

# Effect of Solvent on Dynamic Range and Sensitivity in Pneumatically-assisted Electrospray (Ion Spray) Mass Spectrometry

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Mass-analyzed detector signal and spray current have been measured in pneumatically-assisted electrospray mass spectrometry. The sample was tetrabutylammonium bromide dissolved in water, methanol, acetonitrile, chloroform, dichloromethane or toluene. At low sample concentrations ( $\leq 5 \times 10^{-6}$  M) the ion signal rose with increasing sample concentration. Above  $10^{-5}$  M the ion signal was fixed and independent of sample concentration. Comparison of signals with spray currents for tetrabutylammonium bromide at  $2 \times 10^{-6}$  M in different solvents revealed a strong correlation between ion signal and spray current. Apparently, the abundance of the tetrabutylammonium ion at  $m/z$  242 is fully controlled by the amount of charge on droplets, while other solvent properties such as volatility, surface tension and polarity do not play a role at low tetrabutylammonium bromide concentrations. Thus, water is a poor solvent for electrospray because it does not allow efficient droplet charging, not because it is less volatile and more difficult to spray than organic solvents. The ion signal at  $2 \times 10^{-6}$  M tetrabutylammonium bromide in different solvents is highest for dichloromethane. At high sample concentrations ( $\geq 10^{-5}$  M) the dependence of the ion signal on spray current is lost. It appears impossible to convert a high charge on droplets into sample ions. Creation of droplets having a surface fully covered with sample is assumed to be the cause of ion signal saturation. Increasing the sample concentration will only increase the number of ions inside a droplet. The number of sample ions at the surface escaping into the gas phase is fixed and independent of sample concentration above  $10^{-5}$  M.

In analytical applications of electrospray and ionspray mass spectrometry, the most widely used solvents are water, methanol, and acetonitrile, since these solvents are the most common eluents in reversed-phase liquid chromatography (LC). When electrospray of an aqueous sample solution appears difficult, a sheath flow of methanol, ethanol, acetonitrile, 2-propanol, or 2-methoxyethanol is used to reduce the surface tension of the sample solution and thereby facilitate nebulization.<sup>1,2</sup> Electrospray, assisted by pneumatic nebulization (ionspray<sup>3</sup>), can handle aqueous sample solutions without the need for admixture of an organic solvent. The yield of ions from a sample solution is improved both in ionspray and in pure electrospray if the percentage of organic solvent in water+methanol or water+acetonitrile mixtures is increased.

Interest in other solvents suitable for electrospray mass spectrometry is growing. LC conditions (e.g., normal-phase LC) or sample solubility may require mixtures of organic solvents and some fundamental studies need to be carried out in non-aqueous solvent systems. Van Berkel and co-workers have shown the electrospray ionization of porphyrins to be possible in toluene and dichloromethane. Addition of methanol and acetic acid or trifluoroacetic acid provided an enhanced and more stable signal.<sup>4</sup> The same group has reported the combination of electrochemistry with electrospray in dry dichloromethane in the presence of trifluoroacetic acid or antimony pentafluoride<sup>5</sup> as well as an extensive study of fundamental electrochemical aspects of charged droplet formation from acetonitrile+dichloromethane.<sup>6</sup> Electrochemistry combined with electrospray ionization of fullerenes has been studied in dichloromethane solutions.<sup>7,8</sup> Toluene has been used as the solvent for the

generation of radical cations and radical anions of C<sub>60</sub> and fullerene adducts by electrospray ionization.<sup>9</sup> Henion and co-workers have used a mixture of chloroform and methanol for LC/MS of triglycerides in which electrolytes were added to promote the formation of M, Na<sup>+</sup> or M.NH<sub>4</sub><sup>+</sup> ions.<sup>10</sup> Potassium iodide, dissolved in a mixture of the aprotic polar solvents dimethylformamide and tetrahydrofuran, has been tested in electrospray mass spectrometry and was recommended for samples that decompose in the presence of protic solvents.<sup>11</sup> A number of papers have been published on the use of non-aqueous solvents and on the use of chlorinated solvents for negative-ion electrospray mass spectrometry.<sup>12–15</sup> Here, the main purpose of chlorinated solvents was the capture of electrons in order to prevent the onset of corona discharge between the spray capillary and other parts of the ion source. Guevremont *et al.* have used ethylene glycol as a solvent.<sup>16</sup> They have challenged the widely held assumption that solvent evaporation is crucial in the release of ions into the gas phase in electrospray ionization.<sup>17</sup>

