental data from Kebarle and Tang<sup>11</sup> for two tetraalkylammonium salts. Fit parameters:  $[Q] = 5.7 \times 10^{-6}$  M,  $C_E = 1 \times 10^{-5}$  M,  $K_A/K_E = 2.0$ , and  $K_B/K_E = 14.3$ .

$[Q]$ . The ratio of the  $K$  values of the analytes was calculated from the ratio of concentrations in the saturation region (7.15). Keeping the ratio of the  $K$ 's at 7.15, the value of one of the  $K$ 's was changed until the solution to eq 19 gave the best values for the surface concentration in the region between linear response and saturation. Again, the equation obtained from this model accurately fits the experimental data over the complete concentration range. As Tang and Kebarle have demonstrated, this cannot be done with eq 1.

A dramatic view of the effect of the presence of the second analyte on the response to the first is shown in Figure 7. In these plots, the electrolyte concentration is kept at the value of  $[Q]$  while the analytical concentration of the second analyte is varied for different values of  $K_B/K_E$ . With the analytical concentration of analyte  $A^+$  at  $10^{-7}$  M (Figure 7a), the response factor remains essentially unity until the analytical concentration of analyte  $B^+$  is almost  $10^{-6}$  M. Thereafter, the response factor falls off gradually

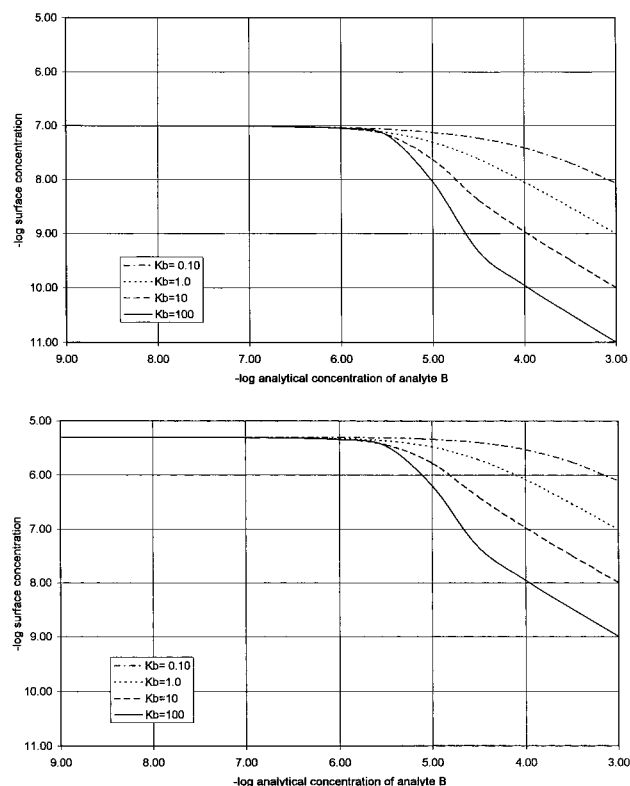


Figure 7. Response to analyte  $A^+$  as a function of the concentration of analyte  $B^+$ .  $[Q] = 10^{-5}$  M,  $K_A/K_E = 1.0$  and  $C_E = 10^{-5}$  M. (a)  $C_A = 10^{-7}$  M and (b)  $C_A = 10^{-5}$  M.

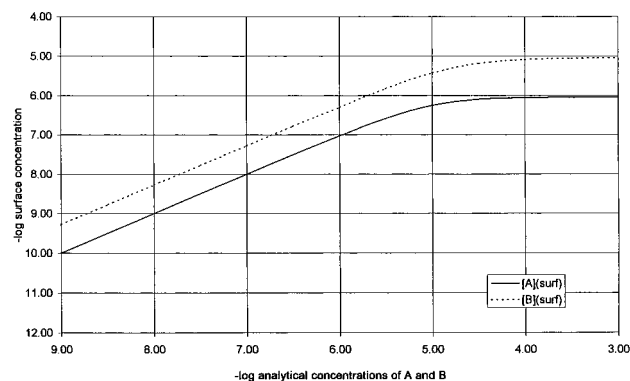


Figure 8. Response function for two singly-charged analytes.  $[Q] = 10^{-5}$  M,  $K_A/K_E = 1.0$ ,  $K_B/K_E = 10$ , and  $C_E = 10^{-4}$  M.

or dramatically depending on the relative values of  $K_B/K_E$  and  $K_A/K_E$ . The same pattern is observed for a higher analytical concentration of analyte  $A^+$  (Figure 7b). In this case, however, the response factor remains at 0.5 throughout the low-concentration range for  $B^+$  because the analyte and electrolyte have the same concentrations and  $K$  values and thus share equally the surface excess charge sites.

