Basics of electrospray ionization

Lutz Alder
Outline

The ionization of the liquid (in positive ESI)
- Formation of droplets with excess of positive charges ions
- Parameters influencing the total ion current

The mechanism of Coulomb explosion
- Properties of the charged “primary” droplets (e.g. size, excess charge)
- Mass and charge balance of formation of secondary and ternary droplets

Consequences of the limited charge in the ESI droplets
- The theoretical model without consideration of matrix
- Influence of buffer on sensitivity and linearity
- The theoretical model with consideration of matrix
- Relationship between of matrix concentration and suppression by matrix
- Matrix effects and linearity

Summary
Processes during ionization of an HPLC eluate with electrospray

ESI source with capillary and orifice without high voltage:
Number of anions and cations in the liquid are identical!
Processes during ionization of an HPLC eluate with electrospray

After switching on the high voltage:
$2 \text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ or
Oxidation of soluted anions to neutrals or
oxidation of metal atoms to cations (e.g. Fe$^{++}$)

typically 4000 V
Processes during ionization of an HPLC eluate with electrospray

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Oxidation of soluted anions to neutrals or oxidation of metal atoms to cations (e.g. Fe\textsuperscript{++})

Formation of the Taylor conus in the electrical field (approx. 1.000.000 V/m)

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Processes during ionization of an HPLC eluate with electrospray

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Oxidation of soluted anions to neutrals or oxidation of metal atoms to cations (e.g. Fe^{++})

Formation of the Taylor conus in the electrical field (approx. 1.000.000 V/m)

Creation of droplets with an excess of positive charges

Typically 4000 V

What influences the total ion current??
Parameters influencing the total ion current

GC-MS with electron impact ionization

0.1 µg/mL hexachlorobenzene

1.0 µg/mL hexachlorobenzene

10 µg/mL hexachlorobenzene

The total ion current depends on the concentration of the analyte!
Parameter influencing the total ion current in a ESI source
(number of excess charges per time interval)

\[ I_{ESI} = A_{Solv} \times E^\varepsilon \times V_f^\nu \times \sigma^n \]

- \( A_{Solv} \): solvent dependent term
- \( E \): field strength in the source (V/m)
- \( V_f \): flow rate (µl/min)
- \( \sigma \): conductivity of the eluent
- \( \varepsilon, \nu, n \): exponents (all \( \leq 0.5 \))

The number of excess charges in the droplets is rising with:
- the ESI voltage
- the flow rate
- With the concentration of buffer in the eluent (conductivity!)

The total ion current does not depend on the concentration of the analyte!

Parameter influencing the total ion current (TIC) in a ESI source

LC-MS with electro-spray ionization

0.1 µg/mL $\rightarrow 2.4 \times 10^8$
fluazifop-butyl

1.0 µg/mL $\rightarrow 2.6 \times 10^8$
fluazifop-butyl

10 µg/mL $\rightarrow 3.9 \times 10^8$
fluazifop-butyl

The concentration of the analyte has a small influence on the total ion current!
Why the TIC is not influenced by analyte concentration?

Reference:

ESI droplets with a fixed number of excess charges on the surface

- $0.1 \mu g/mL = 3 \times 10^{-9} \text{ mol/L}$
- $1 \mu g/mL = 3 \times 10^{-8} \text{ mol/L}$
- $10 \mu g/mL = 3 \times 10^{-7} \text{ mol/L}$
First summary of important aspects of electrospray ionization

1. The electrospray ion source permanently produces a fixed number of ions (about $3 \times 10^{-6}$ mol/l) independent from analyte concentration. These “excess charges” are located on the surface of the droplet.
Outline

The ionization of the liquid (in positive ESI)
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Consequences of the limited charge in the ESI droplets
  • The theoretical model without consideration of matrix
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Summary
Geometry of a charged primary droplet