In spite of the list of publications given above, there is little detailed knowledge on the effect of solvents other than water, methanol and acetonitrile on the electrospray mass spectra of simple, well-defined samples. Interestingly, in the earlier experiments on electrospray nebulization, a number of different solvents were tested: some with success, some without. Examples of suitable solvents are water, methanol, acetone, chloroform and diethyl ether, whereas toluene and benzene could not be dispersed by electrospray.<sup>18</sup> A further observation is that dynamic range is limited in electrospray mass spectrometry. When the sample concentration rises above  $10^{-5}$  M, the number of sample ions arriving at the detector of the mass spectrometer remains nearly constant.<sup>19,20</sup> We have observed that the dynamic range is somewhat wider in methanol than in acetonitrile.<sup>20</sup>

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Electrospray mass spectrometry can be broken up into three steps: dispersion of a sample solution into electrically charged droplets, release of ions from droplets, and transport of ions from the atmospheric pressure ionization source into the vacuum system and mass analyzer. In principle, dynamic range limitation could take place in one or more of these steps. Ion transport into the vacuum, however, has been ruled out as the limiting factor.<sup>21</sup> There is controversy about the role of electrolyte concentration in sample solutions and about the limiting effect of droplet charging. Kebarle and Tang take the viewpoint that a linear range of ion signal versus sample concentration can be observed if excess background electrolyte is present in the sample solution, while the upper limit to the ion signal can be attributed to reduced efficiency of droplet charging.<sup>22</sup>

For the majority of applications in LC/MS and protein chemistry, formation of charged droplets takes place by electrochemical removal of counter ions.<sup>23</sup> Electrospray is a special case of an electrolysis cell.<sup>6,23</sup> The amount of positive charge on droplets can be measured as the current due to negative ion removal when droplets leave the sprayer, or as the current collected when droplets are discharged against a counter electrode. Electrospray current is a function of conductivity of the solution fed into the electro-sprayer:

$$I_{\text{spray}} = \text{constant (conductivity)}^n, \quad \text{where } n < 1$$

Conductivity is a function of total electrolyte concentration. All electrolytes contribute to conductivity: acids, bases and salts present in the eluent from a liquid chromatograph in LC/MS, electrolytes present as impurities in solvents and the sample, if present as ions in solution. In most analytical applications of electrospray ionization, the sample is present in a much lower concentration than other electrolytes that have been added to promote ionization of the sample or to improve the separation in an LC column. Under such conditions, conductivity and spray current are independent of sample concentration. Solutions of samples in very clean solvents on the other hand do show a dependence of conductivity and spray current on sample concentration.<sup>20,21</sup> Kebarle and Tang have compared spray current measurements with ion signals for samples dissolved in reagent grade methanol.<sup>22</sup> At low sample concentration ( $<10^{-5}$  M), impurities in reagent grade methanol (e.g. sodium salts) are present at a higher concentration than the sample. Conductivity of a sample solution in reagent grade methanol is largely due to impurity electrolytes, so that the spray current is independent of sample concentration. Above approximately  $10^{-5}$  M sample concentration, the sample becomes the dominant electrolyte. Conductivity increases with increasing sample concentration and the spray current rises weakly. The conclusion drawn by Kebarle is that sample ion signal is proportional to sample concentration as long as other electrolytes dominate in solution, which is the case up to  $10^{-5}$  M sample concentration in reagent grade methanol. In this low sample concentration, high background electrolyte concentration regime the spray current is constant. As the fraction of sample ions increases, the sample ions can take a proportionately increasing share of the charge on a droplet, and generate a signal proportional to sample concentration. Above  $10^{-5}$  M sample concentration, the charge on droplets increases only weakly with increasing sample concentration. In this regime sample ions dominate in solution. Charge on droplets is almost constant and nearly independent of sample concentration in Kebarle's experiment, so that the amount of charge that can be turned into

sample ions that leave droplets increases only slightly with increasing sample concentration. As a result, the sample ion signal remains at a constant level, in spite of an increase of sample concentration. In short, the upper limit of linear dynamic range is determined by the level of background electrolytes in solution and is, in fact, due to a limited amount of charge available on a droplet.<sup>22</sup> One would expect that dynamic range would be even more limited if the concentration of background electrolyte were to be reduced. When working with very clean solvents, using rigorously cleaned hardware, we were not able to confirm this prediction. We found the linear dynamic range to be still limited to  $10^{-5}$  M sample concentration. We have not been able to correlate the upper limit of the sample ion signal to a limit in droplet charging since, in our experiments, spray current keeps rising significantly when the ion signal abruptly reaches its upper limit.<sup>20,21</sup>

Experiments presented in this paper are aimed at the investigation of sensitivity, dynamic range and droplet charging in different solvents. As mentioned above, experiments in different solvents published thus far have been done by other authors with solutions of peptides and proteins or other smaller molecules in the presence of acids or other electrolytes. Solutions of samples plus electrolytes are too complex for a meaningful comparison of ion abundances and spray currents. Quaternary ammonium salts are soluble in water and in a wide range of organic solvents and do not require the addition of acids or bases in order to generate ions in solution. Furthermore, quaternary ammonium ions can neither be formed nor be lost by gas-phase proton transfer reactions, that might interfere with measurement of the abundance of ions released from charged droplets in electrospray ionization.

