

Mass Spectrometry

Fundamental LC-MS

Electrospray Ionisation – Theory



Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.

Aims and Objectives

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Aims

- Introduce the mechanism of electrospray Ionisation (ESI)
- Present the main theories that explain the ion production process in Electrospray Ionisation
- Indicate the when the technique should be used and its advantages and limitations

Objectives

At the end of this Section you should be able to:

- Describe the working principles associated with electrospray Ionisation
- Explain the difference between positive and negative ESI, and when to use each mode
- List and describe the most important components of an electrospray Ionisation
- List the main instrument variables affecting electrospray Ionisation
- Explain the two most popular theories for electrospray ion production

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Introduction

Electrospray is a method by which ions present in a solution can be transferred to the gas phase. The process involves the application of an electric field across an interface which acts to form an electrochemical cell in the interface.

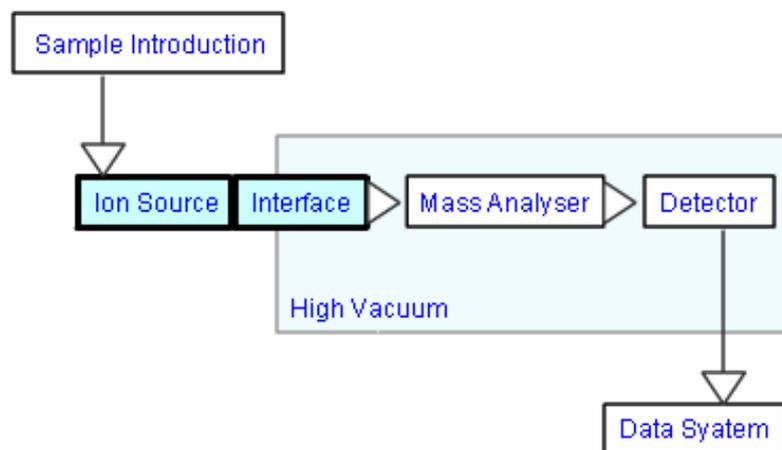
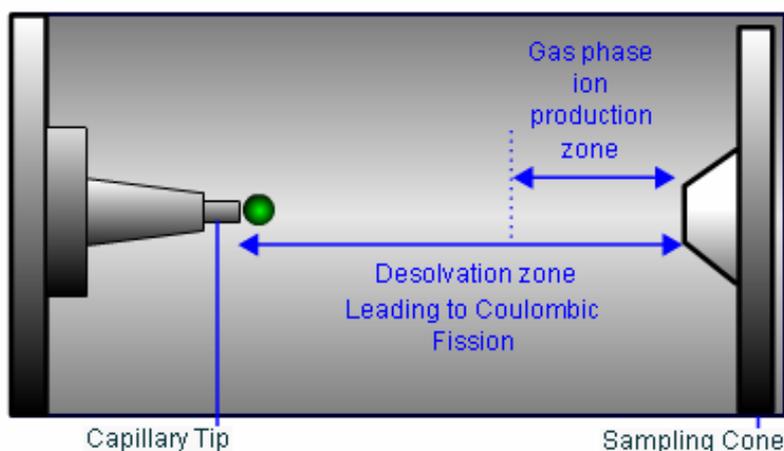
In electrospray ionisation there are three important processes that occur in order to transfer sample molecules from the HPLC eluent into gas phase ions within the mass spectrometer. These processes are:

- Production of charged droplets at the capillary tip
- Shrinkage of the charged droplets: leading to coulombic fissions
- Production of gas phase ions from small / highly charged droplets

Electrospray ionisation is highly compatible with analytes possessing the following characteristics:

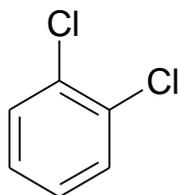
- Moderate to highly polarity
- Up to 100,000 Dalton
- Ionise in solution perhaps with multiple charge

Electrospray ionisation favours the analyte in the ionised form, in eluent solution prior to introduction into the API interface.



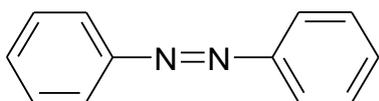
Suitable analytes for ESI

Analyte species that are capable of carrying charge in solution include:^[1-7]



1,2 di-chloro benzene

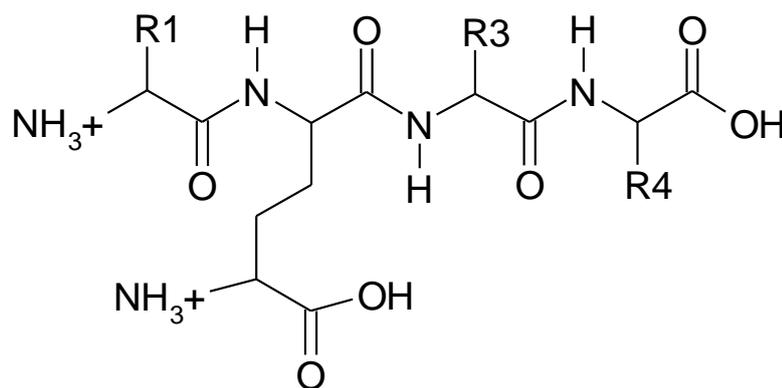
Those containing hetero-atoms (i.e. chlorinated, brominated etc.) –the hetero-atom within the molecule is able to carry a proton in solution by association with unpaired electrons.



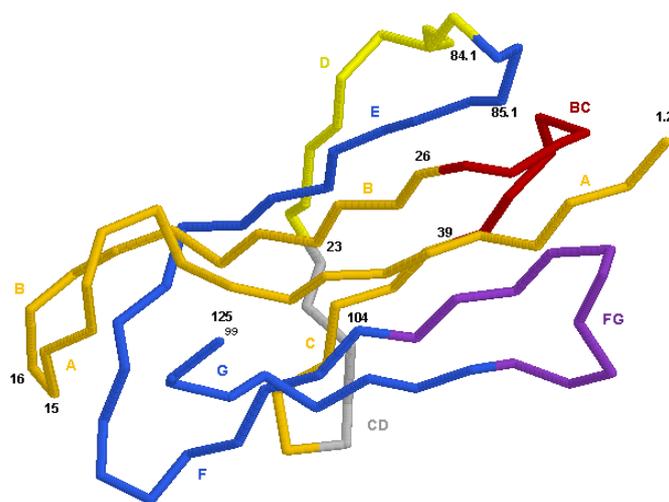
azobenzene

Compounds producing a charge through inductive effects (i.e. azo-compounds, phthalates, opiates etc.).

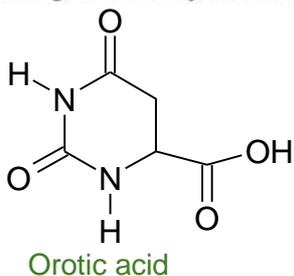
Compounds capable of holding multiple charge (i.e. proteins, peptides etc.) –multiple basic amino acid sites on these compounds are all capable of ionisation in solution given correct pH conditions.



Polipeptide

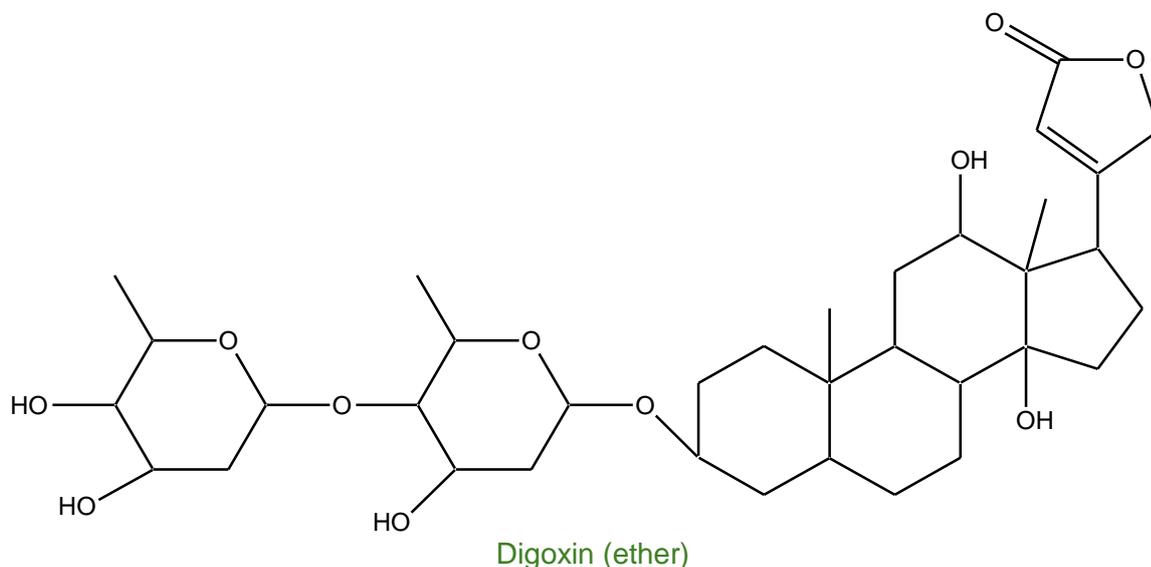


β 2 microglobulin (11.8 kDa) –Protein



Compounds that are ionisable (acids, bases etc.) – adjusting pH can cause the compound to favour the ionic state in solution.

Compounds that have a strong dipole moment but do not ionise under conditions suitable for HPLC analysis (aldehydes, some ethers etc.) – ESI droplets may be charged by electrostatic effects and the compound carries charge by association with adducting species such as Na, K or MeCN ions in solution.



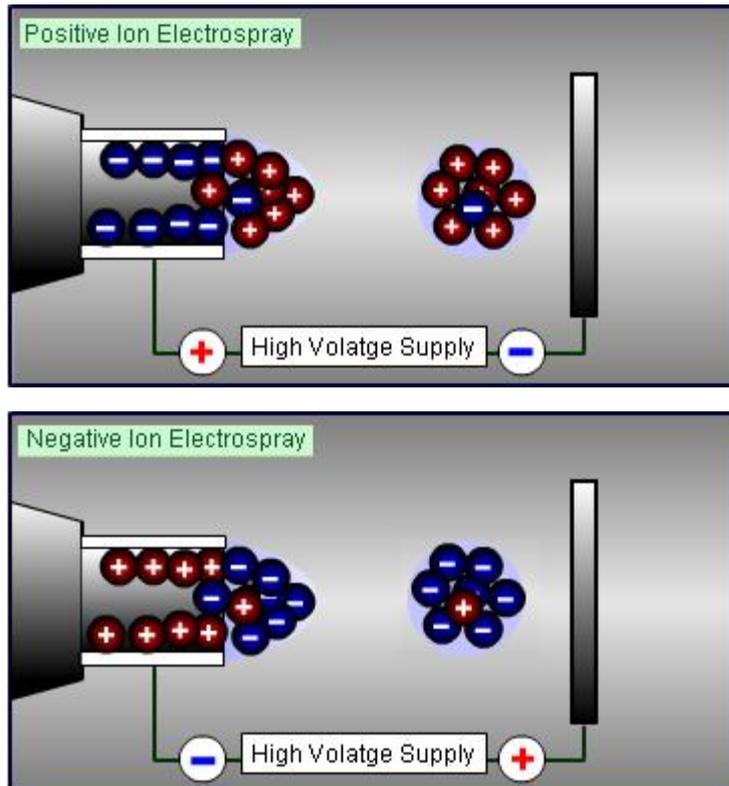
Production of charged droplets at the capillary tip

The first stage in Electrospray ion production is the production of charged eluent droplets at the tip of the 'sprayer'. The sprayer is fed by the HPLC eluent (at a suitable flow rate) and the resulting spray is directed into the desolvation chamber of the Atmospheric Pressure interface.