Volume of a ball = \( \frac{4}{3} \pi r^3 \)

volume if diameter is 1 \( \mu \)m: \( 5,2 \times 10^{-19} \) m\(^3\)
volume if diameter is 10 \( \mu \)m: \( 5,2 \times 10^{-16} \) m\(^3\)

Surface of a ball = \( 4 \pi r^2 \)

surface if diameter is 1 \( \mu \)m: \( 3,1 \times 10^{-12} \) m\(^2\)
surface if diameter is 10 \( \mu \)m: \( 3,1 \times 10^{-10} \) m\(^2\)

1000 droplets with 1 \( \mu \)m diameter have the same volume as one droplet with 10 \( \mu \)m. But the surface area of these 1000 droplets is 10 times higher.

Small droplets are able to carry a higher percentage of charge!
Calculation of the number of elementary charges in the droplet

Calculation is possible with the help of the ESI current

ESI current \( I_{\text{ESI}} \): \( 1 \times 10^{-6} \) A (1 µA)
Flow rate \( V_f \): 200 µl/min = 3.3 µl/s

Amount of charge per µl = \( \frac{I_{\text{ESI}}}{V_f} = 3 \times 10^{-7} \) As/µl

Elementary electric charge \( e \): \( 1.6 \times 10^{-19} \) As
Elementary charges (e) per µl = \( \frac{I_{\text{ESI}}}{V_f} / e \)

\[ = 1.800.000.000.000 \text{ charges (e) per µl} \]

Elementary charges in a droplet of 3 µm (3\( \times \)10\(^{-6}\) m) diameter

volume of droplet \( V_{Tr} \): \( 1.41 \times 10^{-17} \) m\(^3\) = \( 1.41 \times 10^{-8} \) µl \( (V_{Tr} = \frac{4}{3} \pi \times r^3) \)

\( (I_{\text{ESI}} / V_f) / e \) x \( V_{Tr} \) = 25.000 charges/droplet

„Concentration“ of elementary (excess) charges

1.800.000.000.000 e/µl = \( 1.8 \times 10^{12} \) e/µL = \( 1.8 \times 10^{18} \) e/L

\( 1.8 \times 10^{18} \) e×L\(^{-1}\) / \( 6.02 \times 10^{23} \) ×mol\(^{-1}\) = \( 3 \times 10^{-6} \) mol excess charges per L (3 µmol/L)
Number of molecules in a primary droplet

Number of solvent molecules in case of

- water: 470.000.000.000
- methanol: 210.000.000.000
- acetonitrile: 160.000.000.000

Number of soluted analyte molecules (assumed molecular weight: 500 g/mol)

- at 10 ng/ml (= 0.02 µmol/L): 284
- at 100 ng/ml (= 0.2 µmol/L): 2.840
- at 1 µg/ml (= 2 µmol/L): 28.400
- at 10 µg/ml (= 20 µmol/L): 284.000

Remind: We have only 25.000 excess charges (c = 3 µmol/L) in the primary droplet!

At > 1 µg/ml the concentration of analyte molecules is higher than the concentration of excess charges (= 3 µmol/L) in the primary droplet!! Saturation may occur!
Evaporation of a charged primary droplet

Initially the surface tension is much stronger than the repulsion of the excess charges on the surface.

As soon as the diameter is reduced to 50% the repulsion of ions on the surface exceeds the surface tension.

After diameter reduction 15 % of solvent are remaining, only! Due to this solvent evaporation the concentration of analytes (and buffer or matrix) rises by a factor of 7 (=100% / 15%)

This evaporation requires less than 0.4 ms (400 µs).