## EXPERIMENTAL

### Chemicals

Tetrabutylammonium bromide (TBABr) (puriss., >99%) was from Fluka (Buchs, Switzerland). Stock solutions ( $10^{-2}$  M and  $10^{-3}$  M) were prepared in methanol. The  $10^{-3}$  M stock solution in methanol was diluted with the desired solvent to give sample solutions containing  $1 \times 10^{-6}$  M,  $2 \times 10^{-6}$  M and  $5 \times 10^{-6}$  M TBABr. The  $10^{-2}$  M stock solution in methanol was diluted with the desired solvent to give sample solutions containing  $1 \times 10^{-5}$  M,  $2 \times 10^{-5}$  M and  $5 \times 10^{-5}$  M TBABr.

Solvents were purchased from Merck (Darmstadt, Germany). Methanol and acetonitrile were gradient grade; chloroform, dichloromethane and toluene were analytical grade. Water was taken from a MilliQ purification system.

### Liquid delivery

A Familic-100N micro syringe pump (Jasco, Hachioji City, Tokyo, Japan) equipped with a Rheodyne (Cotati, CA, USA) 7010 injector was used for liquid delivery. The pump syringe, sample syringe, sample loop, injector rotor and other hardware that is in contact with sample solutions were cleaned by repeated sonication in gradient grade methanol. The solvents and the sample solutions were degassed before use by sonication for 15 min and the liquid delivery system was flushed for ca. 1 h with the sonicated solvent before analysis. The solvent in the syringe pump was identical to the solvent of the sample solutions: methanol solutions were injected into a methanol stream, chloroform solutions into a stream of chloroform, etc. Blank injections were made to

check the cleanliness of the sample loop, syringes and sample vials. A flow rate of 10  $\mu\text{L}/\text{min}$  combined with sample injection from a 20  $\mu\text{L}$  loop caused 2 min-wide flat-topped peaks, broad enough to show the stability of the system and minimize errors in ion signal and spray-current measurements. Gas-tight syringes having a smooth glass barrel and a teflon-tipped plunger were used for sample handling. By exercising extreme cleanliness as described here, it is possible to record reproducible spray currents for very dilute sample solutions.

As an exception, the samples in toluene were not introduced by loop injection but by infusion from a teflon tube (1 m long, 0.3 mm i.d.) connected to a sprayer made of 0.3 mm o.d., 0.15 mm i.d. platinum capillary, since TBABr dissolved in toluene adsorbed strongly on the walls of the stainless steel sample loop, the injector rotor and fused silica transfer line.

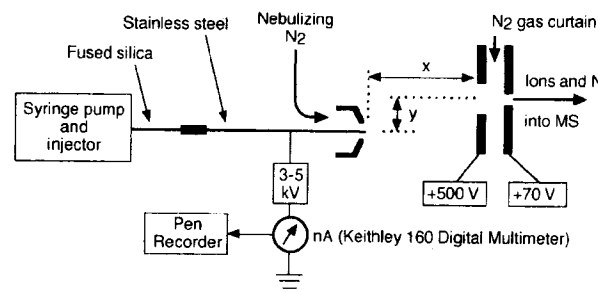
### Mass spectrometer and spray system

The instrument was a Nermag (Argenteuil, France) R 3010 triple quadrupole mass spectrometer equipped with a custom-made atmospheric pressure ionization (API) source and spray-current measurement system, described in detail earlier.<sup>20,21</sup> Dry nitrogen was used as the curtain gas. The mass spectrometer was scanned from  $m/z$  70 to 600 at a rate of 7 s/scan.

The ionspray interface used is also described in the earlier study. Stainless steel or platinum capillary tubes (10 cm long, 0.3 mm o.d., 0.15 mm i.d.) were used as sprayers protruding 0.5 mm out from the polypropylene nebulizer tip. Nitrogen (99.8%) was used as the nebulizing gas and the gas pressure was adjusted to 3 bar. The sample injector was connected to the sprayer by means of fused silica capillary (1 m long, 50  $\mu\text{m}$  ID). The tip voltage of 3 kV was used for all solvents except for toluene, for which best sensitivity was achieved at 4.4 kV. The position of the sprayer was adjusted to achieve maximum sensitivity and stability for each solvent (see Fig. 1 and Table 1).