The effect of higher electrolyte concentrations is shown in Figure 8. This plot should be compared with that of Figure 5c where the electrolyte concentration is at the minimum possible value of  $[Q]$ . The response factors for both analytes is depressed to a degree that depends on their  $K$  values. Also, the hump in the response curve for the less favored analyte is gone; this effect only occurs when the electrolyte concentration approaches  $[Q]$ .

**Processes Affecting the Solution Composition before Droplet Formation.** While the equilibrium model may accurately predict how the ionic species in solution will partition between

the interior and surface excess charge phases, it is important to recognize that the composition of the solution undergoing this partition is not simply the sum of the solution ingredients. As several researchers have pointed out, the electrochemical process that occurs between the solution and the metal capillary can have a significant affect on the solution composition.<sup>13</sup> In the case of positive ion formation by ESI, the electrochemical process is oxidation. The oxidation must be of a species that exists at the metal/solution interface. The effect of this oxidation may be to create an ionized species from a neutral molecule or atom and thus make it observable in the mass spectrum. Kebarle demonstrated this by using a lead capillary tip and showing that  $Pb^{2+}$  cation appeared in the spectrum (as  $Pb^+$  through charge loss while still in the air) in equivalent amounts.<sup>10</sup> More recently, Van Berkel has used the electrochemical process to change neutral organic molecules to ionized forms so that they may be observed.<sup>13</sup> More commonly, it is the solvent or capillary material that is involved in the electrochemical process.

In order to assess the effect of the electrochemical process on solution composition, the possible reactions will be divided into two types: those that increase the cation concentration and those that deplete the anion concentration. Examples of the former are  $Fe(s) \rightleftharpoons Fe^{2+} + 2e^-$  and  $2H_2O \rightleftharpoons 4H^+ + O_2 + 4e^-$ , and an example of the latter is  $4OH^- \rightleftharpoons 2H_2O + O_2 + 4e^-$ .

The extent of the increase in cation concentration can be calculated from the electrolysis current  $I$ , the solution flow rate  $\Gamma$ , and the Faraday constant  $F$ .<sup>10</sup> In fact the calculation is exactly the same as that of eq 5. Thus, the electrochemical process increases the cation concentration in the original solution by exactly  $[Q]$  equivalents per liter (or decreases the anion concentration by the same amount). This process can produce the requisite concentration of electrolyte (at least  $[Q]$ ) with no salts added to the solvent. If analyte molecules are being oxidized with 100% current efficiency, the concentration of the oxidized form would be  $[Q]$ . Under the most favorable conditions ( $C_E = [Q]$ ), this concentration is already at the "saturation level". If the current efficiency for the analyte oxidation is much less than 100%, the slope of the working curve would follow the oxidation rate as a function of concentration.

Many researchers have noticed a pitting of the capillary material at its tip, indicating that the tip is the site of the electrochemical reaction. The solution between the Taylor cone and the site of the electrochemical reaction is negatively charged due to an excess of the counterion. The field that creates the surface excess charge has driven these counterions away from the cone surface. The solution must therefore contain these "excess" counterions up to the point that the electrochemical reaction either produces an equivalent amount of cation to neutralize their charge or the equivalent amount of anion is consumed. To minimize diffusion and because of the excess negative charge of the solution, this process will occur close to the Taylor cone.

The fact that the oxidation products are created close to the Taylor cone means that they are in the solution phase for a relatively short time. This fact has been taken advantage of to observe ions that are stable in solution for only a short time.<sup>13</sup> Conversely, some researchers have successfully used a short section of glass capillary for ESI, locating the necessary metallic contact many centimeters from the tip.<sup>10,13</sup> The above discussion does not preclude this phenomenon but it does require that the



entire solution between the contact and the tip contain an excess of the counterions. The excess counterions will remain close to the capillary wall and must flow upstream at the rate required by the needle current.

Whatever processes affecting the chemical composition of the test solution that may occur before the droplet formation, it is the ions present in the resulting solution that undergo the partitioning introduced by the formation of the surface charge phase at the Taylor cone.

**Processes Affecting Relative Ion Abundance after Droplet Formation.** In the above discussion, it has been assumed that the ions forming the surface excess charge on the droplets are those that are seen in the mass spectrum, and in the same relative abundance. However, there are several phenomena that can occur after the droplet formation that may result in exceptions to this assumption. There may be some selectivity in the desolvation process as the ions forming the surface excess charge are separated from the droplet, there are a variety of chemical reactions the desolvated ions may undergo before they are mass analyzed, and there may be some mass selectivity in the ion-transfer and mass analysis processes. These effects and the use of the proposed model in studying them are discussed briefly in this section.