L. Alder, LAPRW 2011, May 8th to May 11th 2011, Montevideo/Uruguay
Parameter influencing the stability of charged liquid droplets

Rayleigh-Limit:

\[ q^2 = 64\pi^2 \varepsilon \sigma r^3 \]

- \( q \) = maximum of charge in a stable droplet (As)
- \( \varepsilon \) = Permittivity (dielectric constant) of the vacuum
  \((8.86 \times 10^{-12} \text{ As}^2 \text{ N}^{-1} \text{ m}^{-2})\)
- \( \sigma \) = surface tension of the liquid
  (e.g. methanol: 0.023 N m\(^{-1}\))
- \( r \) = radius of droplet (in m)


John William Strutt
3rd Lord Rayleigh
* 12.11.1842
† 30.06.1919
Nobel price in physics in 1904
Process of Coulomb explosion – The first photograph taken 1994

„Flash shadowgraph“ of a primary droplet forming secondary droplets.
Reference:
Mass and charge balance of Coulomb explosion

- **Evaporation**
  - d = 3 µm
  - m = 12 pg
  - 25,000 x e

- **“Explosion”**
  - d = 1,60 µm
  - m = 1,69 pg
  - 25,000 x e

- **Formation of ternary droplets**
  - d = 0,16 µm = 160 nm
  - m = 0,0016 pg
  - 20 x 190 x e

- **20 secondary droplets**
  - In total 2 % of mass and 15% of charge
  - 50% of Rayleigh limit

- **Up to 15 “explosions” of the primary droplet after further evaporation**
  - d = 1,59 µm
  - m = 1,66 pg
  - 21,200 x e

- **98 % of mass**
  - 85% of charges

- **20 droplets á:**
  - d = 16 nm

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Properties of a charged secondary droplet

Number of solvent molecules in case of

- water: 71.000.000
- methanol: 32.000.000 0.015 %
- acetonitrile: 25.000.000

Calculated number of soluted analyte molecules

- at 10 µg/ml (= 20 µmol/L): 280
- at 1 µg/ml (= 2 µmol/L): 28 0.10 %
- at 100 ng/ml (= 0.2 µmol/L): 3
- at 10 ng/ml (= 0.02 µmol/L): 0.3

Only if **analytes** are able to occupy more surface than the **buffer**, they will occur in higher concentrations in the secondary droplets!

If the secondary droplet “explodes” we get …
The ternary droplet: ready for ion evaporation

typical diameter: 10 nm

Calculated number of solvent molecules in case of
water: 18.000
methanol: 8.000
acetonitrile: 6.000

Rayleigh-Limit: ≤ 50 elementary charges

Ratio between charges (protonated analytes) and solvent (water) molecules:
18 / 18.000 = 1 / 1.000

Ratio between protons and water molecules in one liter of $10^{-1}$ mol/L HCl (= pH 1):
0.1 mol / 50 mol = 1 / 500

During solvent evaporation and preferred transfers of ions from the surface during Coulomb explosion droplets become extremely acidic!
The ternary droplet: ready for ion evaporation

Calculated number of solvent molecules in case of:
- water: 18,000
- methanol: 8,000
- acetonitrile: 6,000

Rayleigh-Limit: < 50 elementary charges

100 water molecules are sufficient to fill the circumference

Circumference = \( \pi \times d = 3.14 \times 10 \text{ nm} = 31.4 \text{ nm} \)

Diazinon

Saturation with analyte means, 25 to 50 quasimolecular ions of such size are spread over the surface.

But which analytes may better occupy the surface and evaporate as ions?
Effect of polarity of analytes on the efficiency of ionization

Relation between response and ”nonpolar surface area” of six similar tripetides

Reference:
Effect of polarity of analytes on the efficiency of ionization

97 pesticides; concentration generally 100 ng/ml

- High water content in eluent: elution of polar pesticides
- Elution of less polar pesticides with higher surface activity
Second summary of important aspects of electrospray ionization

1. The electrospray ion source permanently produces a fixed number of ions (about $3 \times 10^{-6}$ mol/l) independent from analyte concentration. These “excess charges” are located on the surface of the droplet.

