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Review

Mechanistic aspects of electrospray ionization

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Abstract

Electrospray ionization (ESI) mass spectrometry can be divided into three steps: Nebulization of a sample solution into electrically charged droplets, liberation of ions from droplets, and transportation of ions from the atmospheric pressure ionization source region into the vacuum and mass analyzer of the mass spectrometer. A sample solution is fed through a capillary tube and a high electric field at the tip of the tube pulls positive charge towards the liquid front. When electrostatic repulsion becomes stronger than the surface tension, a small electrically charged droplet leaves the surface and travels through the surrounding gas to the counter-electrode. Under the majority of experimental liquid chromatography–mass spectrometry and capillary electrophoresis–mass spectrometry conditions, positive charge on droplets is generated by the removal of negative charge via electrochemical discharge of negative ions against the metal wall of the spray capillary. When the ESI source is set up for the detection of negative ions, all power supplies are at reversed polarity. Removal of positive ions inside the tip of the spray capillary provides droplets depleted of positive charge. The supply of negative charge to the solution may also take place; electrons released from the spray capillary can be captured by sample molecules having a high electron affinity. Droplet size decreases and charge density at the droplet surface increases after droplet disintegration and solvent evaporation. When the electric field at the surface of a droplet has become sufficiently high, ions are emitted from the droplet surface into the surrounding gas and are sampled by the mass analyzer. Sample ion intensity is dependent on ion structure and is affected by solvent composition and presence of additives. ESI behaves as a concentration sensitive detector for chromatography. When the sample concentration is increased above 10 μM, the sample ion signal saturates, which can be explained by the assumption that the surface of ion-emitting droplets is full at 10 μM. Sample ion abundance over a wide m/z range is further affected by inherently mass-dependent efficiencies of ion transportation, ion separation and ion detection.

Keywords: Reviews; Electrospray ionization; Mass spectrometry

Contents

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1. Introduction

Electrospray ionization (ESI) has become one of the most important ionization techniques for the on-line coupling of liquid phase separation methods with mass spectrometry (MS). It is a simple and elegant method that handles small and big molecules, operates at atmospheric pressure and at a moderate temperature, and is probably the most gentle ionization technique available for MS.

During the history of development of liquid chromatography (LC)–MS coupling, emphasis was put on the different designs of interface between the liquid chromatograph and the ionization technique [1]. For example, the moving belt and particle beam systems are interfaces between the LC and the electron impact and chemical ionization sources. Direct liquid introduction is an interface for a chemical ionization source. The thermospray nebulizer is an interface for electron beam- or electric discharge-induced chemical ionization. The heated pneumatic nebulizer is the interface for LC–MS with atmospheric pressure chemical ionization.

Three LC–MS techniques can be considered as ionization techniques where the ''interface'' is an integral part of the system: Filament-off thermospray, continuous flow fast atom bombardment (FAB) or secondary-ion mass spectrometry (SIMS), and electrospray. Continuous flow matrix assisted laser desorption ionization (MALDI) may become the fourth LC–MS technique of this kind [2].

ESI–MS can be divided into three steps: Nebulization of a sample solution into electrically charged droplets, liberation of ions from droplets, and transportation of ions from the atmospheric pressure ionization source region into the vacuum and mass analyzer of the mass spectrometer.

2. Nebulization

A detailed study of the formation of a mist of fine droplets through the exposure of a liquid to a high electric field was published by Zeleny [3]. Different shapes of sprays at various spray voltages were documented by high speed photography. Renewed scientific interest in electrospray resulted in a series of publications starting in 1952 [4–6]. The theory and applications of electrospray nebulization have been summarized in books [7,8] and in a special issue of the Journal of Aerosol Science [9].

Electrospray nebulization in its simplest form is presented in Fig. 1a. A sample solution is fed through a capillary tube and a high electric field at the tip of the tube pulls positive charge towards the liquid front. When electrostatic repulsion becomes stronger than the surface tension, a small electrically charged droplet leaves the surface and travels through the surrounding gas to the counter-electrode. In Fig. 1a, the capillary is at a more positive potential than the counter-electrode. It is a matter of design or particular constraints whether the spray

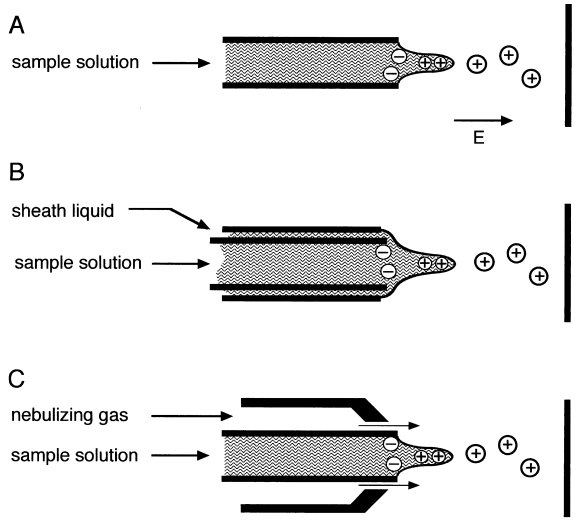


Fig. 1. Nebulization by electrospray. (a) Pure electrospray of a sample solution; (b) electrospray of a sample solution mixed with a sheath liquid; (c) electrospray of a sample solution with assistance by pneumatic nebulization. Reproduced from ref. [39] with permission.

capillary is at a high voltage or at ground potential. A reversal of the electric field in Fig. 1a will result in the production of negatively charged droplets.