## RESULTS

The spray currents measured when neat solvents are nebulized by pneumatically assisted electrospray are given in Table 1. A high spray current reflects a high level of



**Figure 1.** Schematic diagram of sample and solvent delivery system, off-axis ionspray nebulizer, ion sampling orifice (at +70 V) with gas curtain protection, and ionspray power supply with spray-current measurement. The skimmer behind the ion-sampling orifice (not shown) is at +25 V.

background electrolytes in the solvent or electrolytic decomposition of the solvent. The latter process may play a role in water. Spray currents for neat dichloromethane and toluene were below 1 nA. The mass-analyzed ion abundances recorded at the detector of the mass spectrometer are given in Fig. 2 for the tetrabutylammonium ion and for the cluster of the tetrabutylammonium ion with neutral tetrabutylammonium bromide. Above  $10^{-5}\text{ M}$  sample concentration, the ion signals for the tetrabutylammonium ion converge to the same abundance level for all solvents except toluene and water. The abundance of the cluster ion  $\text{TBA}^+\cdot\text{TBABr}$  keeps rising with increasing sample concentration, while the abundance of the  $\text{TBA}^+$  ion has reached a plateau. The cluster ion abundance is strongly dependent on solvent, but does not rise above 10% of the abundance of the tetrabutylammonium ion for any of the solvents, except toluene. The relative abundance of the cluster ion  $\text{TBA}^+\cdot\text{TBABr}$  in ionspray ionization from toluene solution is remarkably high (approximately 25% at the highest sample concentrations), while the absolute abundances of the  $\text{TBA}^+$  ion and the cluster  $\text{TBA}^+\cdot\text{TBABr}$  are the lowest in the case of toluene. The cluster ion abundances in chloroform and dichloromethane are very similar. Solutions in methanol and acetonitrile also show nearly identical abundances of the cluster ion. Water as a solvent gives rise to low absolute abundances of both the  $\text{TBA}^+$  ion and the cluster  $\text{TBA}^+\cdot\text{TBABr}$  while the relative abundance of this cluster ion is lowest for water when compared with all other solvents.

The spray current as a function of sample concentration is

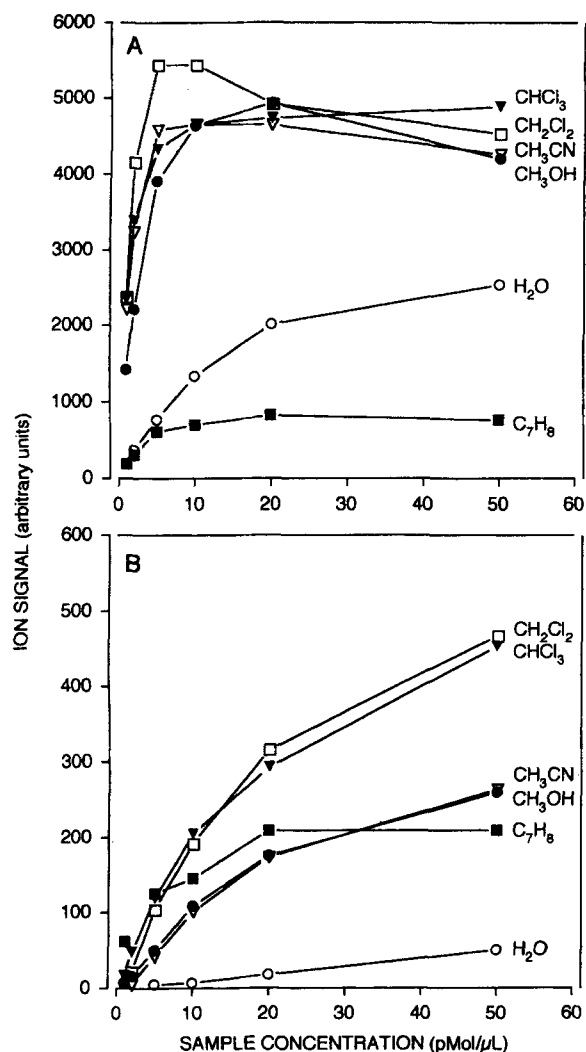
**Table 1. Properties of solvents<sup>a</sup>**