2. If a molecule (analyte) wants to appear in the mass spectrum it must successfully compete for a place on the charged surface of the droplets!
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  • The theoretical model without consideration of matrix
  • Influence of buffer on sensitivity and linearity
  • The theoretical model with consideration of matrix
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Summary
The theoretical model (without co-eluting matrix)

**Basic Assumption:**
Equilibration between the inner part of the droplet and the surface layer

$$[\text{NH}_4^+\text{COOH}^-] + [\text{NH}_4^+] = \text{concentration of buffer (C_B)}$$

$$K_E = \frac{[\text{NH}_4^+]}{[\text{NH}_4^+\text{COOH}^-] \times [\text{H}^+]}$$

A = Analyte

$$[\text{A+H}]^+ = \text{quasimolecular ion of A}$$

$$K_A = \frac{[\text{A+H}^+]}{[\text{A}] \times [\text{H}^+]}$$

$$[\text{A+H}]^+ + [\text{NH}_4^+] = \text{concentration of excess charges (Q)}$$

The theoretical model (without co-eluting matrix)


Basic Assumption:
Equilibration between the inner part of the droplet and the surface layer

\[
\begin{align*}
[\text{NH}_4^+\text{COOH}^-] + [\text{NH}_4^+] &= \text{concentration of buffer (C}_B\text{)} \\
K_E &= \frac{[\text{NH}_4^+]}{[\text{NH}_4^+\text{COOH}^-] \times [\text{H}^+]} \\
A &= \text{Analyte} \\
[A+H]^+ &= \text{quasimolecular ion of A} \\
K_A &= \frac{[A+H^+]}{[A] \times [\text{H}^+]} \\
[A+H]^+ + [\text{NH}_4^+] &= \text{concentration of excess charges (Q)}
\end{align*}
\]
The theoretical model (without co-eluting matrix)

**Equation to calculate the analyte concentration** \([A+H]^+\) **on surface**

\[
a \times [A+H]^+^2 + b \times [A+H]^+ + c = 0
\]

- \(a = K_A/K_E - 1\)
- \(b = -(Q \times (K_A/K_E - 1) + C_A \times (K_A/K_E) + C_E)\)
- \(c = C_A \times Q \times (K_A/K_E)\)

- \([A+H]^+\) concentration of the quasimolecular ion of the analyte
- \([Q = [NH_4^+] + [A+H^+]\) total number of excess charges
- \([C_A = [A+H]^+ + [A]\) total concentration of the analyte; \(0 \ldots 10^{-6}\) mol/L
- \([C_B = [NH_4^++COOH^-] + [NH_4^+]\) concentration of buffer (ammonium formiate); \(10^{-4} \ldots 10^{-2}\) mol/L
- \([K_A = [A+H^+] / [A] \times [H^+]\) equilibrium constant of analyte
- \([K_E = [NH_4^+] / [NH_4^++COOH^-] \times [H^+]\) equilibrium constant of electrolyte

Application of the theoretical model

The influence of buffer concentration on sensitivity and linearity

Buffer concentration: $10^{-3}$ mol/L

Red calibration line:
- analyte with best ability to stay on surface ($K_A/K_E = 1000$)
- analyte with $K_A/K_E = 100$
- analyte with $K_A/K_E = 10$
- analyte with $K_A/K_E = 1$

Buffer concentration: $10^{-4}$ mol/L

Reduction of buffer concentration will enhance sensitivity, but simultaneously this reduction will lower linearity!
The theoretical model (with co-eluting matrix)


Additional parameter and equations:

\[ M = \text{(uncharged) matrix} \]

\[ [M+H]^+ = \text{quasimolecular ion of matrix} \]

\[ K_M = \frac{[M+H^+]}{[M] \times [H^+]} \]

\[ [A+H]^+ + [NH_4^+] + [M+H]^+ = \text{concentration of excess charges (Q)} \]
The theoretical model (with co-eluting matrix)


Additional parameter and equations:

\[ [M+H]^+ = \text{quasimolecular ion of matrix} \]

\[ K_M = \frac{[M+H^+]}{[M] \times [H^+]} \]

\[ [A+H]^+ + [NH_4^+] + [M+H]^+ = \text{concentration of excess charges (Q)} \]

The theory may explain in which extent the analyte concentration on the surface is a function of concentration and surface activity of matrix!