Electrospray is the dispersion of a liquid into electrically charged droplets and, as such, combines two processes; droplet formation and droplet charging. The formation of small, micrometer-sized droplets does not present a problem if the liquid's flow-rate, surface tension and electrolyte concentration are low. An increase in one or more of these variables makes it more difficult for the electric field to produce the desired charged aerosol for MS. The electric field strength at the sprayer tip can be increased to try and overcome the adverse effects of the aforementioned three variables, but too high an electric field will give rise to an electric discharge that accompanies the electrospray process. A discharge can be tolerated in some electrospray nebulization applications, but is detrimental in electrospray mass spectrometry. Electric discharge is particularly troublesome in the formation of negatively charged droplets. In the negative-ion mode, the sprayer tip is at a high negative potential with respect to other parts of the source, and field emission of electrons from the sharp spray needle or from the sharp tip of the solvent front (the Taylor cone, [7–9]) is a facile process. Electrons are accelerated by the electric field between the sprayer and the surrounding source walls and ionize the mixture of gases and solvent vapours in the source. The electric discharge can be quenched by the capture of electrons by means of an electron-scavenging gas, such as oxygen [10], a freon or the vapour of a chlorinated solvent [11].

Modifications to the simple electrospray system, as shown in Fig. 1, are aimed at increasing tolerance towards the adverse effects of high liquid flow-rate, high surface tension and high electrolyte concentration. Dilution of an aqueous solution with an organic solvent reduces the surface tension. Coaxial addition of a sheath flow of methanol, acetonitrile, ethanol, isopropanol or 2-methoxyethanol to the sample solution at the tip of the spray capillary was first used for the combination of capillary electrophoresis with electrospray MS and later also used for sample infusion and liquid chromatographic coupling with electrospray MS [12,13]. In sheath flow-assisted electrospray, it is still the electric field alone that has to disperse and charge the liquid in one operation.

The assistance of a high velocity gas flow is used in electrospray MS [14]. In a simple approximation, the pneumatic nebulizer takes care of aerosol formation, while the electric field charges the droplet. When compared with ''pure'' electrospray, pneumatically assisted electrospray can handle aqueous solutions and higher flow-rates without the need for critical adjustment and can be operated at a lower field strength so that electric discharge is eliminated. Ultrasonic assistance offers the same advantages, but is a more complex and more expensive combination of mechanical and electronic devices [15,16]. Trade names are IonSpray (SCIEX) and Ultraspray (Analytica of Branford).

The electric field, E_c, at the tip of an electrospray capillary can be calculated [17] using the equation

$$E_c = \frac{V_c}{r_c \ln(4d/r_c)} \quad (1)$$

where V_c is the voltage difference between the spray capillary and its counter-electrode, r_c is the radius of the spray capillary and d is the distance between the spray capillary and its counter-electrode.

In spite of higher voltages quoted in the literature for pneumatically assisted electrospray (IonSpray) compared with pure electrospray, the electric field at the tip of the sprayer is lower because the distance from the opposing electrode (called the interface plate or curtain plate) of the ion source is longer and the outer radius of the spray capillary is larger than in pure electrospray ion sources.

A further advantage of pneumatically assisted electrospray is the freedom of positioning the sprayer inside the ion source, since the formation and direction of the spray are controlled by the high velocity gas flow and not by the electric field at the tip of the sprayer. By positioning the sprayer diagonally [18] or at right angles [19,20] with respect to the source axis, stability of operation is improved and penetration of droplets and contaminants into the vacuum system is reduced. Most manufacturers have resorted to pneumatic assistance for ESI-MS.

3. Droplet charging

Fig. 1 is laid out for the formation of positively

charged droplets. From a macroscopic viewpoint, it is sufficient to assume excess positive charge to be present in the liquid front. From a chemical viewpoint, it is necessary to define the mechanism of charged droplet formation and its relationship to the composition of the sample solution, which is, in turn, strongly dependent on the composition of the eluent used for the high-performance liquid chromatographic separation.

Does positive charge on a droplet imply that positive charge was indeed supplied to the liquid front? The supply of positive charge is possible if a metal spray capillary gradually dissolves, with concomitant formation of metal ions. Although this process can indeed take place, it is of minor importance in the practice of on-line LC–MS or CE–MS [21].

Under the majority of experimental LC–MS and CE–MS conditions, positive charge on droplets is generated by the removal of negative charge via electrochemical discharge of negative ions against the metal wall of the spray capillary. Under special conditions, electrons can be removed from sample molecules having a very low ionization energy, for example, porphyrins [22] and polycyclic aromatic hydrocarbons [23]. When positively charged droplets hit the opposite plate in Fig. 1, electrons are consumed to neutralize the positive ions in the droplet. As such, the ESI source is a special case of an electrolysis cell [17]. The electrolytic nature of electrospray has been studied extensively by Van Berkel and Zhou [24], and results have been compiled in a recent review [25].