Solvent	H <sub>2</sub> O	CH <sub>3</sub> OH	CH <sub>3</sub> CN	CHCl <sub>3</sub>	CH <sub>2</sub> Cl <sub>2</sub>	Toluene
Sprayer position <sup>b</sup>						
x (cm)	2.1	4.6	3.8	2.9	2.8	4.1
y (cm)	3.1	2.2	1.8	1.3	1.3	1.8
Spray current from background (nA)	38	28	11	1	0	0
Polarity index <sup>c</sup>	9.0	6.6	6.2	4.4	3.4	2.3
Proton donor <sup>c</sup>	0.40	0.51	0.33	0.28	0.34	0.32
Proton acceptor <sup>c</sup>	0.34	0.19	0.26	0.39	0.17	0.24
Dipole interactor <sup>c</sup>	0.26	0.30	0.41	0.33	0.49	0.44
Dielectric constant	78	33	38	4.8	9.1	2.4
Dipole moment in gas phase (Debye units)	1.85	1.70	3.92	1.04	1.60	0.37
Polarizability ( $10^{-24}\text{ cm}^3$ )	1.45	3.3	4.4	9.5	7	12.3
Surface tension ( $\text{mNm}^{-1}$ )	73	22	29	27	27	28
Viscosity (mPa.s)	1.00	0.55	0.34	0.58	0.44	0.59
Enthalpy of evaporation at 25 °C						
kJ/Mole	44	37	33	31	29	37
kJ/mL	2.4	0.91	0.64	0.39	0.45	0.35

<sup>a</sup> Majority of data taken from the CRC Handbook of Chemistry and Physics, 72nd Edition (CRC Press, Boca Raton).

<sup>b</sup> See Fig. 1.

<sup>c</sup> See Ref. 24.

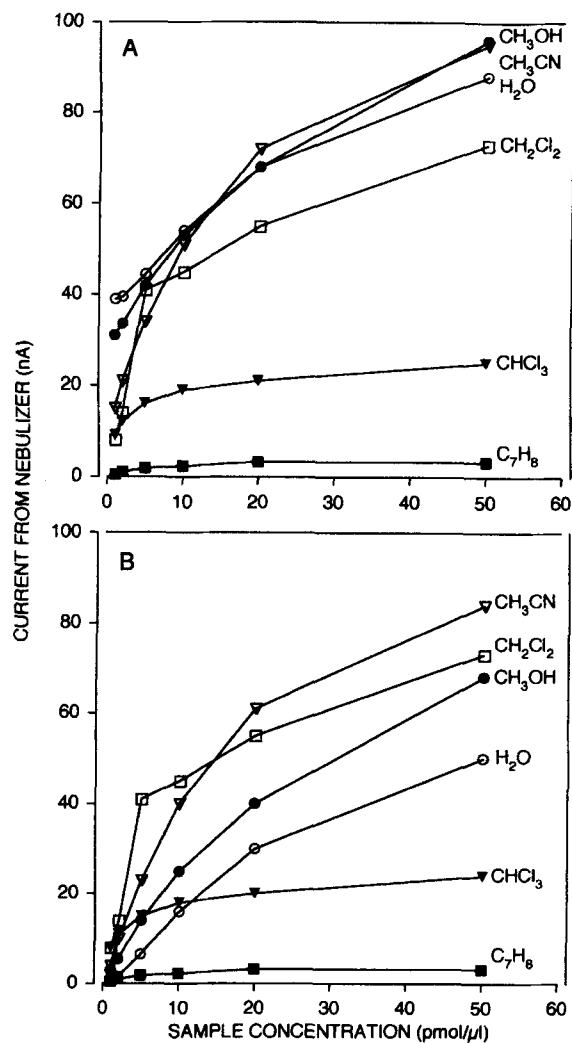


**Figure 2.** Mass analyzed ion signals at the detector of the mass spectrometer as a function of concentration of tetrabutylammonium bromide in different solvents; (a) signal of the tetrabutylammonium ion at  $m/z$  242; (b) signal of the isotopomer at  $m/z$  563 of the cluster ion of tetrabutylammonium with tetrabutylammonium bromide. Signals in arbitrary units of the data system; note the expanded signal scale in (b). Solvents used:  $\circ$  water,  $\bullet$  methanol,  $\nabla$  acetonitrile,  $\blacktriangledown$  chloroform,  $\square$  methylene chloride,  $\blacksquare$  toluene.

presented in Fig. 3. Figure 3(a) displays the total spray currents due to background plus sample at different sample concentrations. In Fig. 3(b) the spray currents are shown as increments above the background current level recorded upon injection of a sample. Above  $10^{-5}$  M the spray currents keep rising with increasing sample concentration for all solvents except toluene and chloroform.

The ion signals and spray current increments at low concentration ( $<5 \times 10^{-6}$  M) are difficult to read from Fig. 2. Ion signals and spray current increments obtained at  $2 \times 10^{-6}$  M in different solvents are given in Fig. 4. The data shown in Fig. 4 are indicative of ionspray behaviour for different solvents well below the concentration level where ion signal saturation sets in. A close correlation appears to exist between spray current increment and sample ion abundance at low sample concentration.