…but the calculation of \([A+H]^+\) becomes more demanding …
The theoretical model (with co-eluting matrix)

Equation to calculate the analyte concentration $[A+H]^+$ on surface

\[
a \times [A+H]^+^3 + b \times [A+H]^+^2 + c \times [A+H]^+ + d = 0
\]

\[
a = K_M - K_A + K_E \left(1 - \frac{K_M}{K_A}\right)
\]

\[
b = C_A \left(2K_A - K_M - K_E\right) + C_B \left(K_M - K_E \frac{K_M}{K_A}\right) + C_E K_E \left(1 - \frac{K_M}{K_A}\right) + Q \left(K_A - K_M - K_E \left(1 - \frac{K_M}{K_A}\right)\right)
\]

\[
c = -C_A \left(Q \left(2K_A - K_M - K_E\right) + C_B K_M + C_A K_A + C_E K_E\right)
\]

\[
d = C_A^2 \times Q \times K_A
\]

concentration of the quasimolecular ion of the analyte

\[
Q = [NH_4^+] + [A+H^+]
\]

total number of excess charges

\[
C_A = [A+H]^+ + [A]
\]

total concentration of the analyte; $0 \ldots 10^{-6}$ mol/L

\[
C_B = [NH_4^+COOH^-] + [NH_4^+]
\]

concentration of buffer (ammonium formiate); $10^{-4} \ldots 10^{-2}$ mol/L

\[
C_M = [M+H]^+ + [M]
\]

total concentration of the matrix substance; $0 \ldots 10^{-4}$ mol/L

\[
K_A = [A+H^+] \times [A] \times [H^+]
\]

equilibrium constant of analyte

\[
K_E = [NH_4^+] \times [NH_4^+COOH^-] \times [H^+]
\]

equilibrium constant of electrolyte

\[
K_M = [M+H^+] \times [M] \times [H^+]
\]

equilibrium constant of matrix substance

The theoretical model (with co-eluting matrix)

The influence of matrix’ ability to occupy the surface on \([A+H]^+\)

**electrolyte:**
- \(K_E = 1\)
- \(C_E = 5\) mmol/L

**analyte:**
- \(K_A = 1000\)
- \(C_A = 300\) ng/ml

**matrix:**
- \(K_M = 10 \ldots 1000\)
- \(C_M = 0,0001\ldots10\) µg/mL

*What we know:*
Matrix’ ability to occupy droplet’s surface is crucial for the extent of matrix effects!
The theoretical model (with co-eluting matrix)

The influence of analyte’s ability to occupy the surface

- **Electrolyte:**
  - $K_E = 1$
  - $C_M = 5 \text{ mmol/L}$

- **Analyte:**
  - $K_A = 1 \ldots 1000$
  - $C_A = 300 \text{ ng/ml}$

- **Matrix:**
  - $K_M = 1000$
  - $C_M = 0.0001 \ldots 10 \mu g/mL$

**Prediction of theory:**

Analyte’s ability to occupy droplet’s surface is important for the sensitivity but not important for the extent of matrix effects!
The theoretical model (with co-eluting matrix)

The influence of analyte’s ability to occupy the surface

electrolyte:
\( K_E = 1 \)
\( C_M = 5 \text{ mmol/L} \)

analyte:
\( K_A = 1 \ldots 1000 \)
\( C_A = 300 \text{ ng/ml} \)

matrix:
\( K_M = 300 \)
\( C_M = 0,0001 \ldots 10 \mu g/mL \)

Prediction of theory:
The similarity of analyte’s behavior does not depend on matrix properties!
The prediction of the theoretical model for dilute and shoot

The influence of matrix concentration on analyt’s response

electrolyte:
\[ K_E = 1 \]
\[ C_M = 5 \text{ mmol/L} \]

analyte:
\[ K_A = 1 \ldots 1000 \]
\[ C_A = 0.003 \ldots 300 \text{ ng/ml} \]

matrix:
\[ K_M = 300 \]
\[ C_M = 0.0001 \ldots 10 \text{ µg/mL} \]