In normal ESI, the desolvation process is far from complete. Visible amounts of liquid are often formed around the first aperture. Under these circumstances, if there were any differences among the species forming the surface excess charge in the energy required to become fully vaporized, those most easily vaporized would have higher response factors. As the fraction of the surface charge that is evaporated increases, the selective effect would be decreased. In the limit of the evaporation of all the surface excess charge, there would be no evaporative selectivity effect. On this basis, I would expect the degree of the selectivity introduced by the evaporative effect to be significantly smaller in the case of the low-flow techniques where the fraction of the solvent evaporated is much larger than in normal ESI.

Some of the desolvated ions may undergo various ion-molecule reactions with the neutral gases while the ions are still in the relatively high-pressure regions of the source. These effects are well documented and include ion fragmentation,<sup>22–24</sup> charge exchange<sup>9,10,13</sup> and ion molecule reactions.<sup>25</sup> In all of these cases, a fraction of the ionic species originally in the surface excess charge phase is showing up in another form in the spectrum. In terms of the equilibrium model presented here, the response predicted should be roughly the sum of the responses of all the product  $m/z$  values the partitioned analyte ion has in the spectrum.

## CONCLUSIONS

The model that has been put forth above explains in quantitative detail many of the behaviors observed in electrospray ionization operation with respect to the effects of electrolyte and other analytes on the response curves for singly-charged analytes. It is based on the conceptually simple model of an equilibrium competition among the ionic species present in the solution for the limited number of excess charge sites that exist on each

droplet at the time of its detachment from the Taylor cone. The ionic species present in the solution are the intentionally added analytes and electrolytes as well as solvent impurities and any electrochemically generated species.

The success of the equilibrium model in accurately predicting observed behavior indicates that the "ionization" step is the creation of the surface excess charge at the Taylor cone and that the species that make up this charge are the ones that we are able to see in the mass spectrum. This means that, regardless of the mechanism of desolvation or ion expulsion, it is the initial excess charges (or their reaction products) that survive.

Another conclusion resulting from the success of the equilibrium model is that the diffusional processes required to establish equilibrium are fast enough to do so. The initial diffusion that establishes the composition of the surface excess charge can occur in the region between the electrochemical step and the surface of the Taylor cone. The short diffusional distances and microsecond-length times apparently allow equilibrium to be established. Thereafter, during the rapid concentration changes that accompany the desolvation process, the composition of the excess charge may not change significantly. It seems plausible that those species that preferred initially to be paired with countercharge ions rather than be surface excess charges would increasingly do so as the solution concentration increases.

The electrochemical process is seen to play a potentially very important role. If the process is the oxidation of neutral species, the concentration of the electrochemically formed ions is equal to  $[Q]$  equivalents per liter, the total amount of the solution's ions converted to excess charge. If the ions produced electrochemically have a lower  $K$  value than the analyte ions, they will not effectively compete with the analyte. If their  $K$  value is lower than other electrolyte ions as well, they may have no apparent effect on the analyte response.

For effective analytical operation, the model suggests that the total analyte concentration remains well below  $[Q]$  and that the electrolyte concentration be as close to  $[Q]$  as possible. Working in this region minimizes the matrix effects.

I believe it will be possible to experimentally develop a table of  $K$  values for a variety of analyte and electrolyte ions in commonly used solvents. In combination with the equations developed above, these constants would enable an accurate prediction of the effectiveness of ESI for given combinations.

Finally, it is possible to postulate about the basis of the differences between normal electrospray and low-flow electrospray ionization techniques. In low-flow techniques, the solution flow rates are up to 1000 times less than with conventional electrospray but the needle currents are comparable. Reference to eq 1 indicates that, at such flow rates, the value for  $[Q]$  will be as much as 1000 times greater than for normal electrospray, or  $\sim 10^{-2}$  M. This would extend the linear operating range to much higher concentrations and greatly reduce the depressive effect of higher electrolyte concentrations. However, it also means that the electrolyte formed electrochemically will also be  $\sim 10^{-2}$  M. Improvements in sensitivity in the low-flow ionization techniques for analyte concentrations below  $10^{-5}$  M cannot be ascribed to greater conversion of the analyte to surface excess charge. Rather, the improvement must be due to a greater efficiency in the conversion of the surface excess charge to vapor-phase ions.

Our group is currently working on extensions of the above model to doubly-charged analytes and electrolytes, ionic species

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formed by protonation and adduct formation, and multiply-protonated molecules.

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