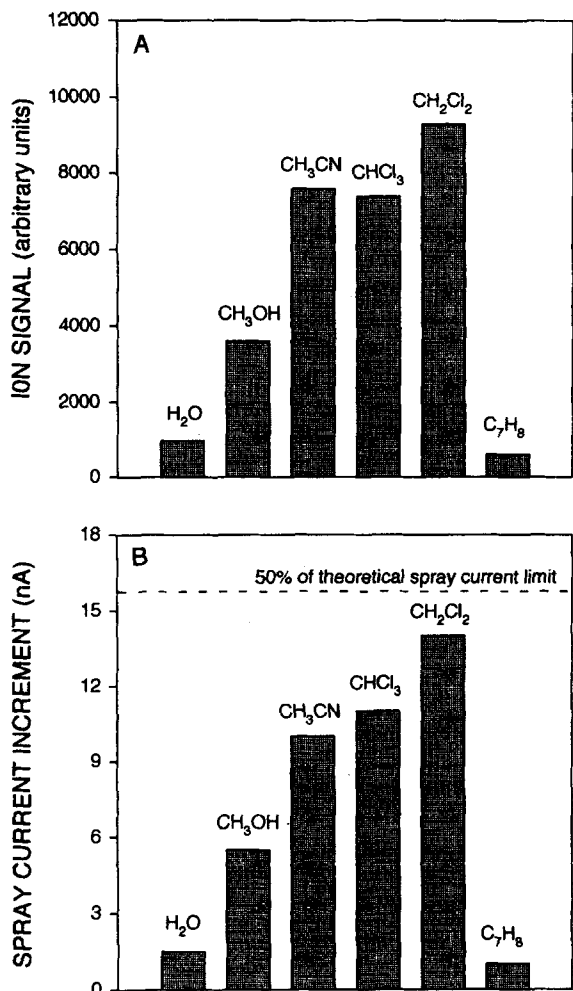
Droplet charging by complete removal of bromide ions from a  $2 \times 10^{-6}$  M TBABr solution introduced at  $10 \mu\text{L}/\text{min}$  would theoretically be measured as a 32 nA spray current increment. In the case of methylene chloride as solvent (Fig. 4(b)) the real spray current increment is approximately 44% of the theoretically expected value.



**Figure 3.** Spray current from the ionspray nebulizer as a function of concentration of tetrabutylammonium bromide in different solvents. (a) Total spray current from sample plus background electrolytes in solvents; (b) spray current increment recorded upon injection of a sample. Solvents used:  $\circ$  water,  $\bullet$  methanol,  $\nabla$  acetonitrile,  $\blacktriangledown$  chloroform,  $\square$  methylene chloride,  $\blacksquare$  toluene.

## DISCUSSION

Since droplet charging in electro-spray nebulization is dependent on the presence of charge carriers in solution, the spraying of very pure or less polar solvents may be difficult or even impossible.<sup>18,22</sup> In pneumatically assisted electro-spray (ionspray) a high velocity gas stream produces the aerosol, and the generation of a stable spray is not dependent on a sufficient number of charge carriers in solution. For example the spray current recorded for ionspray of neat chloroform, dichloromethane, or toluene is  $\leq 1$  nA, so that the droplets can be considered to be uncharged. Since the solvent front emerging from the pneumatic sprayer is exposed to a high electric field, removal of counter ions from solution at the sprayer tip takes place by the same electrochemical processes as in 'pure' electro-spray. Earlier studies have revealed that 'pure' electro-spray and pneumatically-assisted electro-spray have essentially identical characteristics as far as the generation of sample ions from the liquid phase is concerned, but show differences in ruggedness with respect to 'difficult' solvents and liquid flow rates. Because pneumatically-assisted electro-spray does not require such a high potential at the tip of the spray capillary as 'pure' electro-spray, there is less



**Figure 4.** Simultaneous measurement of detector signal and spray currents at low sample concentration; (a) mass analyzed ion signals of the tetrabutylammonium ion at  $m/z$  242 at the detector of the mass spectrometer for  $2 \times 10^{-6}$  M tetrabutylammonium bromide solutions in different solvents; (b) spray current from the ionspray nebulizer for  $2 \times 10^{-6}$  M tetrabutylammonium bromide solutions in different solvents.

chance of a corona discharge from the spray capillary, so that reliable spray current measurements can be made in the low nA range.

The sample ion abundance at high sample concentration is identical for four out of the six solvents. One might conclude that an upper limit exists to the number of sample ions that can be transported into the mass analyzer. In an earlier paper however, we have ruled out this explanation for the upper limit in dynamic range.<sup>21</sup>

Spray current and ion abundance show a clearly different relationship with sample concentration. At high sample concentration, an increasing amount of droplet charge cannot be converted into tetrabutylammonium ions. The 'missing charge' is converted, in part, into the emission of the cluster ion  $TBA^+ \cdot TBABr$ . The abundance of this latter ion keeps rising with increasing sample concentration while the abundance of  $TBA^+$  remains at a constant level. The amount of charge converted into the cluster ion is, however, much smaller than would be expected from the spray current measurement.

We have proposed that the upper limit of the dynamic range may be due to limited space on the surface of offspring droplets separated from the primary aerosol droplets.<sup>21</sup> In a simple calculation, the number of sample ions plus sample ion pairs in a  $1 \mu\text{m}$  radius droplet at

$10^{-5}$  M concentration is 24 000. Let us assume that, in a simplified sequence of events, a number of  $0.1 \mu\text{m}$  radius offspring droplets are formed, which shrink by evaporation to a radius of  $0.01 \mu\text{m}$  ( $10 \text{ nm}$ ), and that release of ions takes place from the latter  $10 \text{ nm}$  radius droplets. We have neglected evaporation of solvent from the primary aerosol droplet which leads to a limited size reduction, but otherwise this simplification differs little from earlier models.<sup>22</sup> It is further assumed that sample ions and ion pairs reside on the surface of the primary droplets. The offspring droplets are formed from protrusions on the surface of primary droplets. The sample ion and ion pair density on the surface of an offspring droplet may be taken to be almost identical to the density on the primary droplet, since the total droplet surface area of primary and offspring droplets increases only slightly when an offspring droplet separates from the primary droplet. When the offspring droplets shrink by evaporation of solvent, the surface density increases rapidly. The calculated area available to a sample ion or sample molecule in different stages is presented in Table 2. The bottom line is that the droplet surface is indeed almost full at  $10^{-5}$  M after droplet disintegration followed by solvent evaporation.

At a high enough sample concentration, the sample ion abundance for solutions in four solvents is almost identical. Water and toluene behave differently. There is no clear relationship between spray current (i.e. droplet charging efficiency) and ion signal abundance.

In order to try and correlate signal ion abundance with solvent properties we have compiled physical parameters in Table 1. Polarity index, proton donor, proton acceptor and dipole interactor values were derived and ordered on a logarithmic scale by Snyder with the aim of classifying solvents for use in liquid chromatography.<sup>24</sup> There is no apparent relationship between this classification and the sample ion signal in the high concentration range. Nor is there a relationship between sample ion abundance and dielectric constant, dipole moment or polarizability.

Surface tension and heat of evaporation per unit volume (mL) are very similar for methanol, acetonitrile, chloroform, dichloromethane and toluene. Droplet size reduction by disintegration and solvent evaporation, with increase of sample density on a droplet surface are controlled by surface tension and heat of evaporation, and droplet charge. In the case of high sample concentration in methanol, acetonitrile, chloroform or dichloromethane, the size reduction and increase of surface density of sample ions plus

**Table 2.** Area available on a droplet surface at  $10^{-5}$  M sample concentration

<b>Radius of initially formed droplet</b>	$r = 1 \mu\text{m}$
Number of sample ions plus sample molecules	24 000
Area available per sample ion or molecule	$500 \text{ nm}^2$
<b>After fission to</b>	$r = 0.1 \mu\text{m}$
Surface density equal to parent droplet	
Area available per sample ion or molecule	$500 \text{ nm}^2$
Number of sample ions plus sample molecules	240
<b>After size reduction by evaporation to</b>	$r = 10 \text{ nm}$
Area available per sample ion or molecule	$5 \text{ nm}^2$
<b>Radius of average organic ion or molecule</b> (C—C bond length $0.15 \text{ nm}$ )	$\sim 1 \text{ nm}$
Area taken by one ion or molecule	$\sim 3 \text{ nm}^2$

molecules is apparently identical for these four solvents, as reflected by an identical sample ion abundance. One may conclude that droplet charge does not play a role, since sample ion abundance does not show a correlation with spray current (droplet charge).

Droplet charging appears to have its effect if too little charge is imposed. Toluene behaves differently from the other four organic solvents, a fact which can be attributed to the low droplet charging efficiency (very low spray current) in electrospray of toluene solutions of tetrabutylammonium bromide. In the case of water, high surface tension and high heat of evaporation resist disintegration and evaporation. As a result, the offspring water droplets are relatively large, having a lower surface density of sample ions and sample molecules, so that complete coverage of droplet surface has not taken place at the highest concentration measured in our experiments. In this case, the sample ion signal shows a steady increase up to at least  $5 \times 10^{-5}$  M.

A crowded droplet surface at high sample concentration can also explain the abundance of the cluster ion  $TBA^+ TBABr$ . The relative abundance of this ion is lowest in the case of water and is high for those solvents, chloroform and dichloromethane, that have a low surface tension and low heat of vaporization, thus producing the smallest droplets with the highest density of sample on the surface. It is the latter two solvents that show the setting in of a limitation of dynamic range at the lowest sample concentration (see Fig. 2).

At low sample concentrations, the picture is completely different. There is no consistent relationship between sample ion abundance and surface tension, viscosity or heat of evaporation. The correlation between sample ion abundance and spray current increment for all solvents, including toluene, is striking. At low concentration there is enough space at the surface of disintegrated droplets, even after size reduction by solvent evaporation. Correlation with spray current increment implies correlation with the number of bromide ions removed from solution upon charged droplet generation. In the case of methylene chloride for example, spray current increment is 44% of the value corresponding with complete removal of bromide ions, i.e. 44% of the bromide ions have been removed from solution. As a result 44% of the tetrabutylammonium ions are 'free', while 56% are paired with bromide ions inside charged droplets. Thus, gas-phase ion formation from droplets of all six solvents at low sample concentration is controlled by the number of free tetrabutylammonium ions, i.e. not paired by bromide ions, in the droplets. It is surprising that, at low sample concentration, the effects of surface tension and heat of evaporation are not borne out in the efficiency of formation of gas-phase analyte ions: acetonitrile requires more heat for evaporation than chloroform, yet the sample ion abundance at  $2 \times 10^{-6}$  M is nearly identical for both solvents. One would expect that ionization from water would be much less efficient, considering its relatively high resistance towards disintegration and evaporation. Nevertheless, the effect of removal of bromide ions overrides the anticipated effects of relative ease of reducing droplet size of the solvents studied. Apparently a free, unpaired tetrabutylammonium ion has such a high tendency to escape from a droplet, that it is not held back by unfavorable conditions such as relatively large size of droplet or efficient ion solvation. Alternatively, one may argue that droplet disintegration and solvent evaporation are so fast and efficient for all solvents, including water, that the rate of release of sample ions from droplets is not limited by these

two factors.

In discussions about solvent effects in electrospray mass spectrometry the relatively poor sensitivity for analytes dissolved in water is attributed to high surface tension, low volatility and efficient solvation of ions in water. Relatively high sensitivity for analytes dissolved in organic solvents is attributed to low surface tension, higher volatility and less efficient solvation of ions in organic solvents. The data for the low concentration regime presented in this paper show that efficiency of removal of counter ions overrides solvent properties. A question to be answered now is which solvent properties are responsible for efficient electrochemical removal of counter ions, and which properties help to stabilize the unpaired quaternary ammonium ion in solution when a droplet is separated from the spray capillary. Unfortunately, almost no data are available for solvation of quaternary ammonium ions in nonaqueous solutions.<sup>25</sup>

If the sample concentration dependence of the ion signal at  $m/z$  242 is combined with the reasonable approximate figures in Table 2, it is also reasonable to assume that an offspring droplet has to shrink to a radius less than 10 nm to make ion evaporation of a sample ion or the release of a nanodroplet from the surface of the offspring droplet possible. In this picture, a nanodroplet is supposed to contain one sample ion that is set free after evaporation of the remaining solvent molecules. Above  $10^{-5}$  M it is not possible (at least under the assumptions adopted in Table 2) to let droplets shrink to a radius much smaller than 10 nm without having some sample ions inside an offspring droplet rather than at the surface of such a droplet. Sample ions inside a droplet are apparently lost, in the sense that they are not available for ion evaporation or release in a nanodroplet.

In conclusion: at low sample concentration, evaporation and disintegration of droplets probably proceed until ion evaporation takes place or nanodroplets containing one sample ion leave the surface of a droplet having a radius of 10 nm or less. Sample ion signal is proportional to the number of free sample ions in solution at low concentration (below  $5 \times 10^{-6}$  M). Properties of solvents such as heat of evaporation, surface tension and viscosity do not appear to play a role at low sample concentration. At high sample concentration (above  $10^{-5}$  M) droplets cannot shrink to a small enough radius without reaching the condition of a droplet surface fully covered with sample ions and sample-ion/counter-ion pairs; an increasing number of free sample ions remain under the droplet surface and sample signal cannot increase since the number of sample ions at the droplet surface has reached an upper limit. The data presented in this communication have been obtained with very clean solutions of a very simple sample. It remains to be seen whether extrapolation to more complicated systems such as a solution of a protein in a solvent containing acids and other electrolytes can be justified.

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