According to the nature of the analyte of interest, positive or negative ion mode can be selected.

In positive ion mode the capillary is the positive electrode (anode) and the sampling aperture plate is the negative electrode (cathode). The positive ions within the eluent solution are repelled from the inner walls of the sprayer needle and move electrophoretically into the body of the droplet formed at the capillary tip. This mode causes positive ions to predominantly populate the sprayed droplet and is used where the analytes (such as bases for example) form cations in solution.

In negative ion mode the reverse situation occurs. The capillary is the negative electrode (cathode) and the sampling aperture plate is the positive electrode (anode). This mode causes negative ions to predominate the sprayed droplet and is used where the analytes (such as acids for example) form anions in solution.



Charged droplet formation at the capillary tip

Remember:

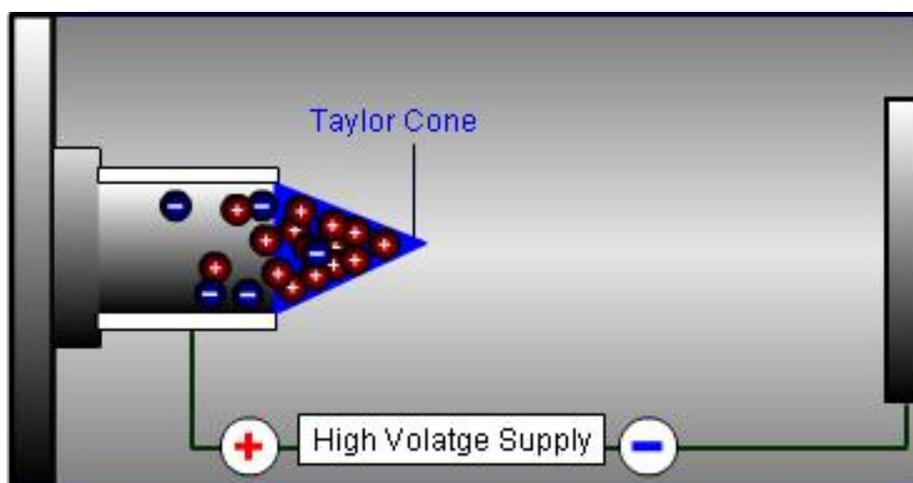
- The capillary is used to introduce the HPLC eluent into the electrospray source, it connects the column or splitting device to the interface.
- In modern instruments the capillary is metallic with an internal diameter of approximately 0.1mm.
- A potential difference (V_c) is applied between the capillary and the sampling cone (usually between 2 to 6 kV). The applied voltage forms an electrochemical cell in the interface which acts to accelerate charged droplets between the sprayer and the sampling orifice as well as forming the basis for an electrophoretic charge separation at the capillary tip
- Because the capillary is thin, the electric field (E_c) in the air around the capillary tip is high (approximately 10^6 V/m).
- The magnitude of the electric field is closely related to the capillary diameter and its distance from the sampling cone and its distance from the sampling cone.

Formation of the Taylor cone

Initially the liquid meniscus is conventionally shaped, contains relatively few ions and has a relatively low charge density.

When the charge density at the liquid meniscus is raised due to the repulsion of anions (negative ion mode) or cations (positive ion mode), the coulombic repulsion forces are also increased. The point at which the surface charge repulsion matches the surface tension of the eluent is termed the 'Rayleigh Instability Limit'.

When the number of ions of like charge is increased, coulombic repulsions overcome the Rayleigh limit and the shape of the meniscus changes to conic in order to relieve charge repulsion. This cone shaped meniscus is referred as the 'Taylor Cone', and upon formation of the cone a stream of droplets containing a vast excess of either cations or anions, will emerge from its surface. This process is termed 'Electrospray'.



Taylor cone

Nebulisation

Overview

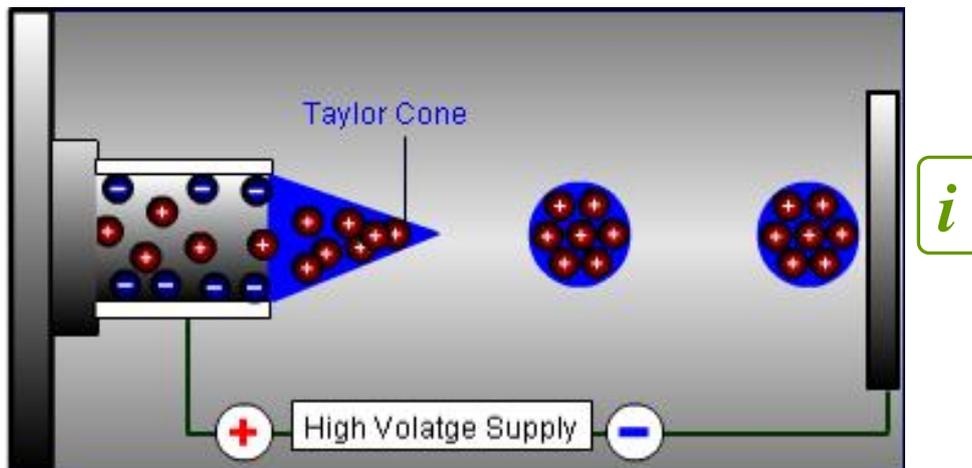
Droplet formation and expulsion into the desolvation region is known as '**Nebulisation**'.

The formation of a stable nebulisation aerosol is dependant upon several factors including:

- The inner and outer diameter, as well as the shape of the capillary tip and the material of construction (metallic, silica etc.)
- The applied potential difference (V_c)
- The flow rate, surface tension and electrolyte concentration of the HPLC eluent
- The flow rate and temperature of any nebulising gas which is applied concentrically to the capillary to assist droplet formation

Establishing a reproducible Electrospray is of primary importance in practical LC-MS and the factors mentioned above act interactively. Of primary importance in establishing a

stable and productive electrospray is the potential difference applied between the capillary and the sampling plate of the API interface.



Nebulisation

Applied Potential Difference

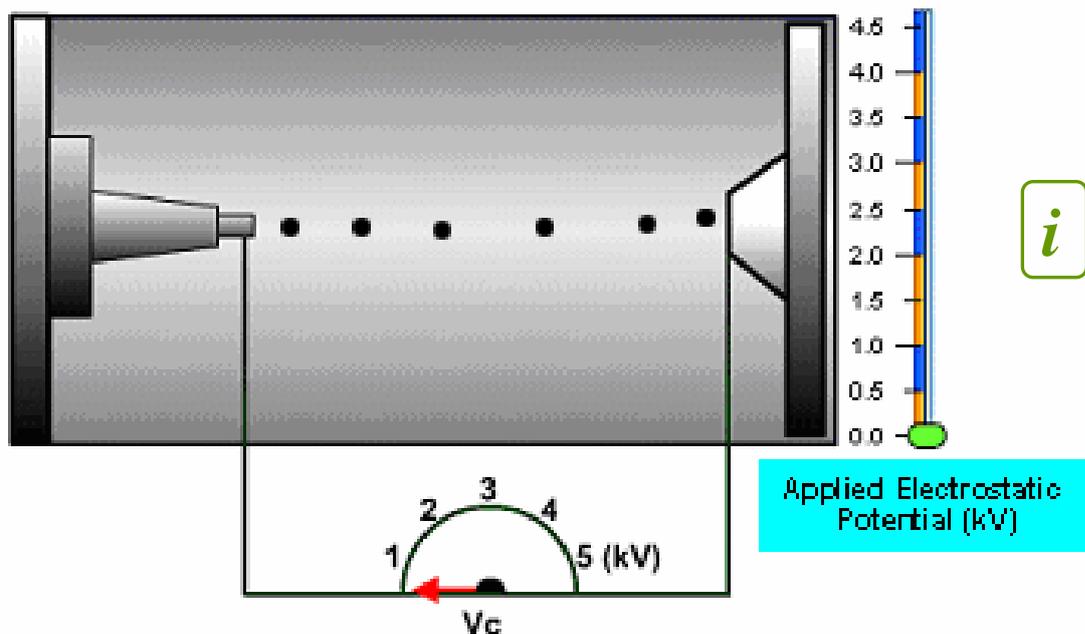
The effectiveness and stability of the nebulisation process is found to be proportional to the magnitude of the potential difference across the system.

As the potential difference across the system is increased, the droplet size reduces and their motion acquires a horizontal component.

Above a certain applied potential difference (experimentally dependant), the Taylor cone is formed and small charged droplets are formed from its tip. This is known as the **Axial Spray mode**. This spray mode will contain the optimum voltage for the experiment (empirically determined).

Further increasing the applied potential will cause a sudden transition to take place –the liquid cone vanishes and a fine mist of droplets is produced from a number of points on the edge of the capillary tip. This is known as **Rim Emission mode**. Whilst an instrument signal will still be produced, it will not be optimised in terms of response and will be irreproducible.

A second transformation occurs at still higher capillary potentials. A **corona discharge** is established between the needle tip and the sampling plate.^[8] Discharge is not a stable or reproducible spray state and the noisy baselines produced results in a lowered **signal to noise (S/N) ratio** which is defined as the ratio of the amplitude of a signal to the amount of unwanted interference (the noise) that has mixed in with it.



In negative ion mode, as the applied potential is increased above a critical limit, a discharge would appear and it corresponds to the emission of electrons from the point of the capillary. In positive ion mode the appearance of protonated solvent clusters such as $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ from water and $\text{CH}_3\text{OH}_2^+(\text{CH}_3\text{OH})_n$ from methanol indicates the presence of a discharge.

Discharge drastically changes the appearance of the resulting spectrum that will now represent the products of ion-molecular reactions. Within a discharge, both positive and negative charge carriers are formed that will recombine within the droplets. The charge on the surface of the resulting droplet will be neutralised and the formation of sample ions can no longer take place by conventional electrospray mechanisms. The net result of this condition is a drastic reduction in the Electrospray signal.

Discharge can be reduced by avoiding highly aqueous eluent systems and performing the experiment at low enough potentials. As can be seen from the instrument response, the spectral signal may appear spiky or erratic and the instrument baseline output may contain 'spikes' that resemble 'electrical noise'.

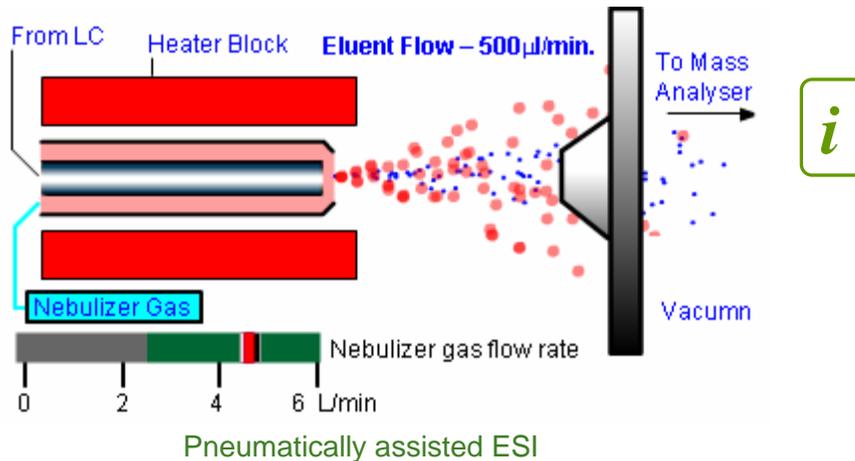


Charge neutralization (corona discharge process)

Factors affecting electrospray production

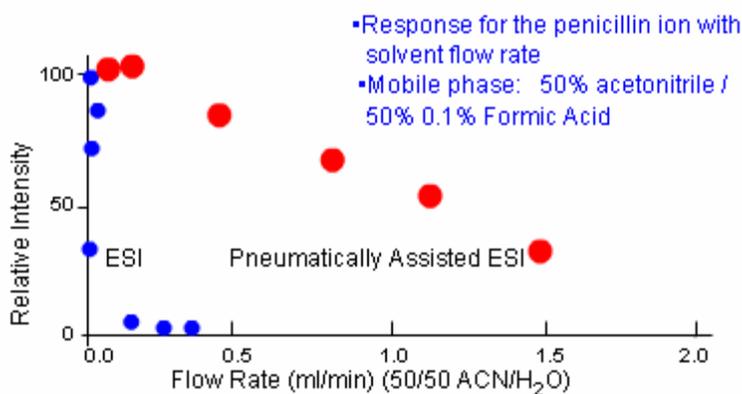
Eluent Flow rate / Pneumatic Assistance

The optimum eluent flow rate is important in all API applications with the optimum electrospray flow rate limited to about 200 $\mu\text{L}/\text{min}$, even when using highly volatile eluent systems and with pneumatic assistance to the spray.



Electrospray is a low-flow-rate technique (limited to a few micro-litres per minute). The instrument response and sensitivity decreases as the eluent flow rate is increased. This behaviour is closely related to the size of the droplets formed at the capillary tip and the number of charges within the droplet. By introducing an axially sprayed gas around the forming droplet (ESI with 'pneumatic assistance') the droplet size is restricted and the droplets are charged more efficiently at higher eluent flow rates.

The eluent flow rate used in LC separations could reach values in the order of 1 mL per minute. Pneumatically assisted ES can operate up to 1.0 mL per minute, but the signal tend to optimize at around 200 $\mu\text{L}/\text{min}$.

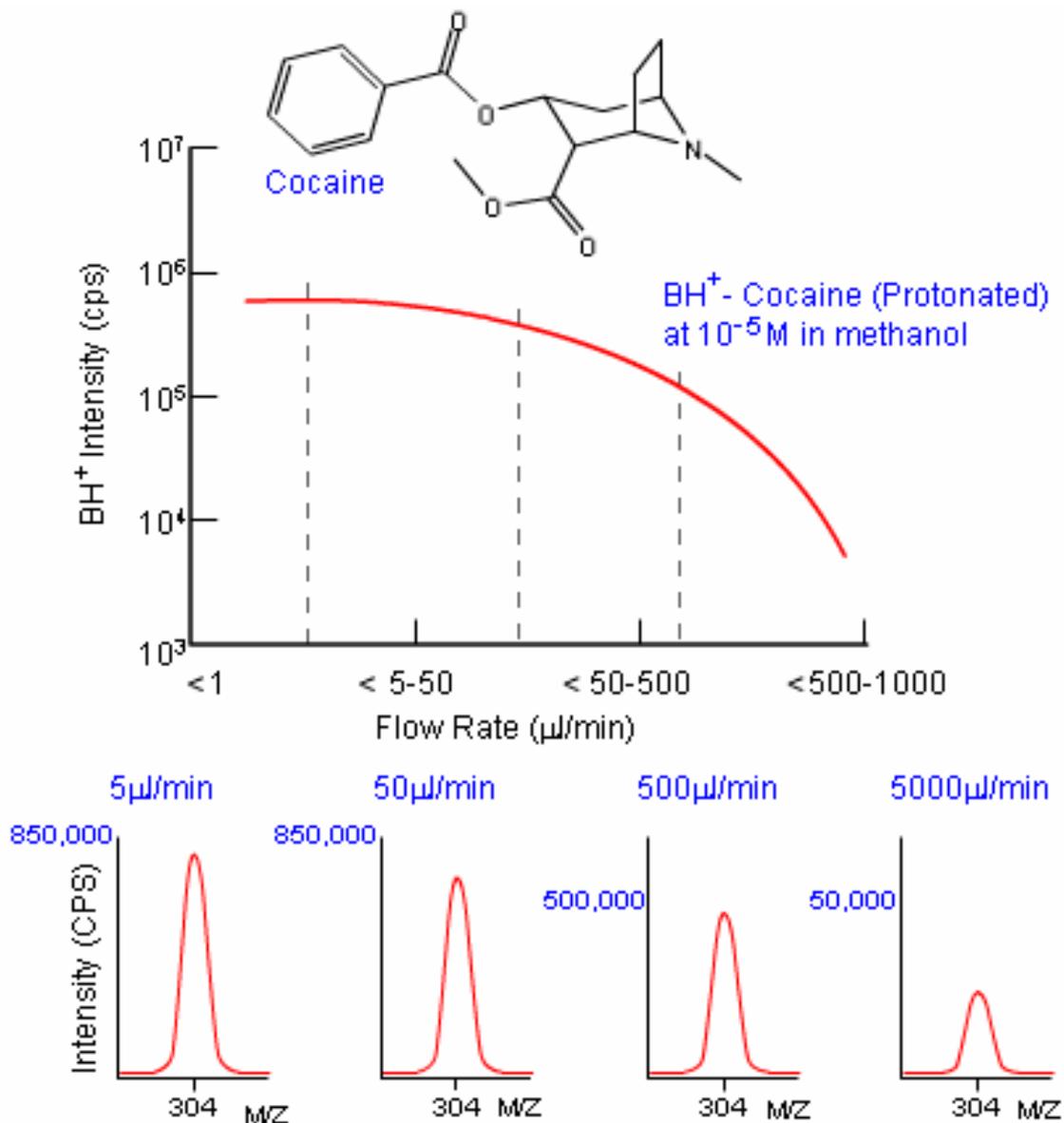


Assisted versus non-assisted ESI

Flow rate

The figure below shows the dependence between the ion intensity of the protonated cocaine molecule and eluent flow rate. Note how the sensitivity of the instrument appears to decrease significantly at higher flow rates.^[9] This observation is related to an increase in the electrospray droplet size at higher flow rates and a resulting decrease in the efficiency of the droplet charging process.^[10]

Effectively, when the column flow rate and the split ratio remain constant, the detector behaves with a concentration dependent response, therefore when the eluent flow rate is increased, the signal should increase proportionally with the amount of analyte, but this is not the case. At increased flow rates where the signal begins to fall away, the processes described by Fernandez de la Mora,^[11] begin to predominate and the larger droplets formed cannot produce sufficient gas phase ions to sustain the constant relationship between capillary current (or signal) and increasing flow rate.



Cocaine ESI response at different flow rates

Solute surface tension

Solvents with low surface tension (i.e Methanol, Iso-propanol etc.), allow for stable Taylor cone formation and hence a stable and reproducible electrospray.

The Rayleigh limit will be overcome at lower potentials and this will tend to lead, on average, to smaller droplets being produced. This aids in the ion formation process and may lead to an increase in instrument sensitivity.

Lower potential differences across the system, will be required for effective spraying when using lower aqueous content, this reduces the risk of spraying in rim emission and lowers the possibility of electrical discharge at the capillary tip.

The addition of a small amount of methanol or iso-propanol (1-2% v/v) to a highly aqueous HPLC eluent, can often bring about an increase in instrument response, as the surface tension is lowered.

Table 1. Physical properties of selected solvents.

Solvent	Surface tension (dyn/cm)	Dipole (D)	Dielectric constant (@ 20°C)	Viscosity (cp)
Methanol	22.55	2.87	32.7	0.59
Ethanol	22.32	1.66	24.55	1.10
Propanol	23.70	3.09	20.33	2.30
Isopropanol	21.79	1.69	19.92	2.40
Butanol	22.98	1.75	17.51	2.98
Acetonitrile	19.10	3.44	37.50	0.38
Water	72.80	1.87	80.10	1.00
DMF	36.76	3.86	36.71	0.92
DMSO	43.0	3.96	46.68	2.24
Acetic acid	13.63	1.74	8.55	0.93
Acetone	23.22	2.69	20.70	0.36
Toluene	28.53	0.31	2.38	0.59
Hexane	17.91	0.08	1.88	0.31
Chloroform	28.12	1.15	4.81	0.57
Cyclohexane	24.98	0.0	2.02	1.00

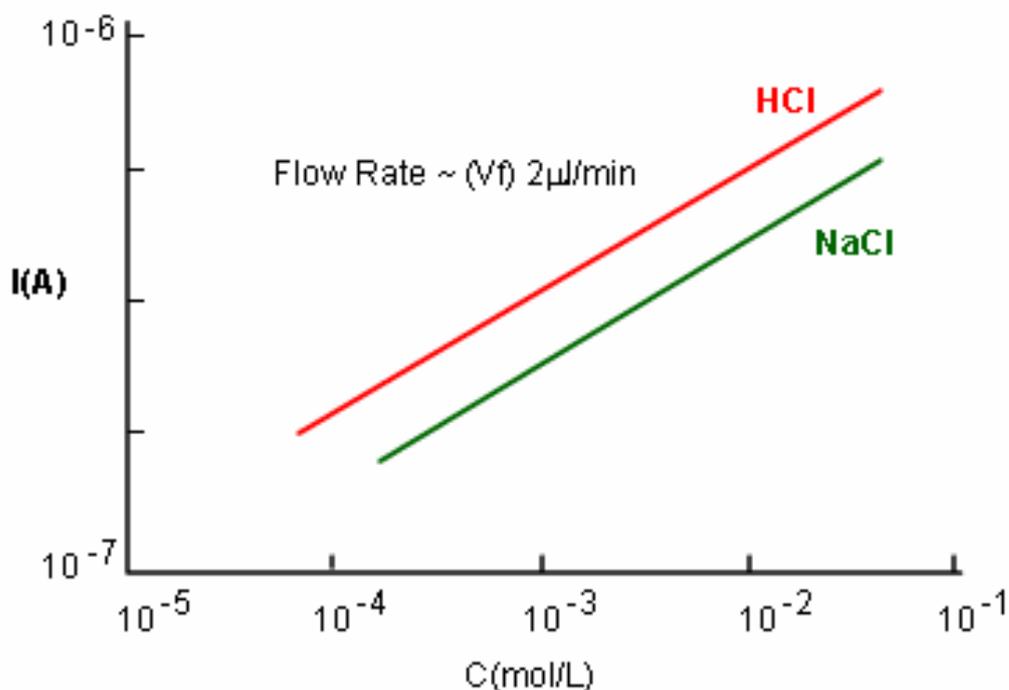
Table 2. Potential differences to overcome Rayleigh limit for selected solvents

Solvent	ΔV (kV)
Isopropanol	2.0
Methanol	2.2
Acetonitrile	2.5
DMSO	3.0
Water	4.0

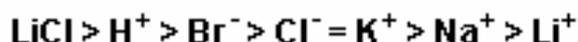
Conductivity and ionic strength of the eluent

The sprayer (capillary or nebuliser) current is dependant upon the conductivity of the eluent solution, which is in turn related to the concentration of ions within the eluent.

In HPLC it is usual to have several electrolyte ions present in a single eluent solution. These may include the analyte and its matrix as well as impurities from the solvents and buffer ions that may have been added. For example, HPLC grade acetonitrile contains a fairly high background level of sodium ions, which gives rise to the background signal. Buffer ions (which usually predominate), have differing conductivities. Ion conductivity is determined by the relative mobility of the electrolyte species in solution at the capillary tip and within the bulk solution of the electrosprayed droplet.



Relative mobility of various electrolyte species in methanol @ 25°C:



From the results shown it can be seen that even at equal concentrations in the eluent solution, HCl and NaCl electrolyte ions produce different capillary currents (and hence different ion intensities), due to their different mobilities in an eluent system of 60%:40% water:methanol.^[9]

The practical implication is that the use of small (low molecular weight), highly charged ions as background electrolytes will give a LOW background signal. This is primarily due to the large sphere of hydration acquired by the smaller ions in solution which renders them less electrolytically mobile in the droplet.

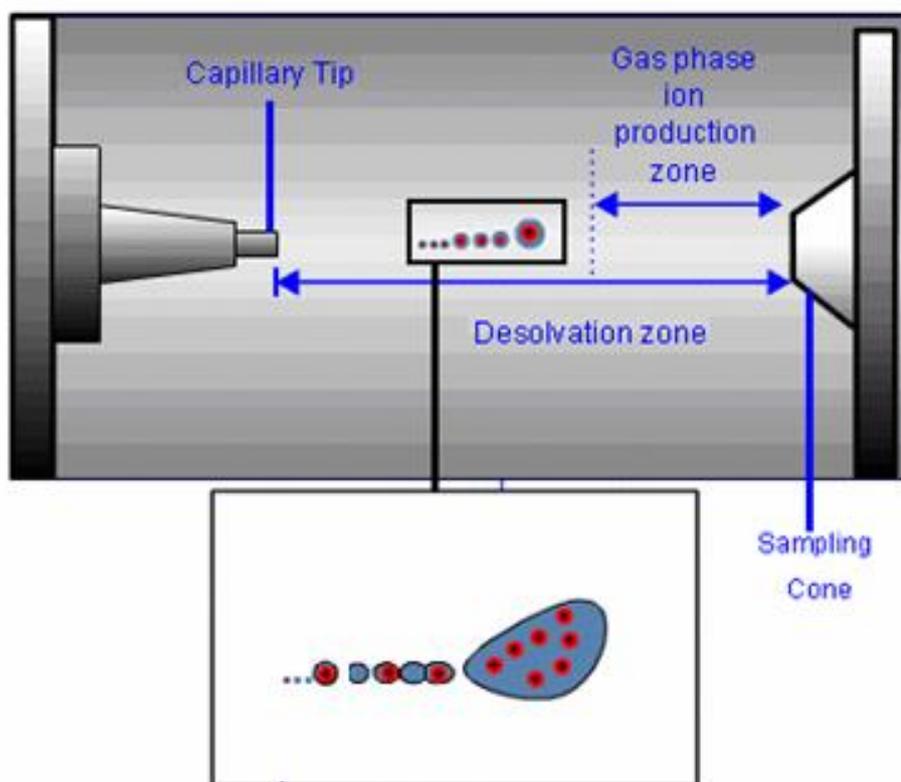
As a general rule, electrolyte ions with LOW mobilities in the eluent solution will interfere less with analyte gas phase ion production.

Droplet desolvation

As the droplet is sprayed into the desolvation zone towards the sampling cone, it will shrink due to solvent evaporation. Solvent evaporation is aided by raising the temperature of the ambient air within the ionisation chamber which is often supplemented by the use of an inert desolvation gas that is pumped into the interface housing. Practically, various manufacturers implement this in a number of ways, however pressurized and heated nitrogen is the preferred choice.

Rayleigh limit

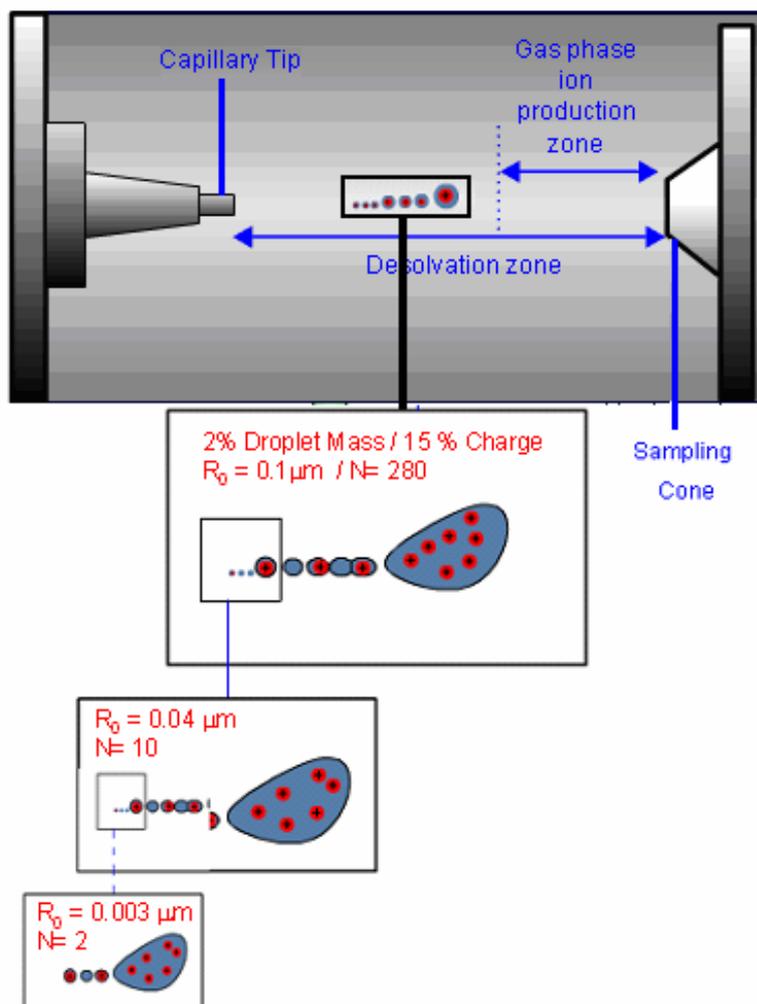
As the droplet shrinks due to solvent evaporation its radius (r) decreases but its charge (q) remains constant –leading to a decrease in the charge separation (inter-ionic distance) at the droplet surface and an increase in repulsion between the surface charges, until the electrostatic repulsive forces become equal to the eluent surface tension (Rayleigh Instability Limit). Further decreasing the radius of the droplet will cause the surface charges to move closer together. The Rayleigh limit will be exceeded and the droplet will undergo a Coulombic Fission (or explosion), in order to reduce the coulombic stress between the the surface charges. The process is often referred to as ‘Coulombic’ or ‘Droplet Jet Fission’.



Jet fission

Jet fission will lead to a reduction of approximately 2% of the parent droplet mass and around 15% of the parent droplet charge.^[12] This predicts that the radius of the offspring droplet will be approximately one tenth of the parent droplet. Mass balance shows that under the experimental conditions being considered, the first Rayleigh jet fission will produce around 20 offspring droplets.

As the resulting droplets hold a greater charge per mass (volume) than the original droplet, the resulting (offspring) droplets will quickly undergo further droplet jet fissions to produce further offspring droplets. The 'cascade' of droplet fission processes lead ultimately to a very small droplet (~10 nm) containing a small number of theoretical charges.



A typical first generation of offsprings will have a radius of approximately 0.10nm and 280 elementary charges ($N=280$). The offspring droplet will reach its Rayleigh instability limit in around 40 μs , when its radius is approximately μm . A second generation of offspring droplets (formed from the first offspring droplet), will have a radius of approximately 0.003 μm and $N=2$ elementary charges.

The whole evaporative process usually occurs within a timescale of between a few hundred microseconds and a few milliseconds –The residence time of the droplet within the desolvation zone.

Production of gas phase ions from small / highly charged droplets

Below an offspring droplet radius of around 10 nm, there are two popular theories on the mechanism by which coulombic stress is relieved and gas phase ions are formed.

Mechanism 1

Dole proposed further droplet fissions until very small droplets containing a single ion each are produced.^[13] Solvent evaporation from these droplets will lead to the formation of a gas phase ions (known as the Charged Residue Theory).

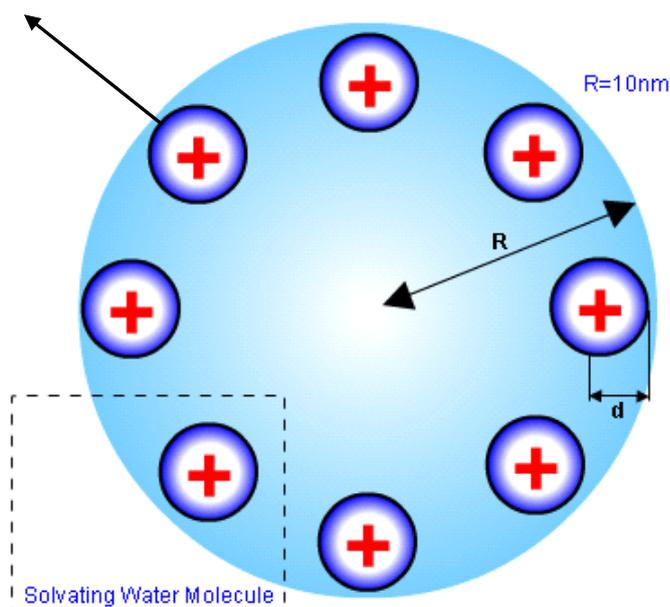


Mechanism 2

Iribarne and Thompson propose that below a droplet radius of 10nm an ion is able to 'evaporate' from within the droplet.^[14,15]

The main supporting evidence comes from ion mobility studies,^[15] which show the production of significant amounts of gas phase ions at times where most of the charged droplets are expected to have relatively large radii and multiple charges. This observation does not support the charged residue model.

The ion mobility studies revealed highly mobile gas phase ions which were spectroscopically identified as ion-solvent cluster molecules of the type $M^+(H_2O)_n$ where $n=3-7$ and M is the analyte species. Currently ion evaporation is the most widely accepted theory.



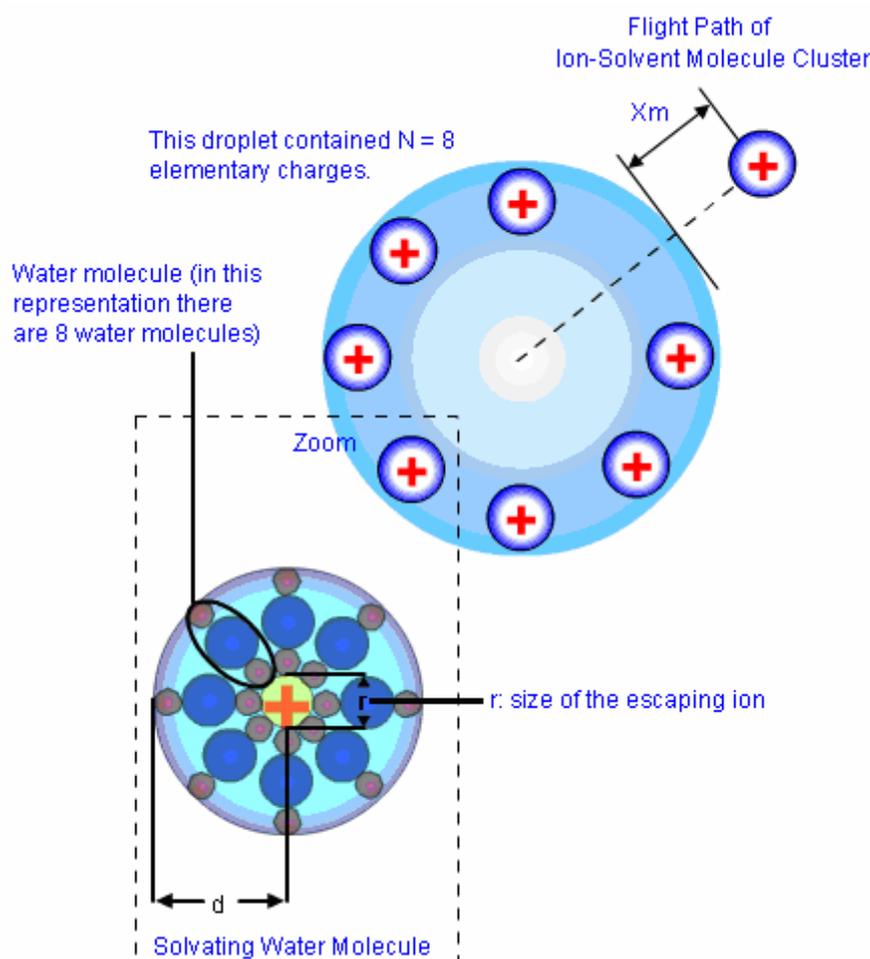
Practical implications

The free energy of activation (ΔG^\ddagger) for droplet emission (i.e. the energy barrier to emission), is found to depend on four parameters:

- The number of elementary charges on the droplet (N)
- The radius of the droplet (R)
- The size and hydration energy of the escaping ion
- The degree of hydration of the ion cluster and its radius (d)

The droplet radius and its number of charges both contribute to the size of the electric field encountered at the surface of the droplet.

The production of droplets with optimum values of R and N should be investigated for each analytical determination and therefore the effects of flow rate, sprayer position relative to the sampling orifice and capillary voltage should each be investigated when developing LC-MS methods that require high sensitivity. Of course the interface (drying gas) temperature and the nature of the solvents used will also have a profound influence on the position within the desolvation zone that meaningful ion production begins. This indicates that when altering the source parameters or eluent composition there may be merit in optimizing the position of the sprayer relative to the sampling orifice to optimize instrument response.



Important:

The rate constant for ion emission to the gas phase is dependent on:

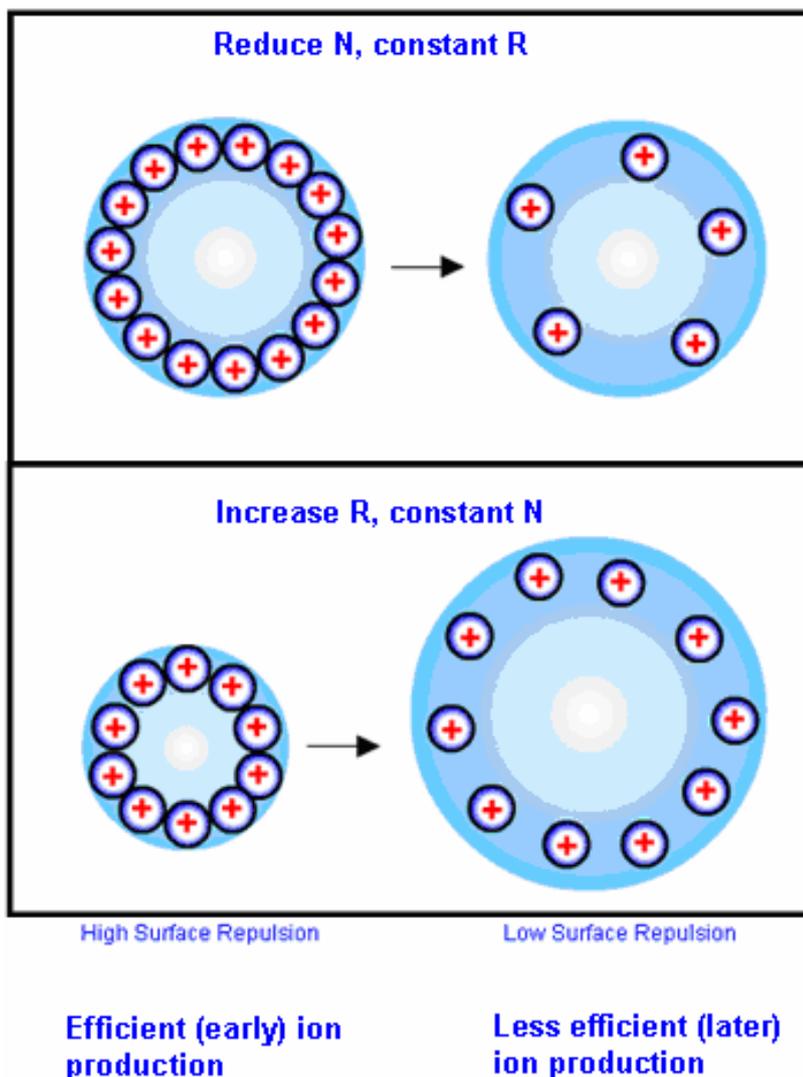
- Coulombic forces acting on the escaping ions
- Repulsive forces arising from interactions with ions of the same sign within the droplet
- Attractive forces due to interaction with ions of opposite sign in the droplet.
- The radius of the hydrated ion cluster (d) describes the distance of the ion charge center from the droplet surface.

The rate of ion production (and the position of ion production onset within the desolvation zone) increases with the number of ions N within the droplet, and decreases with the radius of the droplet R . Decreasing N and/or increasing R is equivalent to a reduction of the surface repulsion forces that are experienced by the droplet i.e. the droplet surface charges are moving further apart. It is less likely that the energy barrier to ion ejection (ΔG^\ddagger) will be overcome, as the attractive forces holding the ion cluster within the droplet will be dominant.

The factors that determine the number of charges within the droplet (N) and the radius of the droplet (R) include:

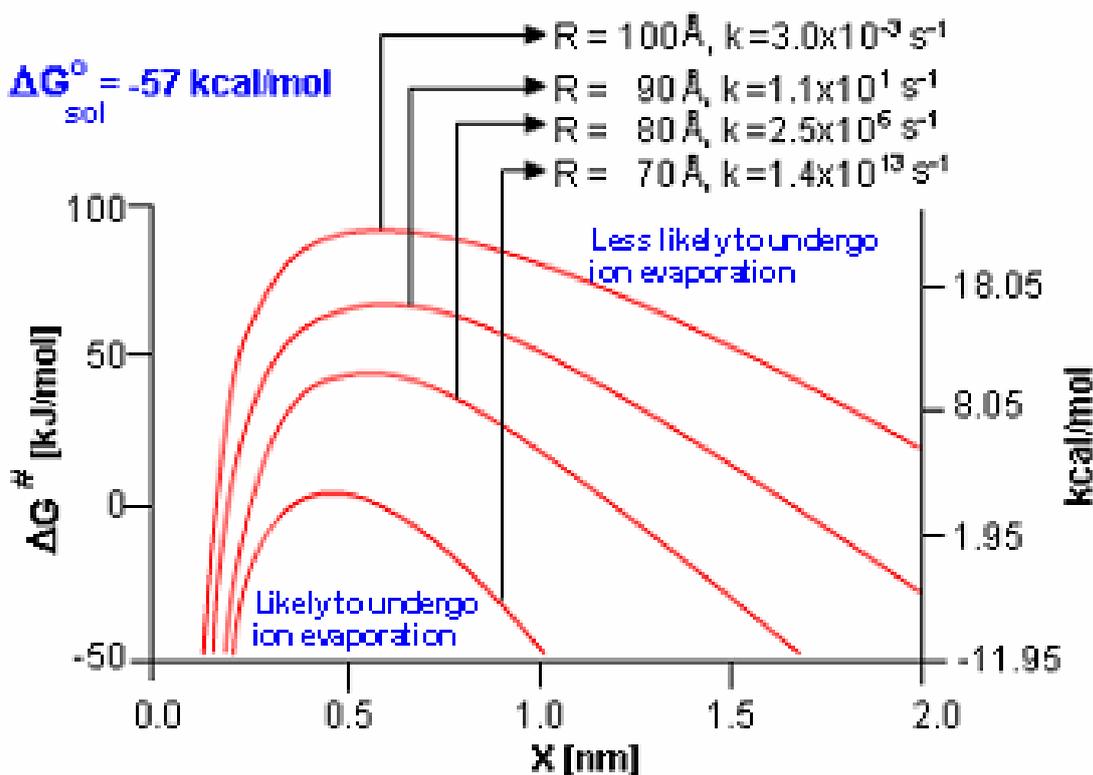
- Eluent flow rate
- Nebulisation type and nebulising gas flow rate
- Eluent composition
- Capillary Voltage
- Drying gas temperature

Reducing the number of ions (keeping the droplet radius constant) or increasing the droplet radius (keeping the number of ions constant) reduces the surface repulsion – making ion evaporation less likely and significantly changes the position of optimum gas phase ion production within the desolvation zone. This may require the re-positioning of the sprayer relative to the sampling orifice if any of the above factors are significantly altered.



For each application, the interface variables will combine to produce a set of optimum conditions for maximum ion production / instrument response, maximizing the signal response can be a complex process. However, almost all combinations will produce a signal, which can be optimized by maximising instrument response for each variable in turn. Capillary voltage, eluent flow rate and sprayer position are usually optimised first in a practical situation.

As can be seen from the data presented below the change in activation energy (ΔG^\ddagger) for the escaping ion changes very rapidly with decreasing droplet radius.^[9] The rate constant (K_i) for emission of ions from the droplets, changes by 16 orders of magnitude with a droplet size reduction of around 30 Å ($R = 100\text{Å}$ to 70Å). Therefore, reducing droplet size produced in the Electrospray, or ensuring rapid and efficient droplet desolvation, is an important concept in producing gas phase ions. For this reason the nebulising gas flow rate, as well as the drying gas temperature are very important parameters in LC-MS optimisation.



The experiment is carried out with constant solvation energy ($\Delta G_{\text{sol}}^0 = -57 \text{ kcal/mol}$), i.e. the same ion is used in each case, and the number of surface charges is approximately constant ($N=70$).

It should be noted that due to the sphere of hydration associated with each ion, it is impossible for the ion to reside at the droplet surface, where mutual repulsion would be maximized and so the energy barrier to ion evaporation smallest. Strongly solvated ions such as Na^+ (i.e. small radius with high charge to mass ratio), are associated a larger number of solvent molecules, thus moving the charge centre further away from the droplet surface and be less likely to undergo ion evaporation.

The larger the value of d , the fewer gas phase ions will be produced because increasing the size of the sphere of hydration will:

- Move the charge further away from the droplet surface (where the electrostatic forces repelling the ions into the gas phase are lowest)
- Shield the charge on the ion

The table shows that the strongly solvated ions Li^+ and Na^+ have larger transfer energies, the Iribarne and Thompson theory predicts larger activation barriers, that is small rate constants k .^[15,16]

Table 3. Energetic parameter for selected solvated ions

M ⁺	Li ⁺	Na ⁺	K ⁺	Cs ⁺	NH ₄ ⁺	(C ₂ H ₅) ₄ N ⁺
$\Delta G_{Sol}^o(M^+)$	122	98.2	80.6	67.5	81	(49)
$\Delta G_{Sol}^o[M^+(H_2O)_m]$	61.2	56.5	55.8	54	55.6	(49)
m	7	7	6	5	~6	~0
R _{ion}	3.82	3.58	3.30	3.29	3.30	(2)
ΔG^\ddagger	13.1	9.30	9.70	7.90	9.50	7.9
k (S ⁻¹)	2.0×10 ³	1.0×10 ⁴	4.9×10 ⁵	9.4×10 ⁶	6.8×10 ⁵	9.8×10 ⁶

$\Delta G_{Sol}^o(M^+)$: Transfer free energy from gas phase to solution for the M⁺ ion [kcal/mol].

$\Delta G_{Sol}^o[M^+(H_2O)_m]$: Transfer free energy from gas phase to solution for the hydrated ion cluster [kcal/mol].

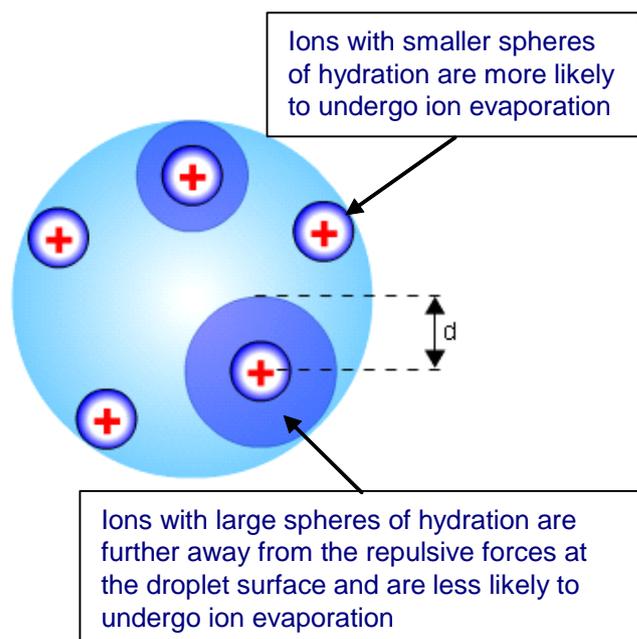
ΔG^\ddagger : Activation energy for ion evaporation [kcal/mol].

m: Number of water molecules in $[M^+(H_2O)_m]$

R_{ion}: Radius of hydrated ions, equal to d parameter in Iribarne equation [Å].

k: rate constant [S⁻¹]

We have already established that small ions (lower molecular weight) with multiple charges (i.e. low mass to charge ratio) will have the larger spheres of hydration.



The closer the ion to the droplet surface, the more likely it is to evaporate from the droplet to give a hydrated gas phase ion. The ion cannot occupy positions at the droplet surface as it is surrounded by a sphere of hydration.

Analyte ions with large spheres of hydration will be the least analytically sensitive in LC-MS –predicting the size of the sphere of hydration may help in optimizing instrument response. Background electrolyte ions (from the solvents, eluent components such as buffers and sample matrix elements), that have smaller spheres of hydration, will form gas phase ions in preference to the analyte. This may also lead to a reduction in analyte signal –a phenomenon commonly known as ‘Ion Suppression’.

Quantitative aspects of Electrospray Ionisation

Sensitivity constants

In order to determine if the analyte or co-electrolyte species will have efficient ion evaporation characteristics it is important to know what factors influence the size of the sphere of hydration, which governs not only the possibility of ion evaporation but also the rate of movement of the hydrated ion from the bulk to the droplet surface. Initial work into experimentally determined sensitivity constants were based on the assumption:^[15]

$$\frac{I_A}{I_B} = \frac{k_A}{k_B} \text{ when } [A^+] = [B^+]$$

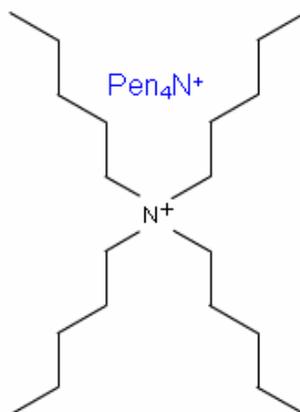
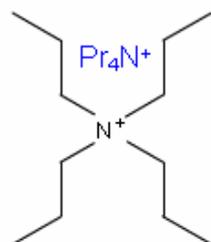
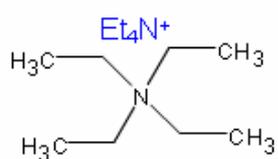
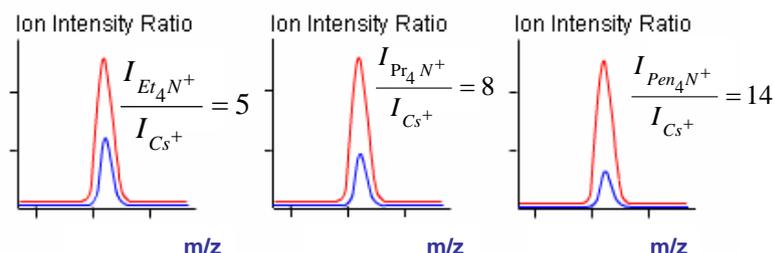
Where:

I_A : Intensity of the specie A.

k_A : Sensitivity constant for the specie A.

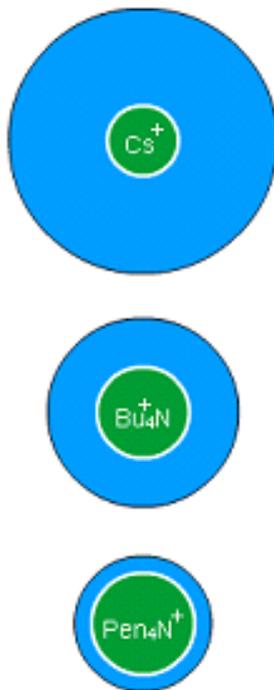
$[A^+]$: Concentration of the specie A^+ .

That is, having equal concentrations of the two electrolytes A and B, the ion intensity ratio will be equal to the sensitivity coefficient ratio. Experimentally, the spectrometrically determined ion intensity ratio I_A/I_B is measured when equal concentrations of electrolytes A^+X^- and B^+X^- are present in the electrosprayed solutions. Determinations are carried out at the same experimental conditions on the same instrument.



The table opposite shows some experimentally determined sensitivity coefficients and all data are relative to k_{Cs^+} .^[9]

Ion	k
Cs ⁺	1
Na ⁺	1.6
NH ₄ ⁺	3
MorphineH ⁺	1.3
HeroinH ⁺	6
Bu ₄ N ⁺	2
Et ₃ N ⁺	5
Pr ₄ N ⁺	8
Pen ₄ N ⁺	14



Relative ion intensities for some selected analytes.^[9]

Relative size of the hydration sphere for some selected analytes.^[9]

NOTE:

1. The charge on the caesium ion is not delocalised, the ion is highly hydrophilic and has a large sphere of hydration
2. The charge on the tetrapentyl ammonium ion is much more delocalised, the ion is more hydrophobic and has a small sphere of hydration –making ion evaporation from the droplet surface much more favourable

As is generally observed in electrospray LC-MS, singly charged ions that have hydrophobic groups, also tend to have high experimental sensitivity coefficients. Ion evaporation theory predicts that this is due to the lower degree of hydration of the more hydrophobic ions (allowing them to more closely approach the repulsive electrostatic forces at the droplet surface), and a lower solvation energy ΔG_{Sol}^O , leading to a lower energy barrier to transition into the gas phase.

A general conclusion that is analogous with mass spectrometry may be drawn here. Where the (singly charged) ion has a larger mass to charge ratio, its sphere of hydration is smaller and it can more closely approach the droplet surface –making it more likely to produce gas phase hydrated ions. This can be useful, for example, when choosing buffers for reverse phase HPLC. Small highly charged species are less likely to interfere with analyte gas phase ion production than larger singly charged species.

Surface Activity

The likelihood of ion evaporation is also dependent upon the ion of interest migrating from the bulk to the surface of the droplet. The relative ease of migration of the hydrated ion to the droplet surface is known as the 'surface activity'.

Tang and Kebarle,^[9] introduced surface activity of the respective ions into their experimental data analysis. Surface activity is a good indication of the ions ability to migrate towards the surface of the electrosprayed droplet and depends upon the hydrophobicity of the ion, as well as the number of charges the ion carries.

The sensitivity coefficient (k) can be shown to depend not only on the Iribarne ion evaporation rate constant (k_i) but also bulk to surface ion equilibrium constant (K_s), as defined by the surface activity.

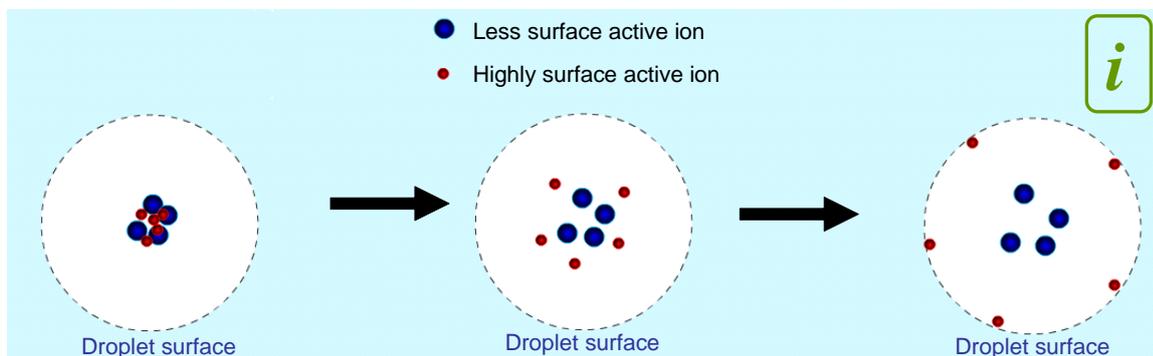
Ions that show high surface activity also stand the greatest chance of transfer to smaller droplets during Droplet Jet Fission, as well as being liberated into the gas phase via ion evaporation processes.

I_A : Intensity of the specie A.

$k_{I,A}$: Iribarne emission constant for the specie A.

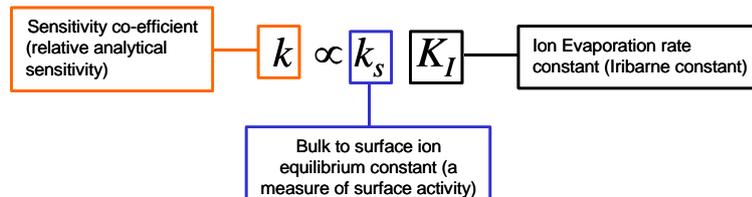
k_A : Sensitivity constant for the specie A.

$K_{s,A}$: Bulk to surface ion equilibrium constant for the specie A.



$$k \propto k_s K_I$$

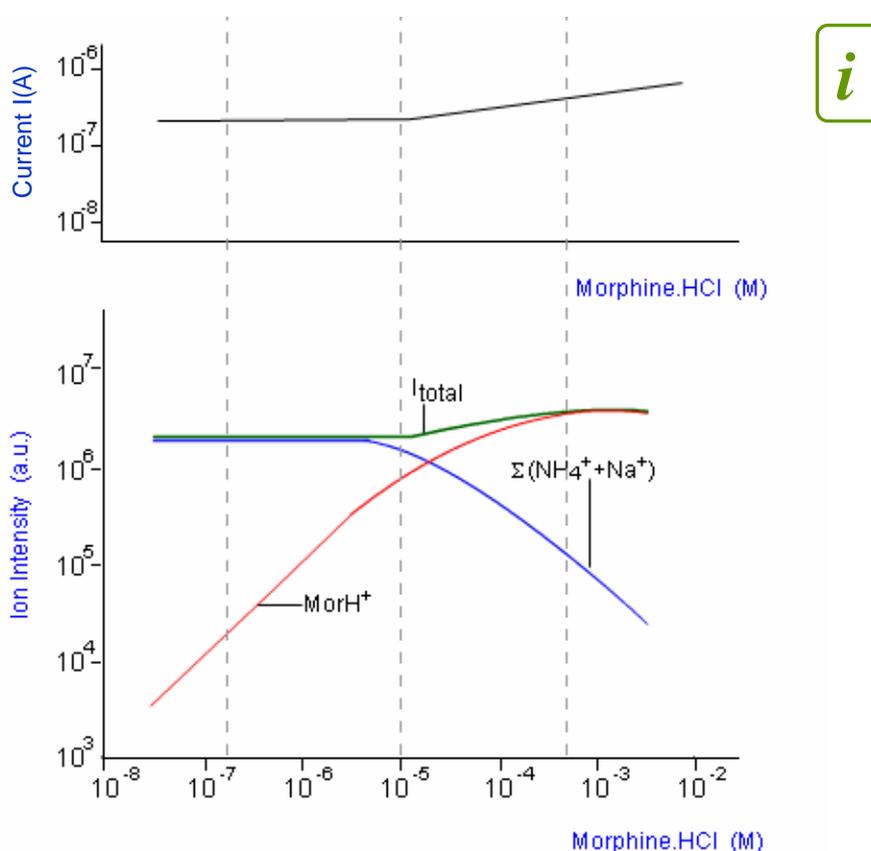
$$\frac{k_A}{k_B} = \frac{K_{s,A} k_{I,A}}{K_{s,B} k_{I,B}}$$



Competing Ions (Ion Suppression and Ion Enhancement)

It is important to understand the relationship between the instrument response (ion intensity) and the concentrations of the analyte and other electrolyte species in the LC eluent system –so that predictions can be made regarding optimum analyte concentration and the removal / reduction of potentially interfering species. Predicting ion intensities for a specific analyte at a given concentration is a common practice when undertaking electrospray LC-MS. Tang and Kebarle modelled the ion intensity (I_m) in the presence of a background electrolyte.^[9]

The solvents typically used for LC-MS will contain impurity electrolytes unless special deionisation techniques are employed. For example, the methanol used in LC experiments will contain Na^+ and NH_4^+ species at around $3 \times 10^{-5}\text{M}$, which serve as a constant concentration background electrolyte. To illustrate the relationship between analyte concentration, capillary current and ion intensity, the electrospray response of the protonated morphine ion in reagent grade methanol has been studied ($[\text{Mor}+\text{H}]^+$ = the protonated Morphine Ion (288 m/z)). The results of these experiments are shown graphically below.



At low $[\text{Mor}+\text{H}]^+$ concentrations (lower than 10^{-7}M), the electrospray capillary current $I(\text{A})$ is solely dependant upon the presence of impurities in the background electrolyte $\Sigma(\text{NH}_4^+ + \text{Na}^+)$. The background electrolyte species will dominate the surface of the electrosprayed droplet as they are in vast excess compared to the $[\text{Mor}+\text{H}]^+$ (analyte) ions. Note how the ion intensity for $\Sigma(\text{NH}_4^+ + \text{Na}^+)$ remains constant, but the $[\text{Mor}+\text{H}]^+$ intensity increases as its concentration is raised.

When increasing the $[\text{Mor}+\text{H}]^+$ concentration (10^{-7} - 10^{-5}M) eventually leads a decrease in the ion intensity of the background electrolyte $\sum(\text{NH}_4^++\text{Na}^+)$ INDEPENDENT OF ITS CONSTANT CONCENTRATION, this is due to a competition effect for the droplet surface with the $[\text{Mor}+\text{H}]^+$ ions. The $[\text{Mor}+\text{H}]^+$ ions are not present in sufficient concentration to displace significantly large numbers of background electrolyte ions from the droplet surface, the capillary current $I(\text{A})$ and the ion intensity will remain approximately constant.

At some point (10^{-5} - 10^{-3}M), the analyte $[\text{Mor}+\text{H}]^+$ begins to dominate and the total electrolyte concentration (as well as the capillary current) begins to increase. The competition for droplet surface between the $[\text{Mor}+\text{H}]^+$ and electrolyte ions becomes significant. The ion intensity for the background electrolyte becomes seriously suppressed by the dominant $[\text{Mor}+\text{H}]^+$ analyte ions.

At high concentrations (10^{-3} - 10^{-2}M), the capillary current is totally dependent on the analyte $[\text{Mor}+\text{H}]^+$ concentration. At very high analyte concentrations a decrease in total ion intensity is observed, this phenomenon has been explained in terms of an increase in droplet size (less specific evaporation area).

The region where the ion intensity of the analyte of interest is proportional its concentration and quantification should be carried out in this concentration range wherever possible. This may require dilution of the sample solution.

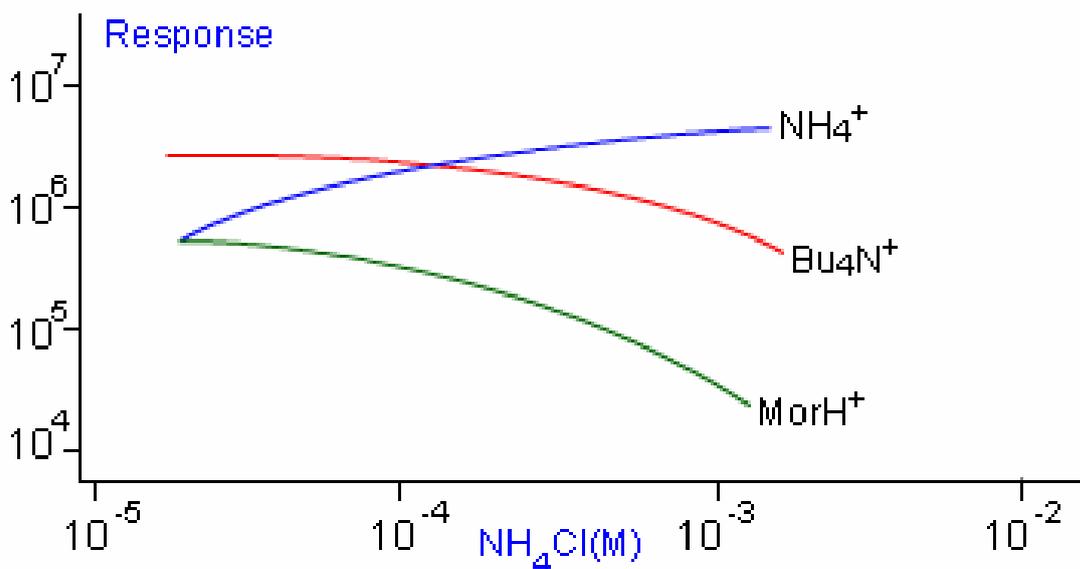
Ion Suppression in Practice

In the presence of a second electrolyte species, the intensity of the analyte can be suppressed, this phenomenon is termed "Ion Suppression". This phenomenon can lead to the artificial and irreproducible reduction in analyte signal when determinations of the analyte at constant concentration are carried out on samples where the background electrolyte concentration varies. Perhaps the most usual presentation of this situation is the analysis in which the nature of the sample matrix changes (i.e. bioanalysis, environmental analysis etc.) The reverse situation can also occur in which analyte response is artificially enhanced –known as Ion Enhancement.

Ion Suppression is a highly practically significant phenomenon in LC-MS because it is so insidious. The unpredictable nature of ion suppression in examples where, for instance, analytes are contained within different matrices, can often render quantitative analysis impossible due to lack of reproducibility.

The magnitude of the suppressive effect is dependent on the sensitivity coefficient of the two ions, which in turn is dependant upon surface activity, solvation energy, degree of hydration and the number of charges held by each ion.

Decrease of analyte ion intensities due to competition with added $[\text{NH}_4]^+$, at constant analyte concentrations $[\text{Bu}_4\text{N}]^+ = [\text{Mor}+\text{H}]^+ = 1 \times 10^{-5}\text{M}$.^[1]



As can be seen from the plot, the [Bu₄N]⁺ and [Mor+H]⁺ ions (which are present at constant concentration), are increasingly 'suppressed' as the concentration of the NH₄⁺ ion (originated from the ammonium chloride buffer) is increased. This is a result of the competitive process between the ions for the limited and fixed number of droplet surface sites.

Sensitivity in ESI

Many factors influence the sensitivity of a particular ion in electrospray ion production

- The number of charges on the ion
- Effective mass to charge ratio (which influences the size of the hydration sphere)
- Ion's ability to migrate through the droplet bulk to the droplet surface
- Surface area of the sprayed droplet
- The proximity of the hydrated ion cluster to the droplet surface (i.e. charge shielding)

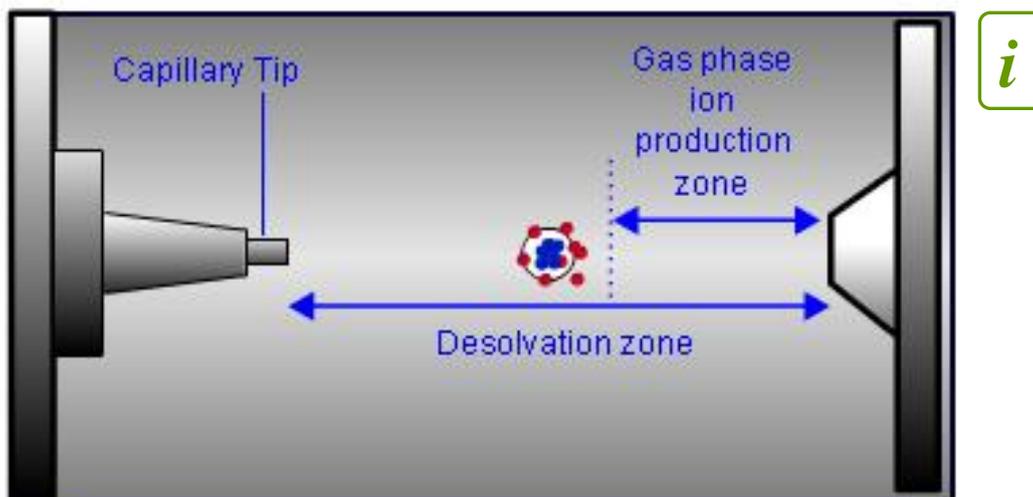
Concentration Dependence of Ion Signal

A further complication in practical terms, is the variability of the function k_A/k_B with analyte concentration –even when both species are present in the electrosprayed eluent in equal concentrations.

For example the ratio $k_{[Pen_4N^+]}/k_{[Et_4N^+]}$ does not remain constant over wide concentration ranges.^[9] At high concentrations the ratio is approximately equal to 7, but at lower concentrations the ratio is approximately 1.

The $[\text{Pen}_4\text{N}^+]$ is more surface active and has a higher sensitivity co-efficient (k) than $[\text{Et}_4\text{N}^+]$, and so it would be expected to be the predominant gas phase ion and give a higher instrument response.

At high concentrations this holds true, as the Pen_4N^+ ions are removed from the droplet surface there are enough ions within the droplet bulk to replace those lost from the surface. The ratio k_A/k_B will be constant across the whole ion production zone within the API interface.

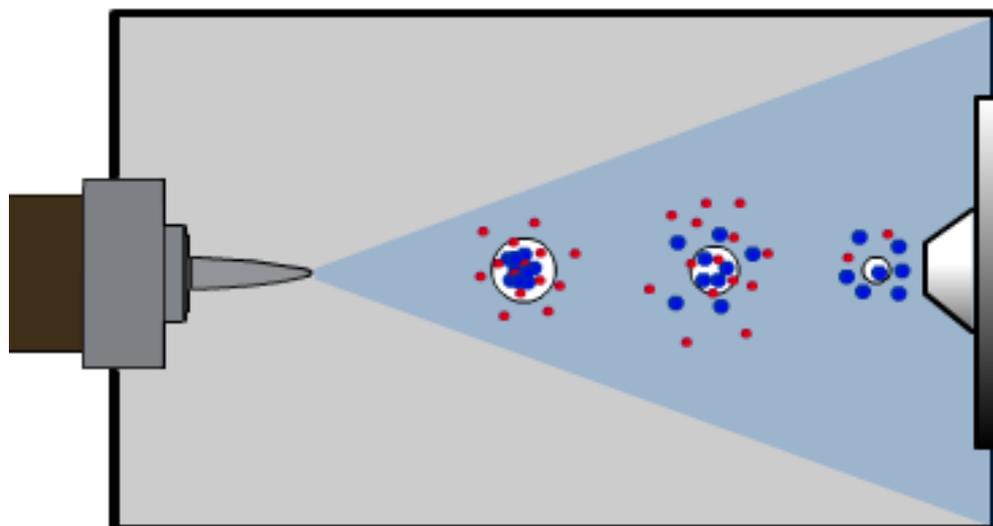


Ion production

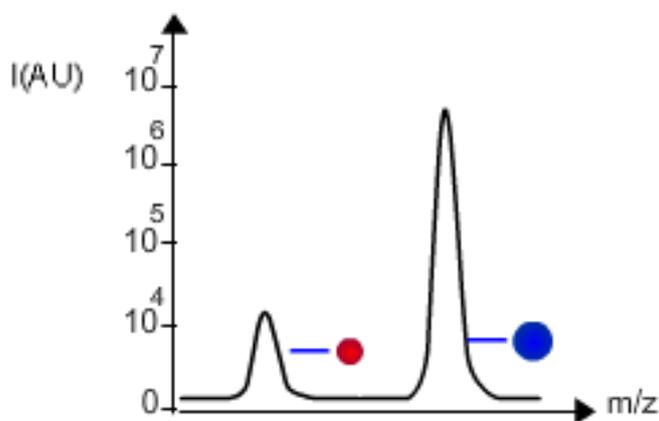
Mass Dependence of Ion Signal

However, at lower analyte concentrations the $[\text{Pen}_4\text{N}^+]$ ions are depleted from the surface, there are not enough ions within the droplet bulk to replenish those lost from the surface through evaporation. The $[\text{Et}_4\text{N}^+]$ ions are lost more slowly which means that at the end these ions will become predominant at the droplet surface. Across the ion production zone, there is a gradient of gas phase ion intensities for both species.

The practical implication is that there will be an optimum distance between the sprayer (Electrospray capillary) and the sampling plate for maximum instrument response for each ion. At lower concentrations, any change in the sprayer will cause the relative response to change.



i

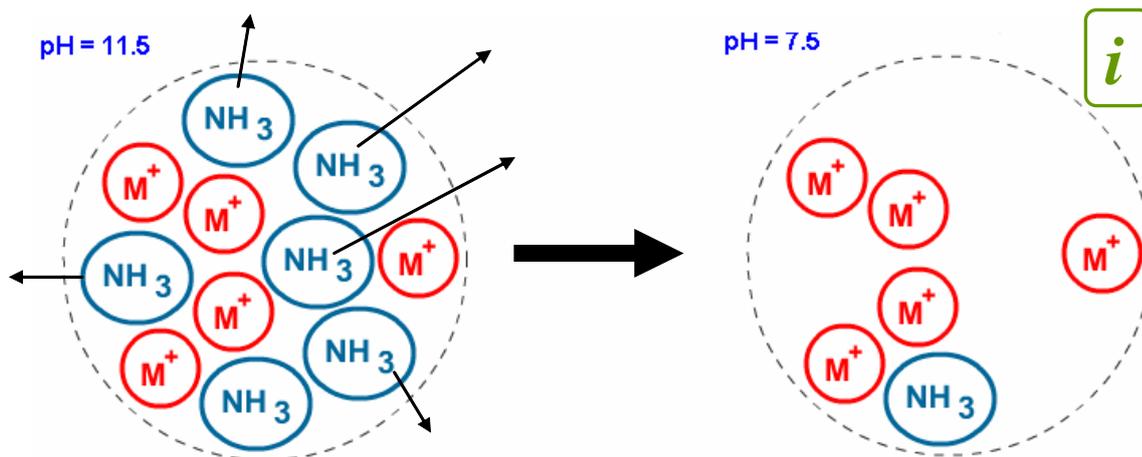


Droplet desorption

Solute Changes in Evaporating Droplets

Solutes and electrolytes whose volatility within the eluent solution differ significantly may expect to undergo changes in relative concentration within the evaporating droplets.

The concentration changes of solutes and electrolytes within droplets are very important as this may affect the physicochemical properties within the droplet solution and also the rate of ion production as has been previously studied. For example, the solute or electrolyte ion may be an acid, base or buffer, which determines the pH of the electrosprayed solution. The electrospray solution pH may influence the mass spectra observed, for example proteins within the eluent may become denatured if the pH of the solution becomes highly acidic which will effect the appearance of the mass spectra. Further, ionisable analytes may be present in the solution, the degree of ionization may change within the API chamber, rendering the analyte effectively neutral and therefore not amenable to transport through the electrostatic systems of the mass spectrometer – drastically reducing instrument sensitivity.



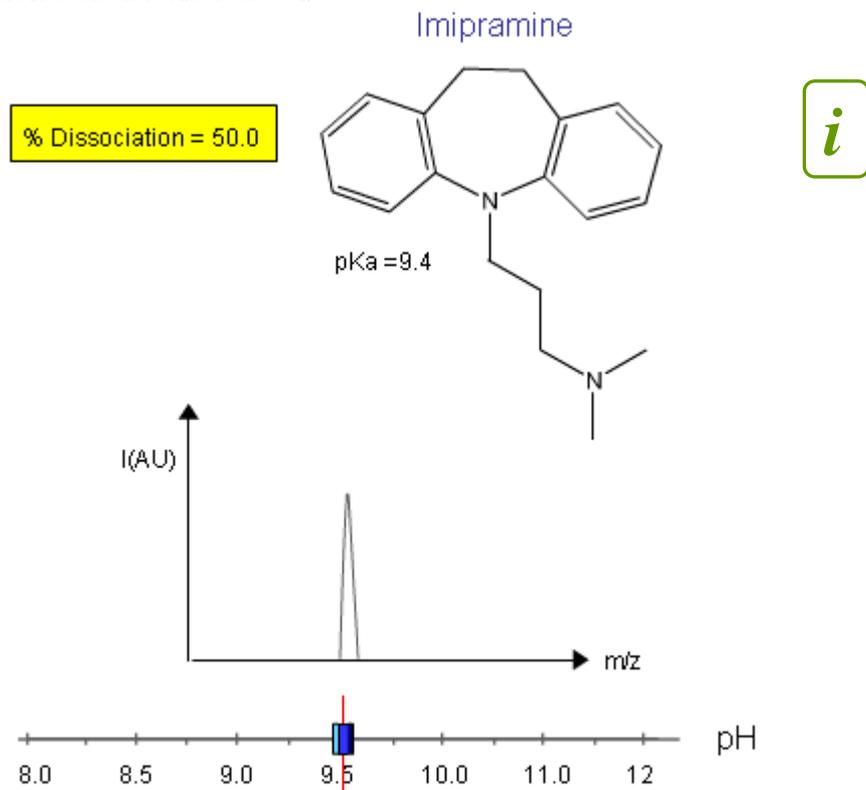
Desorption of basic species leading pH changes

It has been shown that the concentration of volatile ammonia in the presence of formic acid, will decrease in the evaporating droplet, leading to a less basic pH than in the original solution.^[16]

When using acidic modifiers, the concentrations of (for example) formic and acetic acids will increase and this will lead to a more acidic pH in the evaporating droplets, again potentially altering the extent of ionization of the analyte and hence the instrument response.

The pH within the droplet can be expected to change quite drastically. This phenomenon may lead to the suppression of ions within the droplet and therefore may change the quantitative response within the experiment.

The drug molecule shown below (imipramine –a tricyclic antidepressant) has a pK_a of 9.4 (i.e. at pH 9.4 half of the analyte molecules present in solution will be charged). If the pH of the droplet is raised to 10.4 through the loss of any species (for example an ammonia based buffer), then only 10% of the analyte molecules would be charged, significantly decreasing the instrument response.



Conclusions on the Quantitative nature of Electrospray Ionisation

The principles discussed here will apply to most analytes and eluent systems normally studied in LC-MS. To this end, the practitioner should be aware of the limitations of the electrospray technique and should consider the next points.

- Determine the linear working range of the instrument for each analyte
- Choose buffer ions that have low molecular mass, that are highly charged and hydrophilic (highly solvated) –these types of compound are expected to have a low sensitivity co-efficient
- Keep the concentration of the buffer ions to a minimum and work at lower concentrations of analyte (if sensitivity allows), to ensure a stable electrospray and to avoid the problems associated with ion suppression
- If the analyte is expected to have a high sensitivity coefficient and is present at low concentrations, depletion of the species from the droplets may be expected. This will cause the analyte ion to become enriched in the gas phase and may be completely transferred from the droplet. This phenomenon is analytically beneficial but it should be remembered that an alternative electrolyte must be present within the electrosprayed solution in order to carry the charge on the droplet. Further, if more than one analyte needs to be quantified, the use of analogous internal standards (preferably isotopically labelled analogs), should be considered
- Be aware that large changes in the pH solution may occur and analyte species that are sensitive to pH in terms of retaining their charge or molecular conformation may be affected

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