Fig. 2a shows how electrospray current flows and how it can be measured. In Fig. 2b, the current readout of one of the power supplies is used. The current delivered by the electrospray power supply is composed of the real spray current, due to discharge of negative ions, plus the current flowing through the sample solution back into the grounded parts of the high-performance liquid chromatography (HPLC) system. The real spray current at 5 μl/min varies from approximately 20 nA, for a 1-μ*M* concentration of a sample in a clean solvent, to a few hundred nA, for a sample in a solution containing other electrolytes at the millimolar concentration level in a real LC–MS experiment. The current leaking back into the HPLC system through the eluent is dependent on

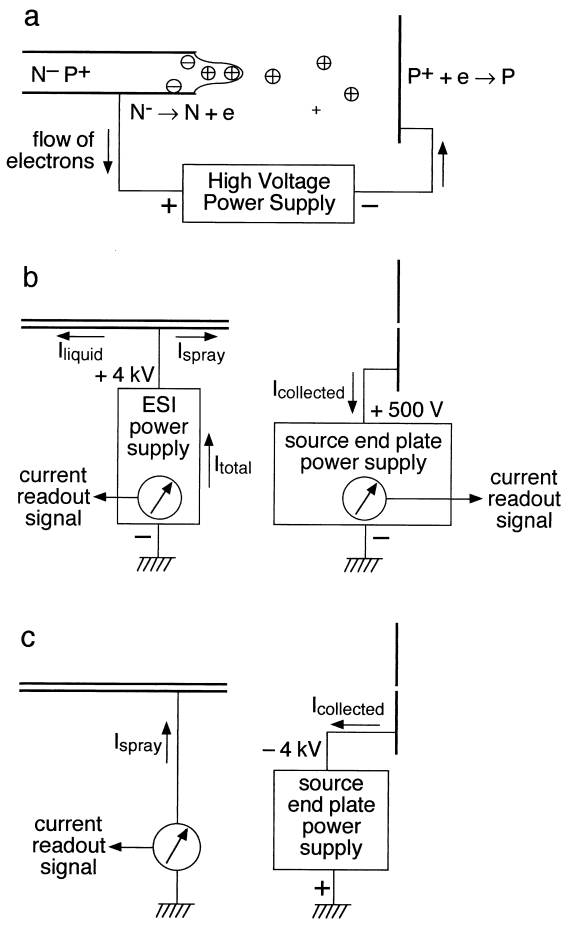


Fig. 2. Droplet charging and spray current measurement. (a) Electrospray as a special case of an electrolysis cell; (b) measurement of currents flowing through power supplies connected to the spray capillary and the source end plate; (c) spray current measurement with the spray capillary at ground potential.

conductivity and can be substantial. We have observed approximately 10 μA for 0.1% trifluoroacetic acid (TFA) in 100% water.

Measurement of the current flowing through the source end plate power supply in Fig. 2b is free from contributions from current leaking through the eluent. The current arriving at the end plate is dependent on the positioning of the sprayer inside the source, since some of the charged droplets may arrive at other walls of the source and never reach the end plate. Spray current measurement is very easy in Fig. 2c where the sprayer is at ground potential while the source end plate is at a high

negative voltage. This voltage arrangement is customary in Analytica of Branford sources on quadrupole mass spectrometers.

Spray current measurement is not usually done with the aim of following electrochemical discharge of ions against the wall of the spray capillary. In practice, the spray current readout provides a means of detecting the onset of an unwanted corona discharge from the sprayer when the spray voltage is increased during tuning of the source to maximize ion intensity. Spray current is normally below 500 nA, while the discharge current quickly rises to 1 μA or more.

When the ESI source is set up for the detection of negative ions, all power supplies are at reversed polarity and current flows in the opposite direction. Removal of positive ions inside the tip of the spray capillary provides droplets that are depleted of positive charge. The supply of negative charge to the solution may also take place; electrons released from the spray capillary can be captured by sample molecules having a high electron affinity.

In the case of positive ion operation of ESI, only some of the negative ions in solution are removed. Under the most favourable conditions, we have observed removal of nearly 50% of the negative ions from a 2-μ*M* solution of tetrabutylammonium bromide in dichloromethane [26]. For 2 μ*M* solutions of quaternary ammonium salts in other solvents, the percentage removal is approximately 25% or less [27,28].

For an increasing concentration of electrolytes in solution, the spray current increases weakly with conductivity [25,29]

$$I_{\text{Spray}} \propto (\text{conductivity})^{n} \quad (2)$$

where $n < 1$.

For a single electrolyte system, conductivity is proportional to electrolyte concentration. In most LC–MS or CE–MS experiments, the concentration of electrolytes added on purpose to the eluent of a buffer solution is orders of magnitude larger than the sample concentration so that sample concentration does not appreciably contribute to the conductivity of the solution that is dispersed by electrospray.

Since *n* may be as small as 0.22, depending on experimental conditions [30], the percentage removal of negative ions becomes smaller with increasing electrolyte concentration.

Spray current also increases weakly with flow-rate

$$I_{\text{Spray}} \propto (\text{flow-rate})^{m} \quad (3)$$

where $m \approx 0.5$ [25,29], so that at higher flow-rates, the percentage of negative ions removed from solution becomes smaller and, as a result, the charge-to-mass ratio of the total amount of droplets generated by electrospray becomes lower. In other words, droplet charging becomes less effective. If the flow-rate of the liquid is reduced, however, this equation predicts that droplet charging should become more effective.

4. Droplet disintegration

When droplets separate from the liquid front at the tip of the spray capillary, electric repulsion has become larger than the cohesive force that keeps the liquid together. During its flight through gas at atmospheric pressure, the droplet undergoes size reduction by evaporation of solvent, so that charge density at the droplet surface increases. Furthermore, the droplets are subjected to shear forces by their flight through dense gas. As a result of both effects, droplets undergo deformation, which leads to local high electric fields at protrusions on the surface. In cases where sufficient deformation and charge density electrostatic repulsion exceed the surface tension, the droplet becomes unstable and falls apart. The upper limit to charge on a droplet is called the Rayleigh stability limit [31]. Local deformation at the droplet surface may turn into a protrusion from which a small jet of microdroplets leaves the original parent droplet [29,32,33], as depicted schematically in Fig. 3.

The radius of primary aerosol droplets in electrospray is of the order of 0.5 to 1 μm. The radius of offspring droplets is estimated to be 0.1 μm. While the time needed for complete evaporation of a 1-μm radius droplet is of the order of milliseconds, the droplets with radii of 0.1 μm shrink within a submillisecond time frame. During evaporation of offspring droplets, a second generation of yet smaller droplets may be emitted from the 0.1 μm droplets.

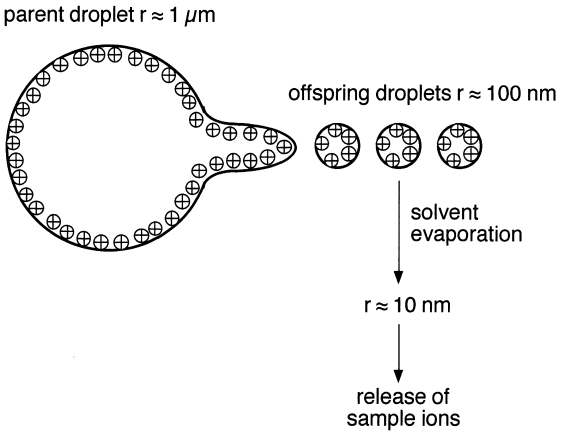


Fig. 3. Droplet disintegration by release of offspring droplets from a protrusion at the surface of a parent droplet, followed by size reduction through solvent evaporation, and release of sample ions from a 10-nm radius microdroplet.

Also, the original 1 μm droplets, having lost part of their mass and charge by release of offspring droplets, will shrink by evaporation and release offspring droplets again.

For a detailed picture, the reader is referred to reviews by Kebarle and Ho [29] and Kebarle and Tang [33].

When offspring droplets have shrunk to a radius of approximately 10 nm, further disintegration to yet smaller droplets is not supposed to take place in order to remove excess charge at the Rayleigh stability limit. Instead, droplet charge is reduced by the release of ions from the droplet surface.

5. Ion emission from droplets

When the electric field at the surface of a droplet has become sufficiently high, ions may be emitted from the droplet surface into the surrounding gas [34]. This process has been investigated by Iribarne et al. [35–37] and was called ion evaporation. Alternatively, an ion with one or more solvation shells may separate from the droplet surface as a nanodroplet [38] that loses its solvent molecules during its flight through the atmospheric pressure ionization source. Either process leads to naked sample ions that can be taken into the mass spectrometer. A distinction between the details of the ion formation processes has little or no influence on the use of ESI for LC–MS or CE–MS. If a sample ion has not been desolvated completely before entering the vacuum system, it can be desolvated by passage through a dry curtain gas, by passage through a heated ion sampling tube or by mild collision-induced dissociation inside the vacuum [39–41].

In analytical applications, the relationship between sample ion abundance observed with the mass spectrometer on the one hand and the concentration of sample and other materials in solution on the other hand is of decisive importance. Furthermore, ESI is increasingly used for the study of weak complexes in supramolecular chemistry, in enzyme and receptor studies, and in research on catalysis. In each application, the question is whether an ESI mass spectrum is a true and quantitative representation of the concentration and association of sample components. Since ESI is based on the release of sample ions from the surface of a charged droplet, it does not necessarily give a true picture of bulk solution chemistry.

The number of ions that escape from droplets is related to the charge on droplets, which can be derived from spray current measurement. Kebarle et al. [29,30] have proposed equations for a two-electrolyte system:

$$I_A = fp\frac{k_A[A^+]}{k_A[A^+] + k_B[B^+]}I_{Spray} \quad (4)$$

$$I_B = fp\frac{k_B[B^+]}{k_A[A^+] + k_B[B^+]}I_{Spray} \quad (5)$$

where $I_A = A^+$ ion signal at the MS detector, $I_B = B^+$ ion signal at the MS detector, f = fraction of charges on droplets that are converted to gas-phase ions, p = fraction of gas phase ions transported into the mass analyzer, k_A = sensitivity coefficient for A^+, k_B = sensitivity coefficient for B^+ and I_{spray} = total droplet current (spray current).

According to Eqs. (4) and (5), the abundance of a sample ion is proportional to the amount of charge on droplets, and proportional to a sensitivity coefficient, k, which is dependent on ion structure. Ionic surface active components have a high k value (approx. ten) and are observed with high sensitivity in electrospray mass spectra. Alkali metal ions have

a low k value (approx. one), and the k values for protonated organic bases are somewhere in between (three to six) [29,30]. No data are available for peptides and other biomolecules. Eq. (4) can be extended for a multi-electrolyte system by extending the denominator with the appropriate number of $k_X[X^+]$ terms.

A number of implications for LC–MS and CE–MS can be derived from Eqs. (2)–(5). First, at constant I_{spray}, the A^+ ion signal is proportional to $[A^+]$ if $[A^+] \ll [B^+]$. This condition is usually met in LC–MS if B^+ is an electrolyte present in the eluent system at the mM level, while the sample concentration is at the μM level.

Second, it may be advantageous to try and increase I_{spray}. According to Eq. (2), one has to increase the conductivity of the solution by adding more electrolyte. Let us assume that $n = 0.5$ in Eq. (2), so that doubling the spray current requires quadrupling the electrolyte concentration. Now, using Eq. (4), we can predict that, although I_{spray} has doubled, I_A is reduced, since $k_B[B^+]$ in the denominator has quadrupled. This is a clear and simple explanation for the observation that ESI efficiency is reduced at the high ammonium acetate concentrations that were customary in thermospray LC–MS. The suppression effect of various electrolytes is different due to differences in sensitivity coefficients and electrolyte dissociation into ions in solution. It has been noted in practice that the suppression effect of salts, such as ammonium acetate, is larger than the effect of volatile acids or bases, such as acetic acid (for positive ion ESI) and ammonium hydroxide (for negative ion ESI). This can be explained by the use of Eqs. (4) and (5) under the assumption that $k_B[B^+]$ is larger for NH_4^+ than for protonated solvent components and clusters, such as $(H_2O)_nH^+$, $(CH_3OH)_nH^+$, $(CH_3CN)_nH^+$ and $CH_3COOH_2^+$.

6. Sample ion signal saturation

Sample ion signal in ESI saturates at a sample concentration of approximately 10 μM, as shown in Fig. 4. At sample concentrations above 10^{-4} M, the ion signal decreases. Sample ion signal saturation might be attributed to insufficient charge on droplets.

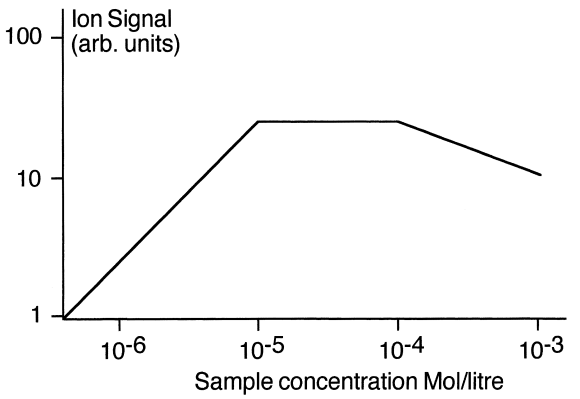


Fig. 4. General picture of sample ion signal at the detector of the mass spectrometer as a function of sample concentration.

In the case of a single component system, made up from tetrabutylammonium bromide in a number of different solvents, it has been shown that the quaternary ammonium signal saturates at approx. 10 μM, while the spray current (droplet charge) keeps rising with increasing sample concentration [26–28]. Thus, insufficient charge on droplets as a cause of sample ion signal saturation is ruled out in this case.

Eqs. (4) and (5) are valid as long as all components in solution have free access to the surface of the droplets with radii of approx. 10 nm that release ions into the gas phase. When the droplet surface is crowded with sample ions, sample molecules and other solutes, the passage for a sample ion from the centre of a droplet via the surface into the gas phase is partly blocked, and a nonlinear relationship between sample concentration and ion signal is expected. In practice, it is found that sample ion signal saturation sets in at a sample concentration of approximately 10 μM.

In a following set of experiments, a simultaneous measurement was made of the concentration dependence of the sample ion signal, the spray current and the current arriving at the skimmer [42] inside the vacuum system. The skimmer current can be taken as a true representation of the total ion current, unaffected by bias due to mass-dependent transmission of ion optics and the quadrupole MS, mass-dependent detector sensitivity and the limited mass range used by a data system for total ion current reconstruction. The result of this simultaneous ion signal and skimmer current measurement is shown in

Fig. 5. Both ion signal and skimmer current reach an upper limit, while spray current (not shown in Fig. 5) keeps rising when the sample concentration is increased above 10^{-5} *M*. Clearly, at this concentration, there is an upper limit to the number of ions that can be liberated from droplets and the sample ion signal no longer follows the concentration dependence predicted in Eq. (4) when the sample concentration exceeds 10^{-5} *M*. The calculated number density of sample ions and neutral molecules at the droplet surface given in Table 1 shows that a crowded droplet surface can explain sample ion signal saturation. Another consequence of crowding at the droplet surface is interaction between sample ions and sample molecules, leading to the formation of ion–molecule complexes. In the case of quaternary ammonium salts, Q^+X^- complexes of the general formula $Q^+(Q^+X^-)_n$ are observed with increasing relative abundance [26,42], while in the case of neutrals that are ionized by protonation, the ion–molecule clusters take the general formula M_nH^+.

The abundance of the sample ion Q^+ or MH^+ remains constant and may even decrease in spite of an increase in sample concentration above 10 μ*M*. At higher sample concentrations, the abundance of clusters increases. Such formation of sample cluster ions may also take place between different components in a mixture. In the case of negative ion electrospray, the formation of $[M-H]^-.M_n$ clusters can be extensive [43]. The observation of cluster ions is such a general phenomenon that proof of association between sample molecules in bulk solution cannot be based on an electrospray mass spectrum alone. In spite of these words of caution, the observation of weak interactions in solution by means of electrospray MS is a very interesting and promising field in the investigation of enzymes, receptors [44] and supramolecular complexes [45,46].

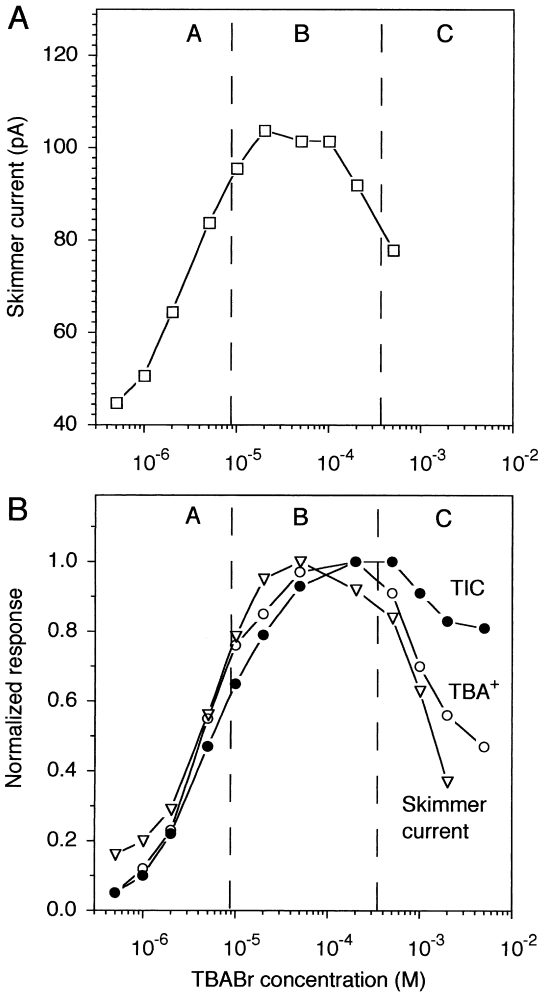


Fig. 5. (A) Skimmer current as a function of sample concentration of tetrabutylammonium bromide in acetonitrile–methanol (95:5, v/v); (B) skimmer current, sample ion signal and reconstructed total ion current, each normalized to its maximum value. Reproduced from ref. [42] with permission.

Table 1
Area available on a droplet surface at a sample concentration of 10^{-5} *M*

Radius of initially formed droplet	$r=1$ μm
Number of sample ions plus sample molecules	24 000
Area available per sample ion or molecule	500 nm^2
After fission to	$r=0.1$ μm
Area available per sample ion or molecule (surface density equal to parent droplet)	500 nm^2
Number of sample ions plus sample molecules	240
After size reduction by evaporation to	$r=10$ nm
Area available per sample ion or molecule	5 nm^2
Radius of average organic ion or molecule (C–C bond length, 0.15 nm)	approx. 1 nm
Area taken by one ion or molecule	approx. 3 nm^2

7. Sample ion formation for electrospray

Since ESI–MS makes use of sample ions present in solution, the question is how to turn a sample into sample ions in solution. A number of compounds exist as ions in solution, e.g. quaternary ammonium salts, phosphonium salts and salts of strong acids such as phosphates, sulfates and sulfonates, to name a few. For other compounds, it may be sufficient to adjust the pH in order to protonate a base or deprotonate an acid, respectively. Acids can be used for the ionization of amines, including peptides and proteins, while a base is used to ionize acidic samples, such as phenols, carboxylic acids, phosphonic acids and sulfonic acids. Suitable acids are acetic acid and formic acid, whereas ammonium hydroxide is a commonly used base for the preparation of a sample solution that is infused into the electrospray mass spectrometer. In HPLC, however, it is mostly undesirable to try and separate protonated amines or deprotonated acid samples on a column. Adjustment of the pH by the post-column addition of a suitable reagent can be used to combine good quality separation with high efficiency ESI. There is some controversy about the use of TFA in LC–MS with ESI. In some ion sources, a strong suppression of the ionization efficiency of peptides is noted and a post-column TFA-fix mixture has been composed [47]. In other ion sources, there is some suppression, but a TFA-fix is not worth the trouble and peptide mixtures can be run routinely [48].

Polar samples that do not contain basic or acidic functional groups cannot be ionized by protonation or deprotonation. As an alternative, these polar molecules can be ''ionized'' by association with another ion in solution. Usually, ammonium and sodium ions are used for positive ion detection of a sample as $M.NH_4^+$ or $M.Na^+$, while chloride, acetate, formate, trifluoroacetate and other negative ions are used for the detection of a sample as $M.X^-$ ions. Examples of this class of sample are amides, polyhydroxy compounds (carbohydrates), esters and ethers.

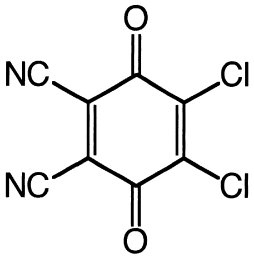
A ''polar'' compound in MS usually is a compound having a fairly high gas phase acidity or basicity. Polar does not mean ''water soluble''. For example, triglycerides are designated as apolar in the chemical literature, but have a moderately high proton affinity in the gas phase.

Apolar samples that cannot be protonated or deprotonated, nor be associated with Na^+ or other positive or negative ions, cannot be ionized by ESI. Atmospheric pressure chemical ionization (APCI) [39,40] might be a better choice, depending on the solvent mixture and the additives used for HPLC separation. If APCI cannot help either, LC–MS should be carried out with a particle beam interface combined with electron ionization or methane chemical ionization.

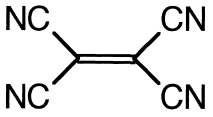
Some classes of sample molecules, such as polycyclic aromatic hydrocarbons and porphyrins, have a very low ionization energy. The removal of an electron from the sample in solution creates a radical cation that can be released from a charged droplet in ESI. Removal of an electron from the sample can take place at the tip of a stainless steel or platinum spray capillary, or upstream inside a separate electrochemical cell [49,50]. Electron transfer can take place straight from the sample molecule towards the spray capillary or the anode of an electrochemical reaction cell. Alternatively, another molecule can be used as an intermediate acceptor of an electron from the sample [23,51]. This latter electron transfer process has been studied extensively in charge transfer complexation in organic chemistry:

$$D + A \rightarrow D^{+\cdot} + A^{-\cdot}$$

Donor molecules, D, are aromatic compounds with electron-donating substituents, while acceptors, A, are molecules having a high electron affinity, such as tetracyanoethylene (TCNE) and dichlorodicyanobenzoquinone (DDQ).



DDQ



TCNE

$A^{-\cdot}$ ions of DDQ are observed in the negative-ion mode [51].

Porphyrins and metalloporphyrins can be detected

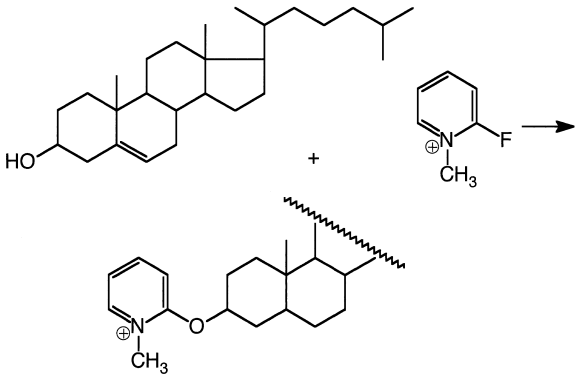


Fig. 6. Derivatization of an alcohol for enhancement of the electrospray ionization response [53].

as radical cations under suitable solvent conditions in ESI-MS [52]. Polycyclic aromatic hydrocarbons can be detected after electrochemical oxidation, or by the addition of DDQ [23,49–51].

Sample derivatization is widely used in GC–MS in order to increase sample volatility, improve chromatographic behaviour and increase the abundance of diagnostic ions. Derivatization is little used in LC–MS, since the avoidance of complexity and reduction of sample handling steps is a major advantage of LC–MS over GC–MS. The introduction of suitable functional groups in alkyl halides, alcohols, phenols, thiols and amines can dramatically enhance ionization efficiency in ESI-MS [53]. Alcohols can be derivatized and converted into quaternary ammonium compounds, as demonstrated in Fig. 6. Very recently, derivatization by the introduction of an electrochemically ionizable moiety into a sample has been reported [54].

8. Concentration-sensitive behaviour of electrospray ionization

The sample ion signal in an electron impact (EI), chemical ionization (CI) or thermospray mass spectrometer is proportional to the number of sample molecules introduced into the source per unit time. In other words, MS is a mass-flow-sensitive detector for chromatography. Electrospray is very different in this respect, since the sample ion signal is proportional to sample concentration, but largely independent from the flow-rate used for sample introduction. ESI acts or behaves as a concentration-sensitive detector for chromatography.

The question is whether ESI is truly concentration-sensitive, or if apparent concentration sensitivity is the result of opposing effects.

ESI-MS is a sequence of nebulization and droplet charging, droplet disintegration and solvent evaporation and, finally, release of ions from very small droplets. The radius of primary aerosol droplets formed by pure electrospray or pneumatically assisted electrospray increases with increasing liquid flow-rate [55]. According to Eq. (3), the spray current increases with approximately the root of the liquid flow-rate, so that the charge-to-mass ratio of the primary droplets decreases with increasing liquid flow-rate. Eq. (3) combined with Eq. (4) predicts that ESI efficiency should decrease with increasing liquid flow-rate.

When offspring droplets separate from primary aerosol droplets, the fraction of sample ions in a 0.1-μm radius offspring droplet relative to sample molecules and sample ions left behind in primary aerosol droplets decreases when the average size of primary droplets is increased. In order to generate free gas phase ions from sample ions that are left behind in primary droplets after a first series of offspring droplets has separated, a second and third series has to be separated [29,30]. However, since coulombic repulsion is the driving force for this separation, and since charge-to-mass ratio decreases with increasing liquid flow-rate, it becomes unlikely that repeating series of offspring droplets separate from primary droplets. As a result, the amount of droplet charge that is finally released as ions into the gas phase decreases.

Since solvent evaporation plays an important role in the mechanism of electrospray, it is not surprising that a high flow-rate, together with a high percentage of water as a solvent component, reduces the efficiency of droplet size reduction and, thus, the release of ions from droplets with a radius of approximately 10 nm. In all systems that have been adapted to high-flow electrospray, the supply of heat is used to support solvent evaporation [56].

In conclusion, a number of factors appear to work together in the reduction of the efficiency of the release of sample ions from the liquid phase if the

flow-rate is increased, and cancel the expected positive effect of increasing the rate of sample mass transportation into the ion source.

The apparent concentration-sensitive behaviour of ESI has a number of practical implications. First, it is advantageous to use microbore HPLC columns that deliver a more concentrated effluent into the ion source than a standard column, if an equal mass of sample is injected into both columns. Second, the major part of the effluent from a column can be split away from the mass spectrometer, without a loss of ion abundance. By the use of a splitter, the load of contaminants on the ion source is reduced, and the major liquid stream can be collected in fractions for further purification and for structure elucidation by other spectrometric techniques. In quantitative LC–MS–MS work in the pharmaceutical industry, the full 200 μl/min effluent from a 2-mm I.D. column is fed into the pneumatically assisted electrospray ion source. A split is avoided mainly to keep the hardware as simple and rugged as possible and to eliminate the risk of blockage of a narrow-bore transfer capillary.

In order to exploit the concentration-sensitive behaviour of electrospray at flow-rates lower than 0.5 μl/min, it is necessary to use special hardware and a small diameter spray capillary [56–58]. Interestingly, Eq. (1) explains why nanoelectrospray works at a low voltage, since both capillary radius and distance to the ion sampling orifice (which acts as the counter-electrode) are small, and a high electric field is created when approx. 600 V is applied.

9. Ion abundance throughout a spectrum

The number of ions arriving at the detector of an ESI–MS system is dependent on ESI efficiency, ion sampling efficiency into the vacuum, and ion transmission efficiency through ion optics and the mass analyzer. Ion sampling and ion transmission are mass-dependent. Ion sampling efficiency decreases for low mass ions [59]. Ion transmission through ion optics also suffers from roll-off towards low mass, and tuning may be mass-dependent.

Since the pressure inside the ion optics stage is so high that many collisions take place, focusing is far from ideal, in particular, for low mass ($m/z<500$) ions in a conventional lens stack. Quadrupoles, hexapoles and octapoles operated at high pressure (>2 mTorr) afford very efficient transportation of ions into the mass analyzer [60]. Nevertheless, discrimination against low-mass ions takes place, albeit below m/z 150 [42], as shown in Fig. 7. At high mass, the transmission of the mass analyzer plays a role. In quadrupole instruments, the transmission of ions decreases with increasing m/z.

The efficiency of ion-to-electron conversion in electron multiplier detectors is dependent on the velocity of ions hitting the first dynode or entrance of a channel electron multiplier. Since all ions arrive with approximately equal kinetic energy, velocity decreases with increasing m/z, and the output signal decreases at high m/z [61]. Manufacturers make use of high energy conversion dynodes to counteract mass discrimination in electron multipliers.

The abundance of ions within a very wide m/z

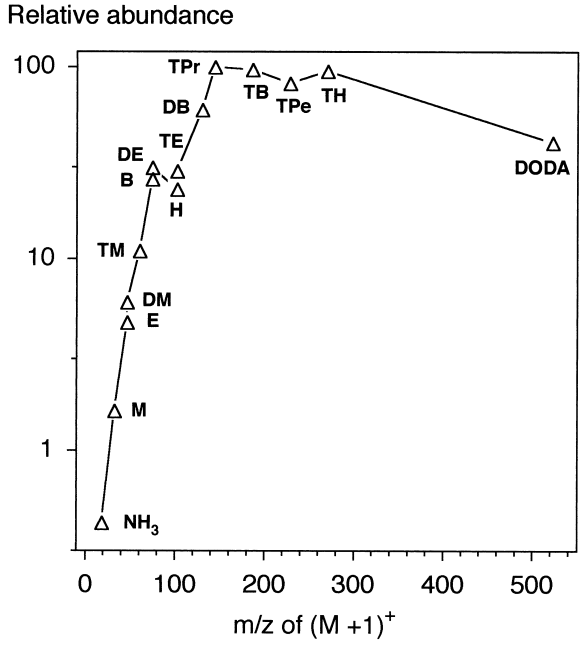


Fig. 7. Combined mass-dependent efficiency of ion sampling, transportation, separation and detection for the atmospheric pressure ionization source and NERMAG R 3010 mass spectrometer at the author's facility. See ref. [42] for details. Reproduced from ref. [42] with permission.

range in a spectrum should be interpreted with caution in a discussion about the mechanism of ESI.

Finally, the ion-to-electron conversion in an electron multiplier with a separate conversion dynode takes place via different processes for detection of negative ions than for positive ions and shows a different mass-dependent efficiency. A direct comparison between positive and negative ion signals is not meaningful, unless mass-dependent gain for both positive and negative ions has been measured.

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