**Prediction of theory:**
During the first dilution steps matrix effect depends on the logarithm of matrix concentration!
What is the prediction of the theoretical model for the influence of matrix on linearity?

electrolyte:
\[ K_E = 1 \]
\[ C_M = 1 \text{ mmol/L} \]

analyte:
\[ K_A = 1000 \]
\[ C_A = 0.3 \ldots 1300 \text{ ng/ml} \]

Do you remember slide 31?
This slide had shown a non-linear calibration line in the absence of matrix.
The prediction of the theoretical model for the influence of matrix on linearity

electrolyte:
\[ K_E = 1 \]
\[ C_M = 1 \text{ mmol/L} \]

analyte:
\[ K_A = 1000 \]
\[ C_A = 0.3 \ldots 1300 \text{ ng/ml} \]

matrix:
\[ K_M = 10 \text{ or } 100 \]
\[ C_M = 10 \text{ µg/mL} \]

Matrix substances (or analytes) with weak tendency to occupy the surface have small influence.
The prediction of the theoretical model for the influence of matrix on linearity

electrolyte:
\[ K_E = 1 \]
\[ C_M = 1 \text{ mmol/L} \]

analyte:
\[ K_A = 1000 \]
\[ C_A = 0.3 \ldots 1300 \text{ ng/ml} \]

matrix:
\[ K_M = 300 \text{ or } 1000 \]
\[ C_M = 10 \mu\text{g/mL} \]

Matrix substances (or analytes) with strong tendency to occupy the surface make the calibration line more linear.

non-linear calibration line in the absence of matrix

fixed y-axis

rescaled y-axis

Matrix substances (or analytes) with strong tendency to occupy the surface make the calibration line more linear.
Last summary of important aspects of electrospray ionization

1. If a molecule (analyte) wants to appear in the mass spectrum it must successfully compete for a place on the charged surface of the droplets!

2. The electrospray ion source permanently produces a fixed number of ions (about $3 \times 10^{-6}$ mol/l) independent from analyte concentration. These “excess charges” are located on the surface of the droplet.

3. Based on theory matrix effects strongly depend on the ability of the matrix to occupy the surface of droplets.

4. Based on theory matrix effects do not seriously depend on the ability of the analyts to occupy the surface of droplets. Analytes behave very similar.

5. The linearity should become better if more buffer is used or if matrix co-elutes with analytes (or if several analytes coelute).
Many thanks for listening!

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Relationship between eluent conductivity and droplet size

\[ R \approx \sqrt[3]{(V_f \times \varepsilon / \sigma)} \]

- \( R \): radius of primary droplets
- \( V_f \): flow rate (\( \mu l/min \))
- \( \varepsilon \): permittivity
- \( \sigma \): conductivity of the eluent

Smaller droplets at higher conductivity!

Smaller droplets means smaller number of excess charges per droplet but \textbf{higher number of charges per mL}!

Process of Coulomb explosion – Development of Rayleigh Jets

Pictures of Coulomb explosion of a large droplet of ethylene glycol ($r = 58\mu m, 20,000,000$ elementary charges) taken with an high speed microscope in combination with an helium-neodym laser.

Evaporated primary droplet

$\Delta t = 140\ \mu s$  \hspace{1cm}  $\Delta t = 150\ \mu s$

Reference:
Process of Coulomb explosion – Development of Rayleigh Jets

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Pictures of Coulomb explosion of a large droplet of ethylene glycol \((r = 58\mu m, 20.000.000\) elementary charges) taken with an high speed microscope in combination with an helium-neodym laser.

\[ \Delta t = 140 \mu s \quad \Delta t = 150 \mu s \quad \Delta t = 155 \mu s \quad \Delta t = 160 \mu s \quad \Delta t = 180 \mu s \quad \Delta t = 210 \mu s \]

Reference: