BfR

Risiken erkennen – Gesundheit schützen

FEDERAL INSTITUTE
FOR RISK ASSESSMENT

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

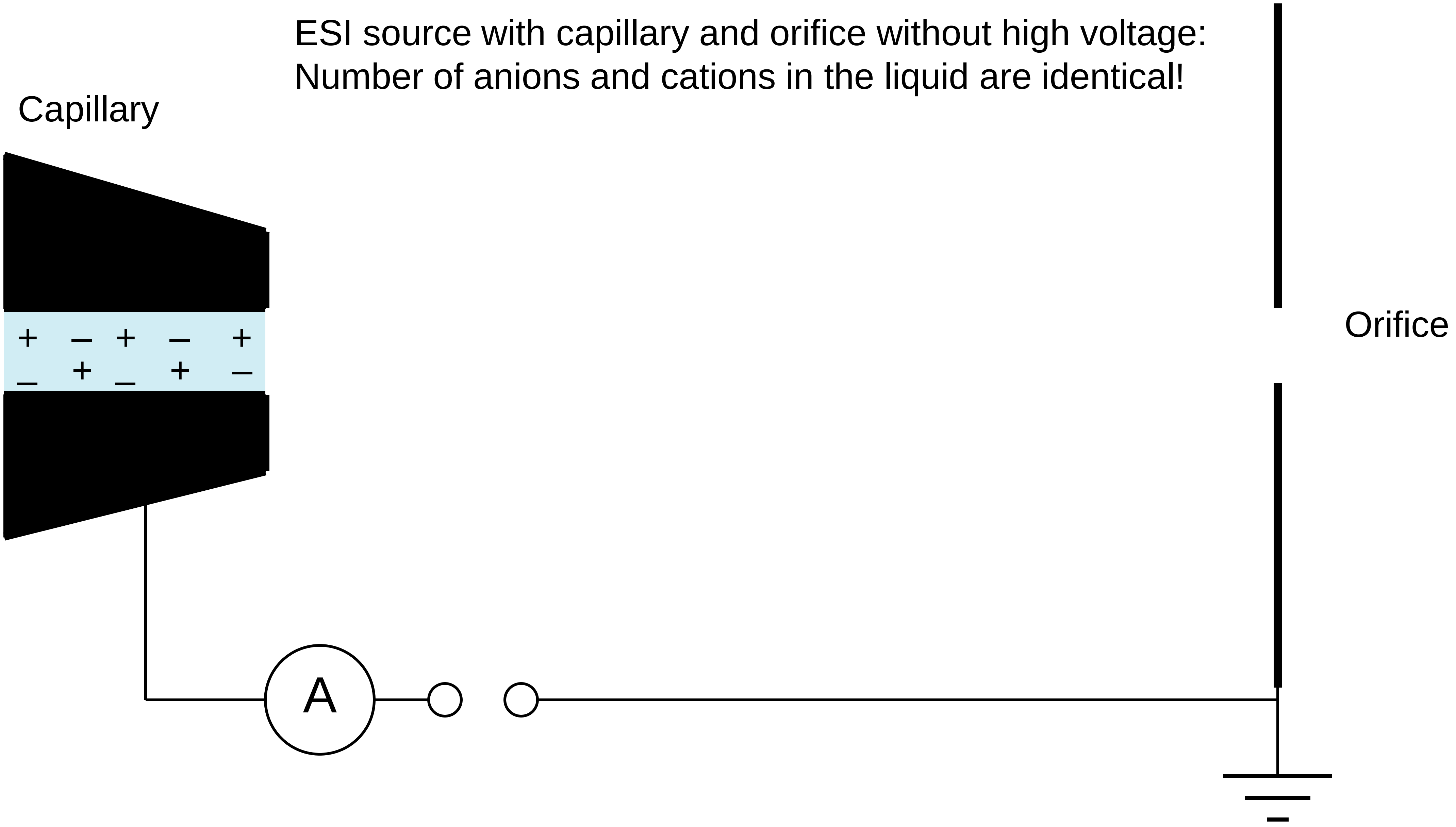
- Properties of the charged “primary” droplets (e.g. size, excess charge)
- Mass and charge balance of formation of secondary and ternary droplets

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

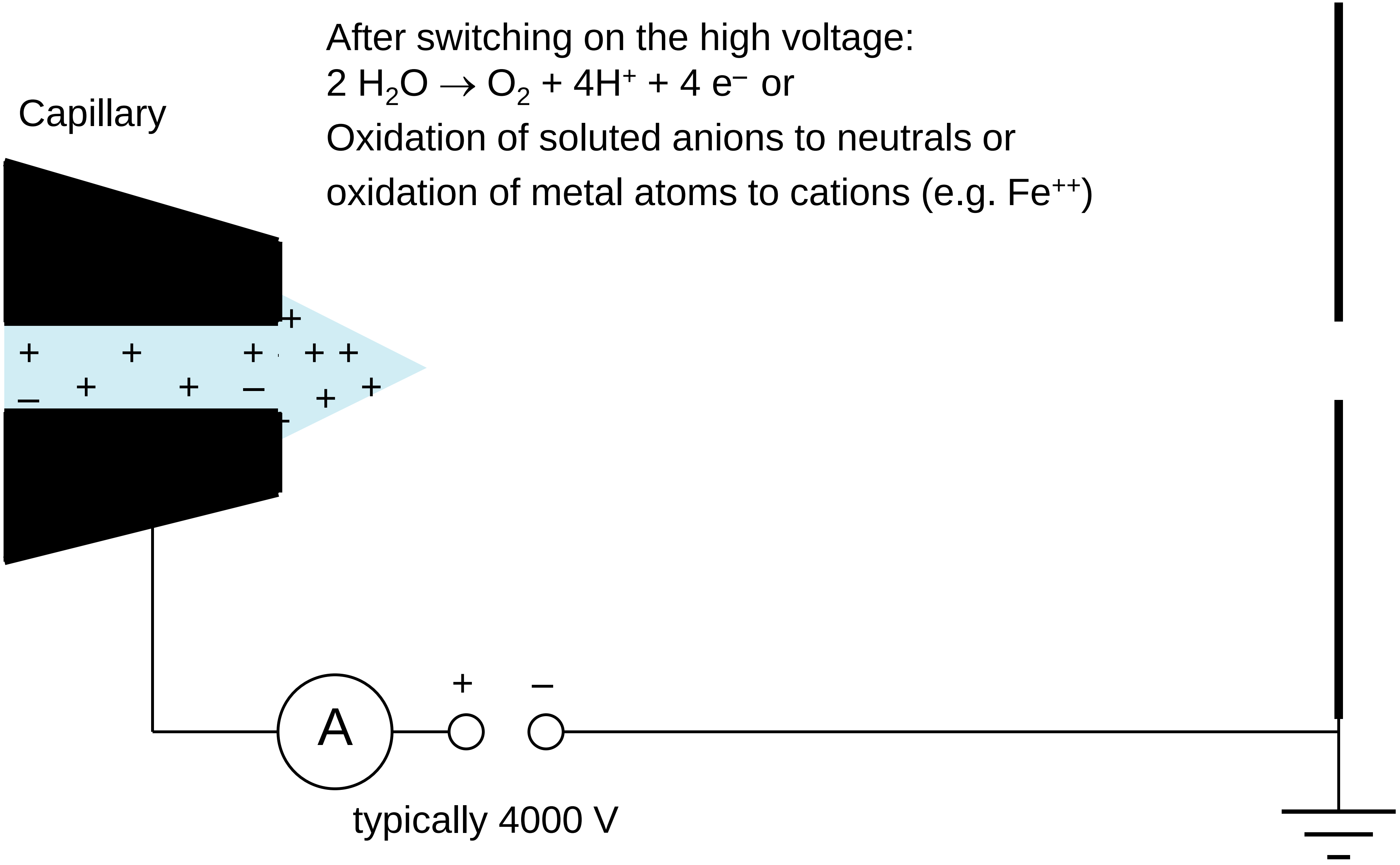
Processes during ionization of an HPLC eluate with electrospray



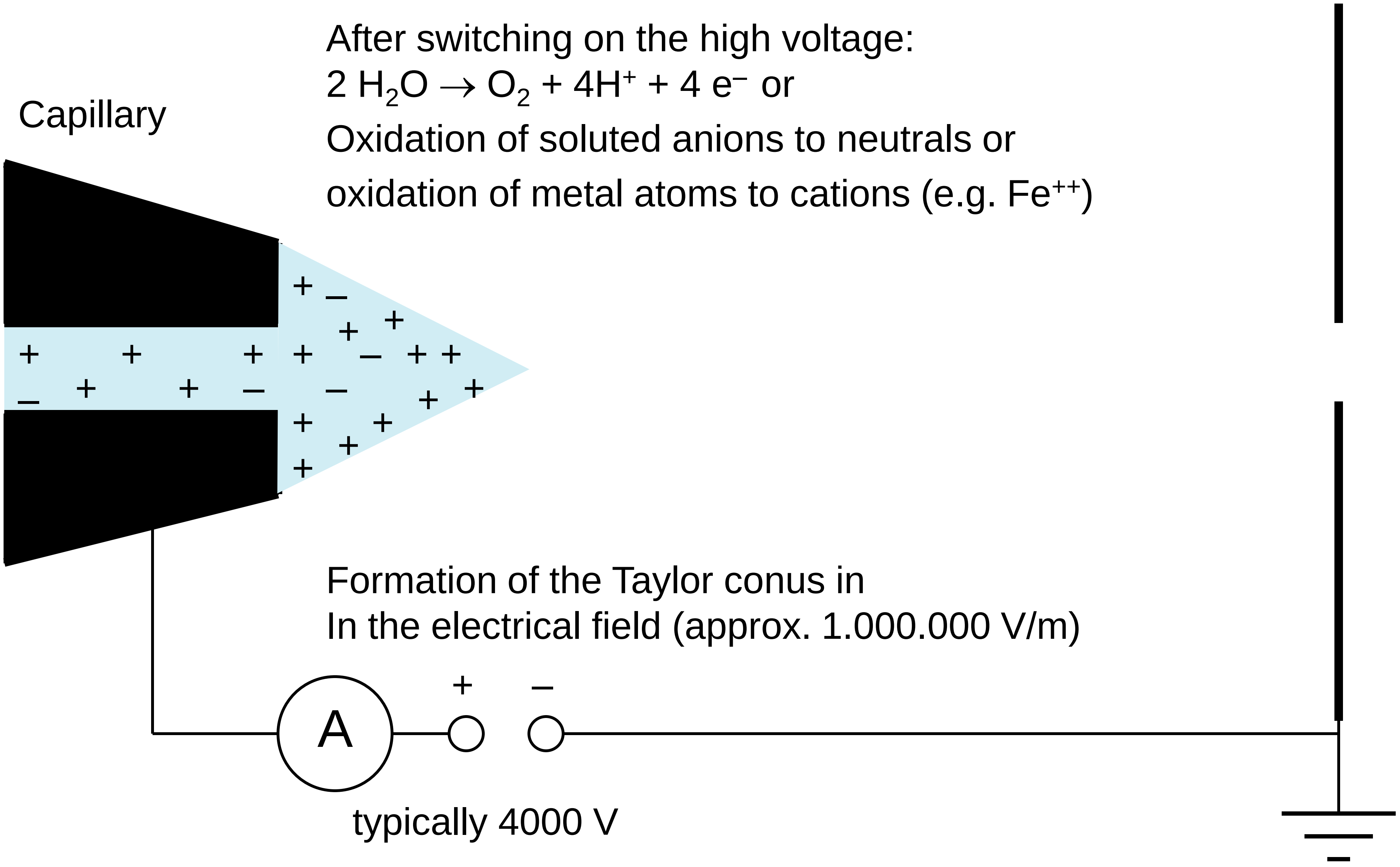
ESI source with capillary and orifice without high voltage:
Number of anions and cations in the liquid are identical!

Processes during ionization of an HPLC eluate with electrospray

After switching on the high voltage:
$2\ H_2O \rightarrow O_2 + 4H^+ + 4\ e^-$ or
Oxidation of soluted anions to neutrals or
oxidation of metal atoms to cations (e.g. Fe^{++})



Processes during ionization of an HPLC eluate with electrospray



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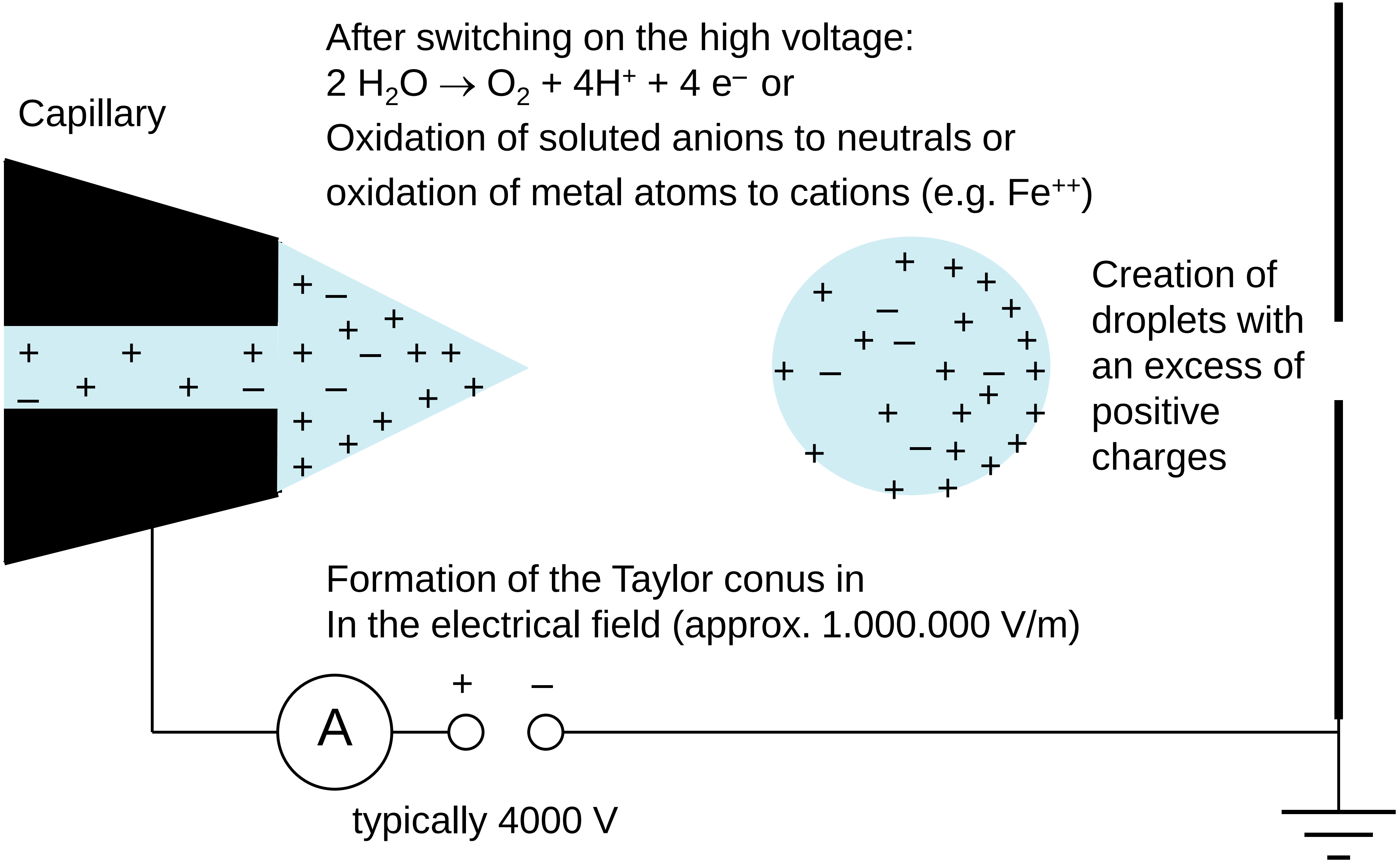
Formation of the Taylor conus in
In the electrical field (approx. 1.000.000 V/m)

Processes during ionization of an HPLC eluate with electrospray



After switching on the high voltage:
$2\ H_2O \rightarrow O_2 + 4H^+ + 4\ e^-$ or
Oxidation of soluted anions to neutrals or
oxidation of metal atoms to cations (e.g. Fe^{++})

Capillary

Creation of droplets with an excess of positive charges

Orifice

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

typically 4000 V

What influences the total ion current??

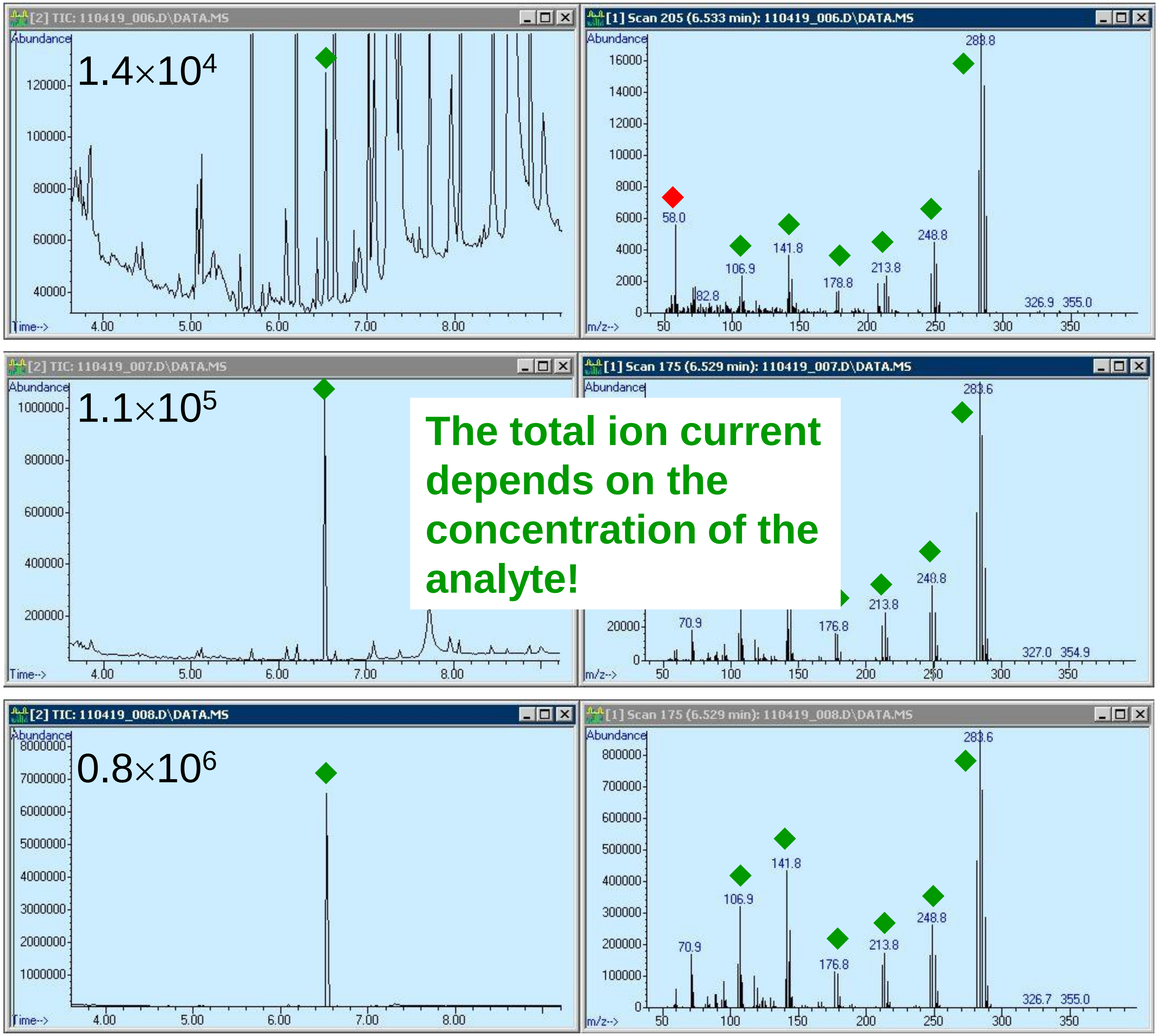
Parameters influencing the total ion current

GC-MS with electron impact ionization

0.1 µg/mL hexachlorobenzene

1.0 µg/mL hexachlorobenzene

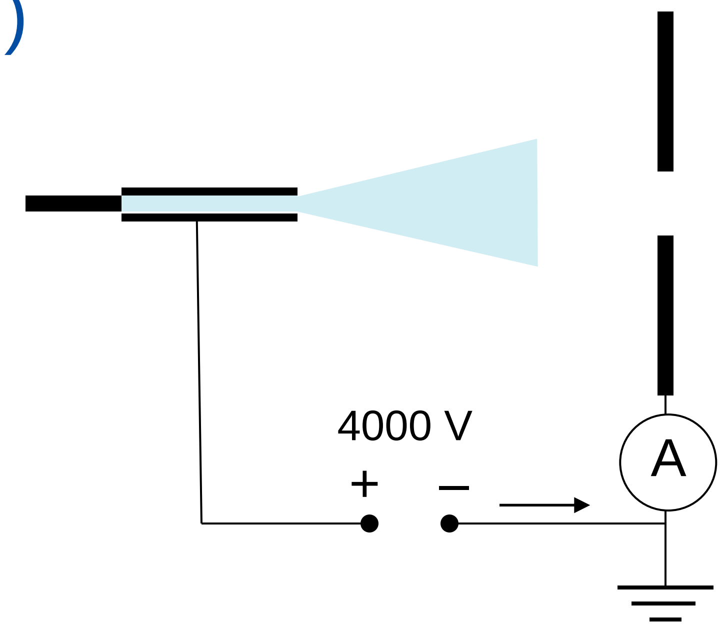
10 µg/mL hexachlorobenzene



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)

$$I_{ESI} = A_{Solv} \times E^{\varepsilon} \times V_f^{v} \times \sigma^{n}$$



A_{Solv}: solvent dependent term
E: field strength in the source (V/m)
V_f: flow rate (µl/min)
σ: conductivity of the eleuent
ε, v, 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!

R.J. Pfeiffer und C.D. Hendricks; AIAA J 6 (1968) 496 (Journal of American Institute of Aeronautics and Astronautics)

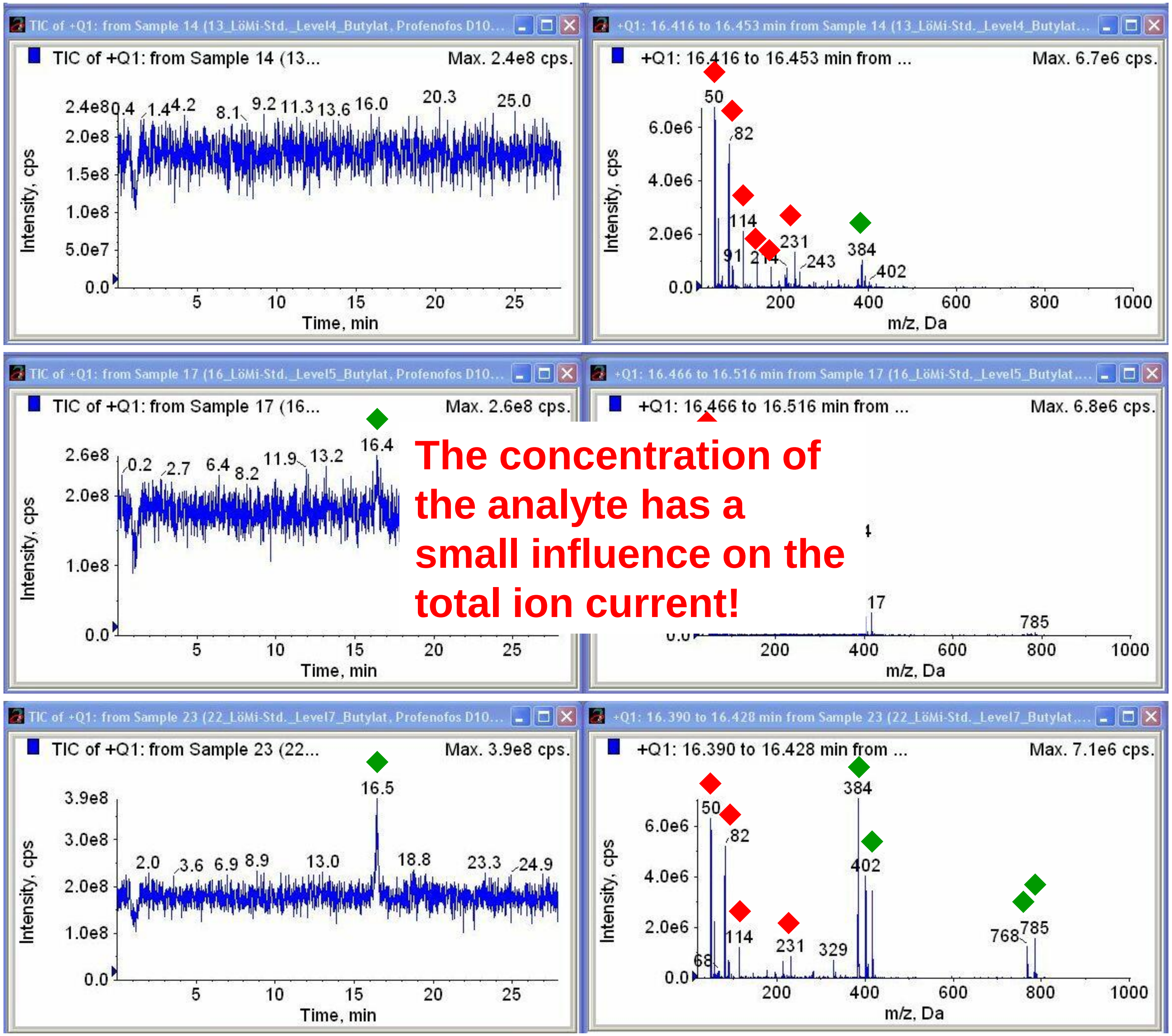
Parameter influencing the total ion current (TIC) in a ESI source

LC-MS with electro-spray ionization

0.1 µg/mL fluazifop-butyl 2.4×10^8

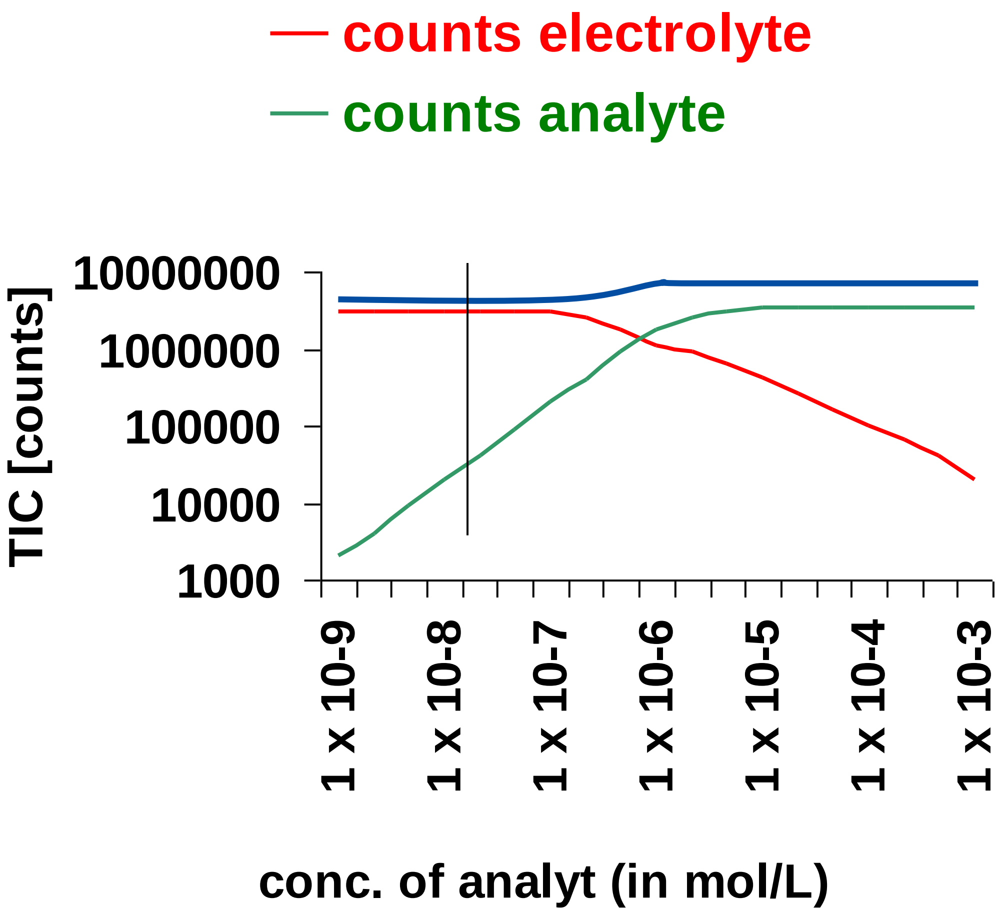
1.0 µg/mL fluazifop-butyl 2.6×10^8

10 µg/mL fluazifop-butyl 3.9×10^8

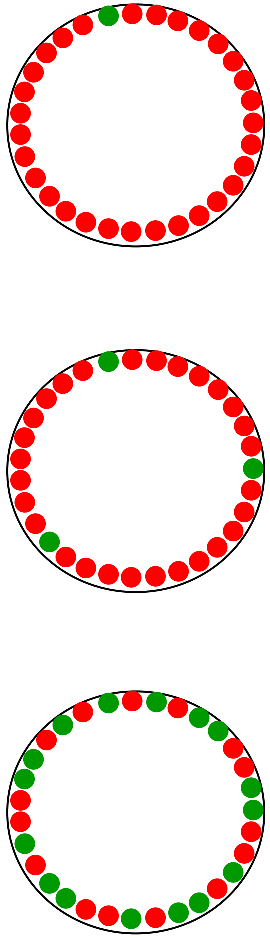


The concentration of the analyte has a small influence on the total ion current!

Why the TIC is not influenced by analyte concentration?



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



0.1 µg/mL =
3×10^{-9} mol/L

1 µg/mL =
3×10^{-8} mol/L

10 µg/mL =
3×10^{-7} mol/L

Reference:
Z Olumee, J.H. Callahan and A. Vertes;
J. Phys. Chem. 102 (1998) 9154

First summary of important aspects of electrospray ionization

1. The electrospray ion source permanently produces a fixed number of ions (about 3×10^{-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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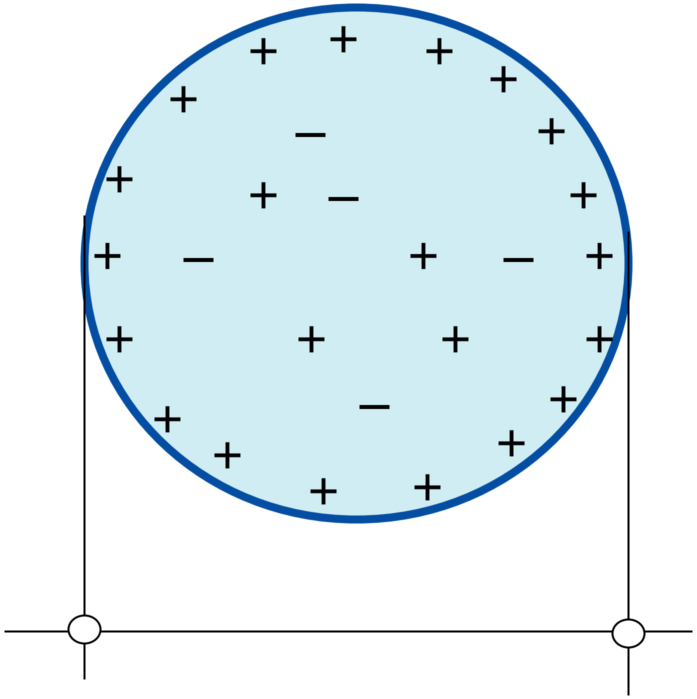
Geometry of a charged primary droplet



diameter: 1 – 10 µm
(small droplets are
formed with narrow
capillaries at low
flow rates)

Volume of a ball = 4/3 $\pi \times r^3$

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

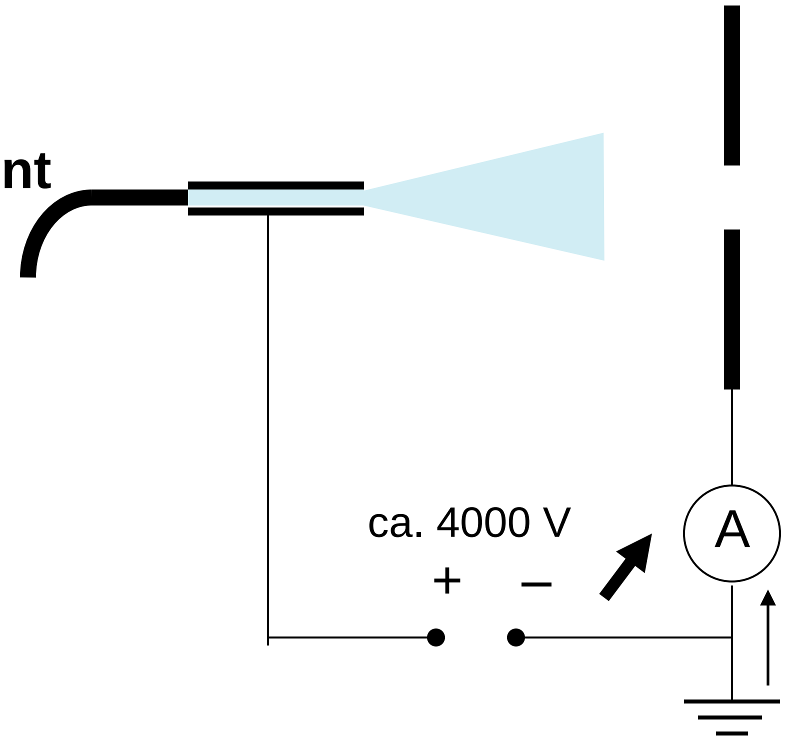
Surface of a ball = 4 $\pi \times r^2$

surface if diameter is 1 µm: $3{,}1 \times 10^{-12}$ m^2
surface if diameter is 10 µm: $3{,}1 \times 10^{-10}$ m^2

1000 droplets with 1 µm diameter have the same volume as one droplet with 10 µm. But the surface area of these 1000 droplets is 10 times higher.

Small droplet 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_{ESI}: 1×10^{-6} A (1 µA)

Flow rate V_f: 200 µl/min = 3.3 µl/s

Amout of charge per µl = I_{ESI} / V_f = 3×10^{-7} As/µl

Elementary electric charge *e*: $1{,}6\times10^{-19}$ As

Elementary charges (*e*) per µl = (I_{ESI} / V_f) / *e*

= **1.800.000.000.000 charges (*e*) per µl**

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

volume of droplet V_{Tr}: $1{,}41\times10^{-17}\,m^3 = 1{,}41\times10^{-8}$ µl ($V_{Tr} = 4/3\,\pi \times r^3$)

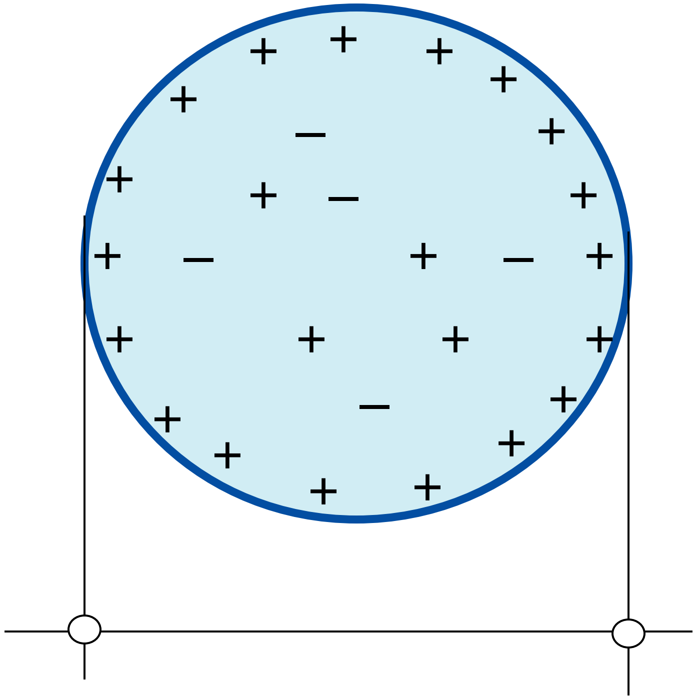
(I_{ESI} / V_f) / *e*) x V_{Tr} = **25.000 charges/droplet**

„Concentration“ of elementary (excess) charges

1.800.000.000.000 *e*/µl = 1.8×10^{12} *e*/µL = 1.8×10^{18} *e* /L

$1.8\times10^{18}\,e\times L^{-1} / 6.02\times10^{23} \times mol^{-1}$ = **3×10^{-6} mol excess charges per L (3 µmol/L)**

Number of molecules in a primary droplet



assumed diameter: 3 µm

Number of solvent molecules in case of

water:	470.000.000.000
methanol:	210.000.000.000
acetonitrile:	160.000.000.000

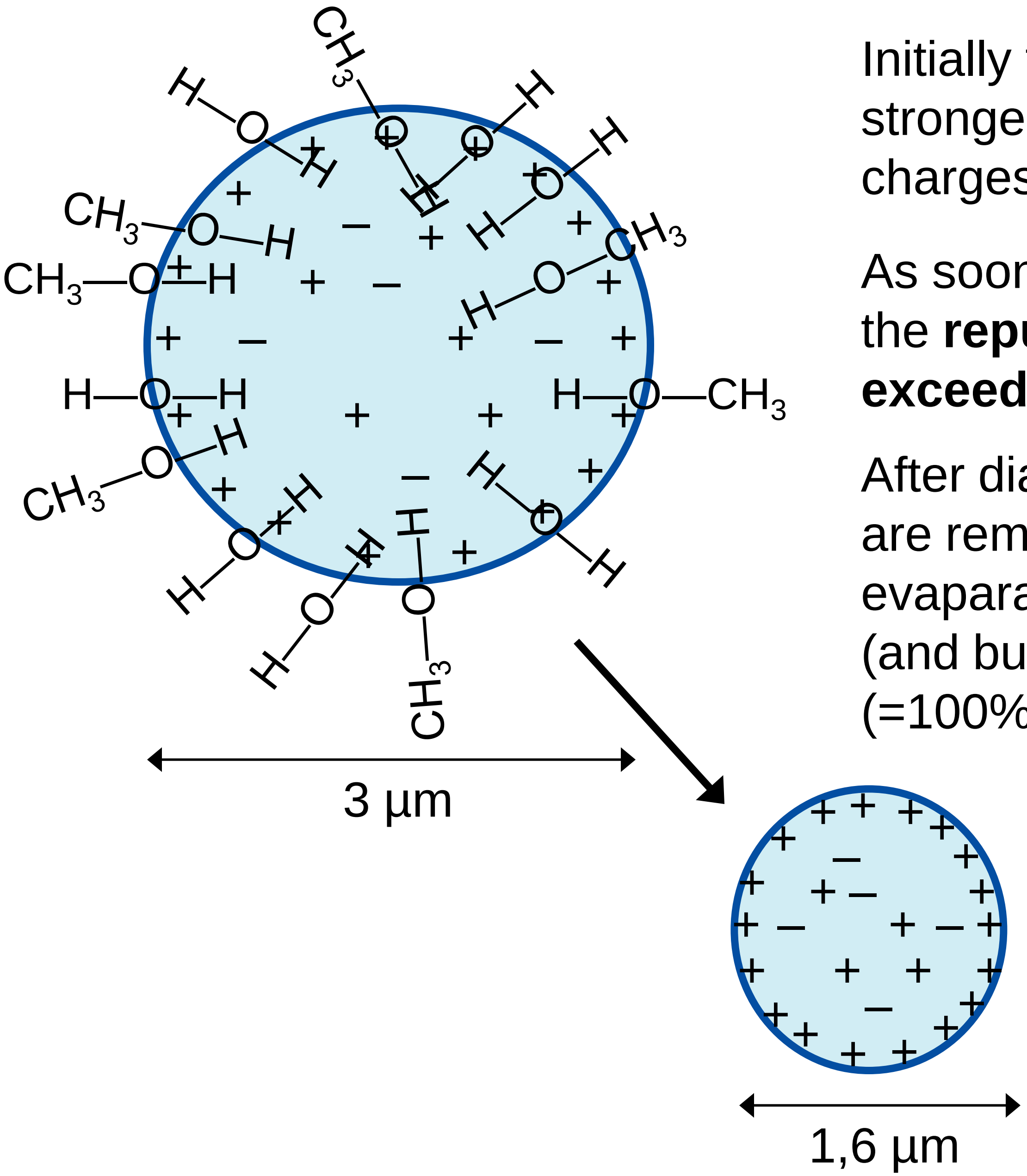
Number of soluted analyte molecules (assumed molecular weight: 500 g/mol)

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 evaparation 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).

Parameter influencing the stability of charged liquid droplets

Rayleigh-Limit:

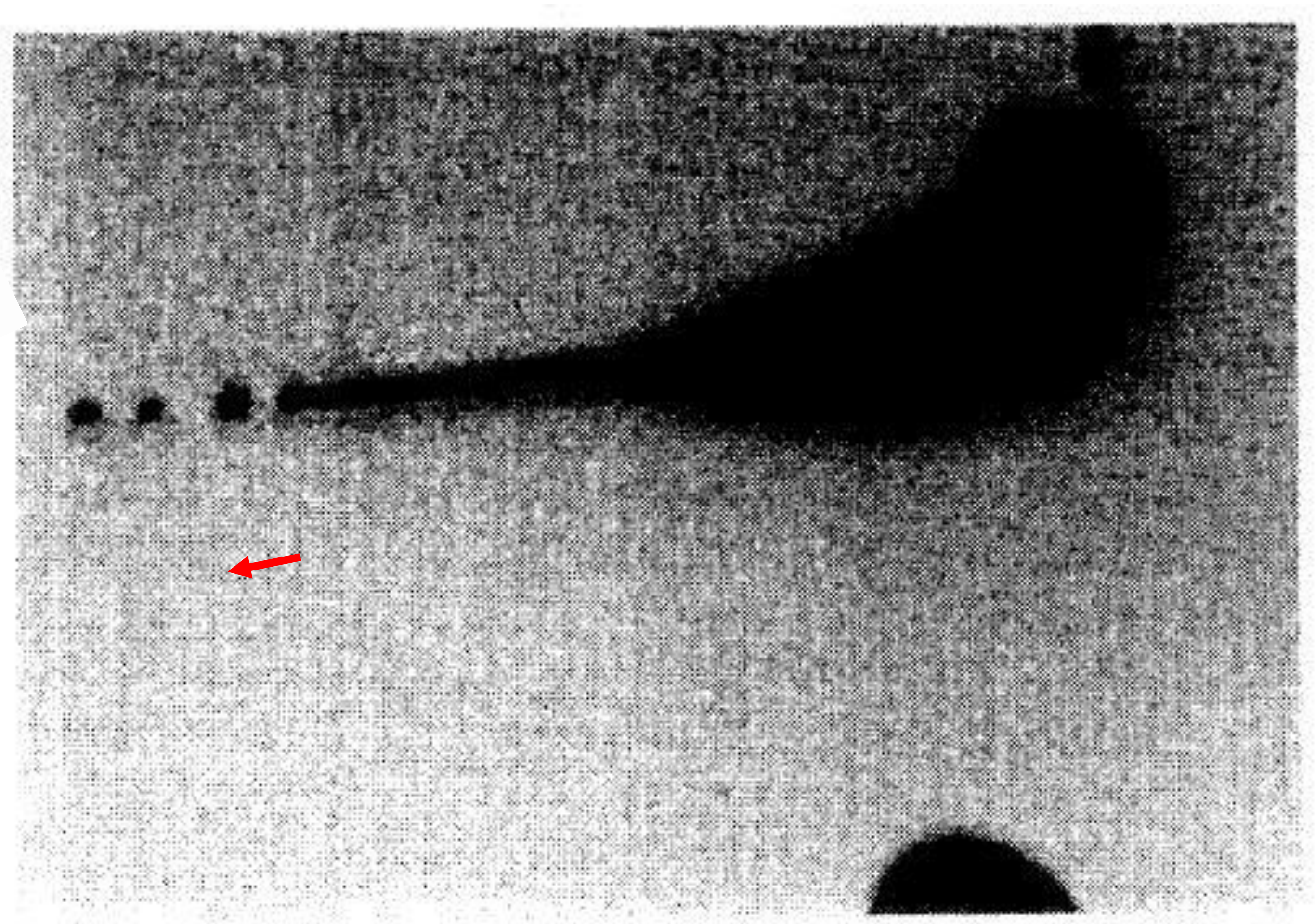
$$q^2 = 64\pi^2 \varepsilon \sigma r^3$$

- q = maximum of charge in a stable droplet (As)
- ε = Permittivity (dielectric constant) of the vacuum ($8{,}86 \times 10^{-12}$ $As^2 N^{-1} m^{-2}$)
- σ = 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

J.W. Strutt: On the Equilibrium of Liquid Conducting. Masses Charged with Electricity. Phil. Mag. 5, 14 (1882) pp. 184-186).

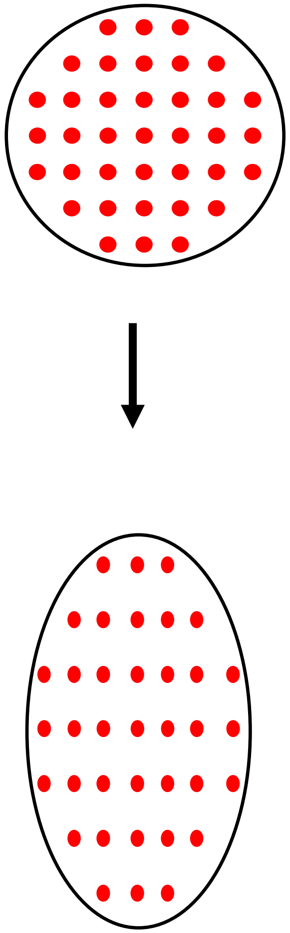
Process of Coulomb explosion – The first photograph taken 1994



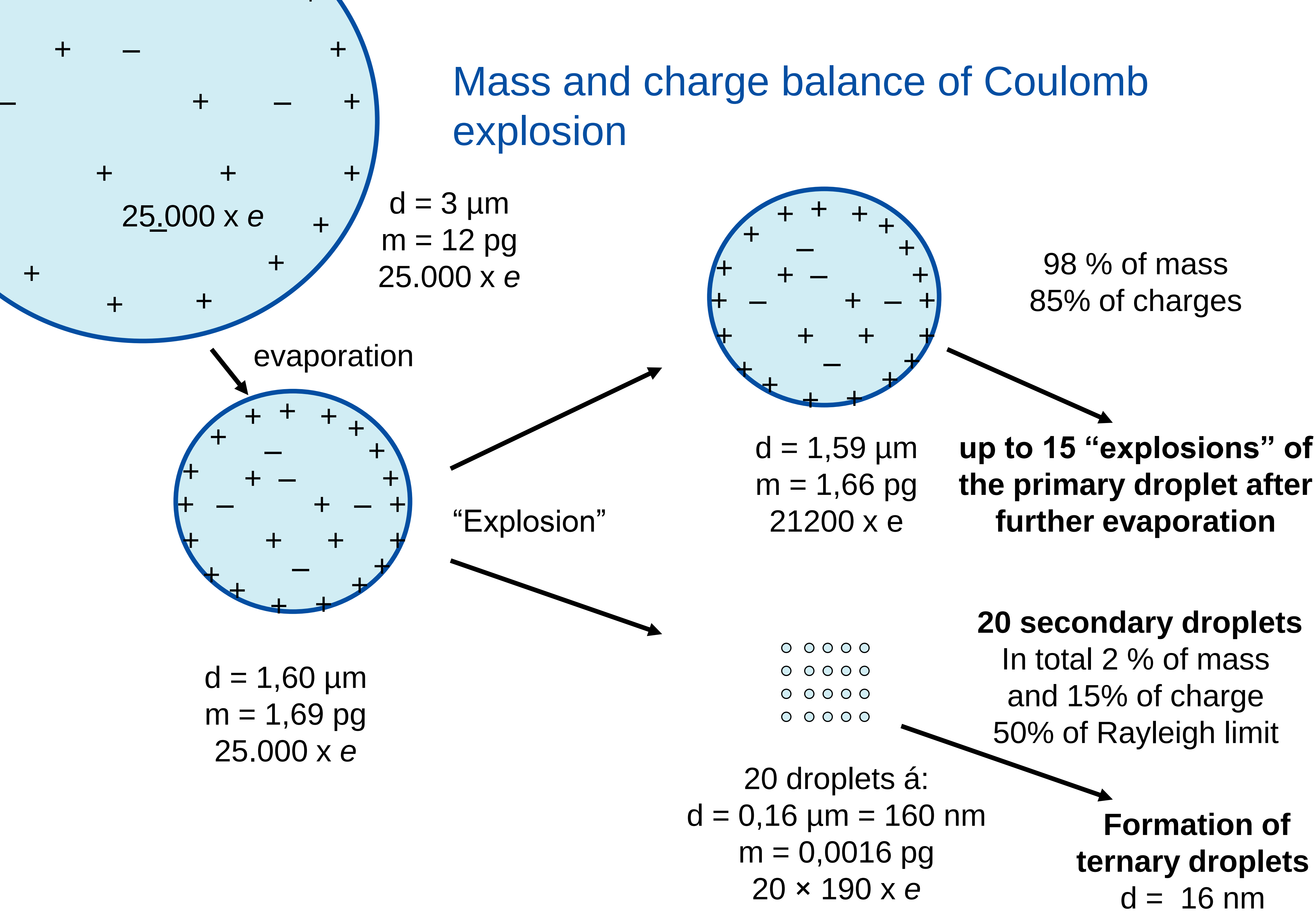
„Flash shadowgraph“ of a primary droplet forming secondary droplets.

Reference:

A. Gomez and K. Tang: Phys. Fluids 6 (1994) 404



Mass and charge balance of Coulomb explosion



25.000 x *e*

d = 3 µm
m = 12 pg
25.000 x *e*

evaporation

d = 1,60 µm
m = 1,69 pg
25.000 x *e*

“Explosion”

d = 1,59 µm
m = 1,66 pg
21200 x e

98 % of mass
85% of charges

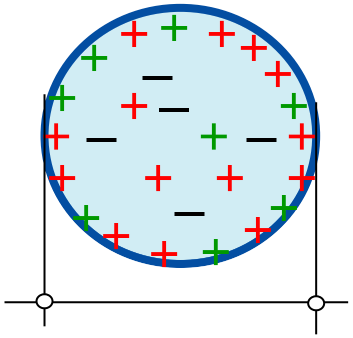
up to 15 “explosions” of the primary droplet after further evaporation

20 droplets á:
d = 0,16 µm = 160 nm
m = 0,0016 pg
20 × 190 x *e*

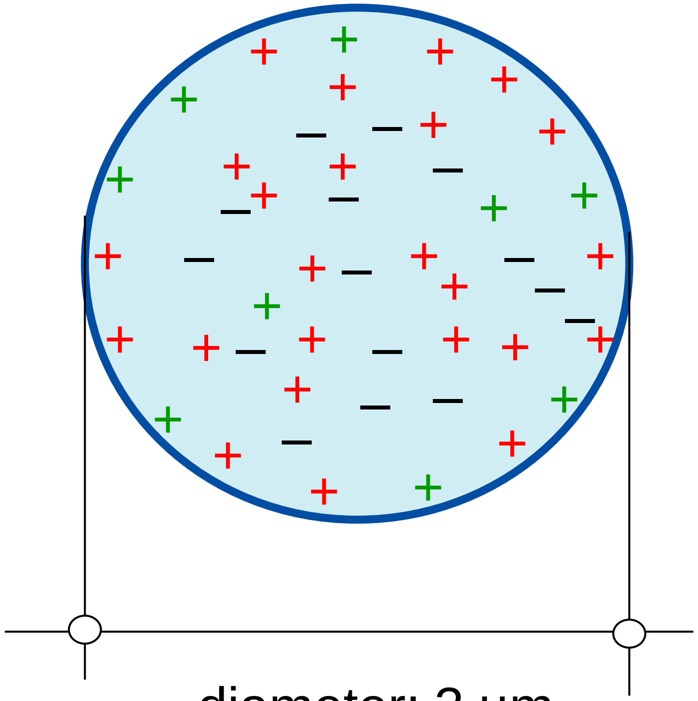
20 secondary droplets
In total 2 % of mass
and 15% of charge
50% of Rayleigh limit

Formation of ternary droplets
d = 16 nm

Properties of a charged secondary droplet



diameter: 0,16 µm
(160 nm)



diameter: 3 µm

Number of solvent molecules in case of

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

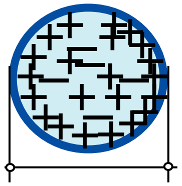
Calculated number of soluted analyte molecules

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

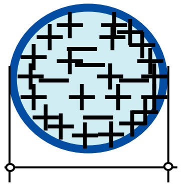
Rayleigh-Limit: ≤ 50 elementary charges

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

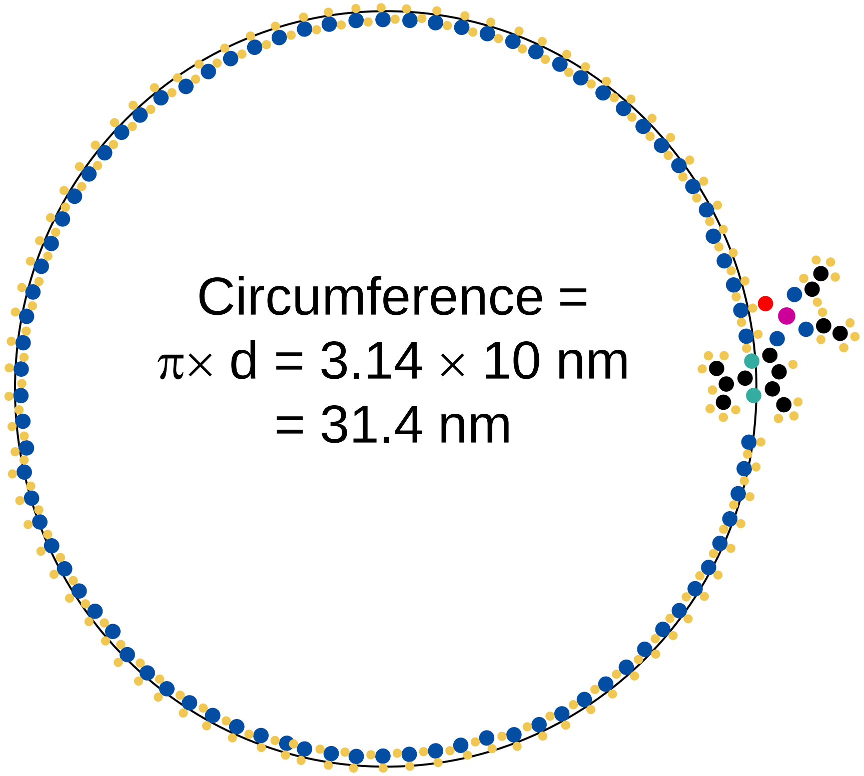
Ratio between protons and water molecules in one liter of 10^{-1} mol/L HCl (= pH 1):
0.1 mol / 50 mol = 1 / 500

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



typical diameter: 10 nm



Circumference =
$\pi \times d = 3.14 \times 10$ nm
= 31.4 nm

Diazinon

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

Rayleigh-Limit: < 50 elementary charges

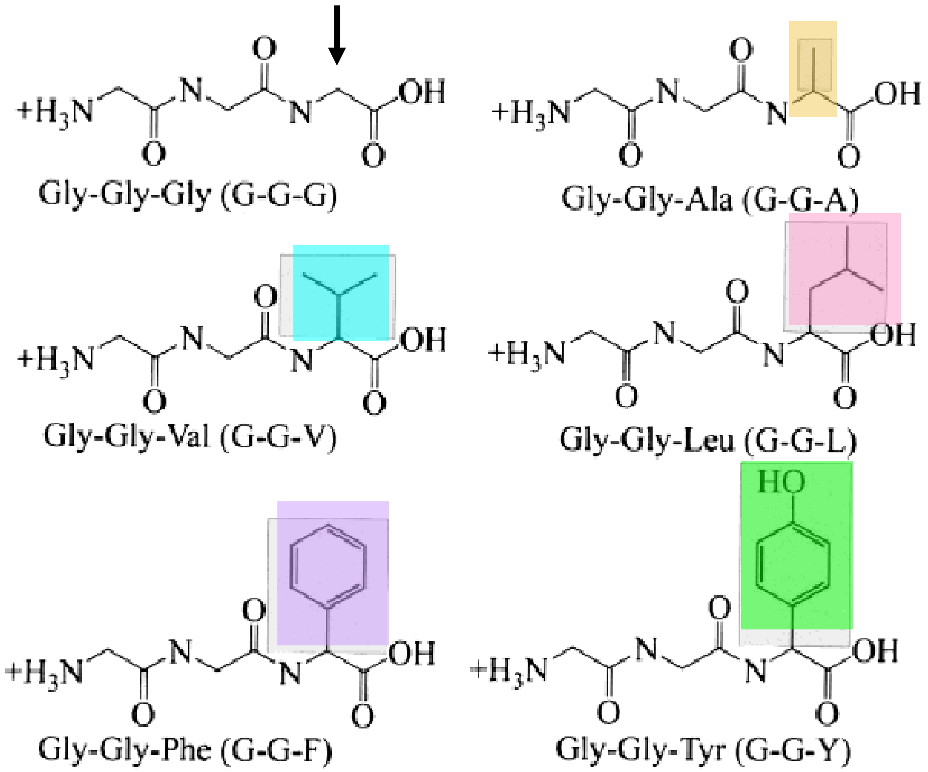
100 water molecules
are sufficient to fill
the circumference

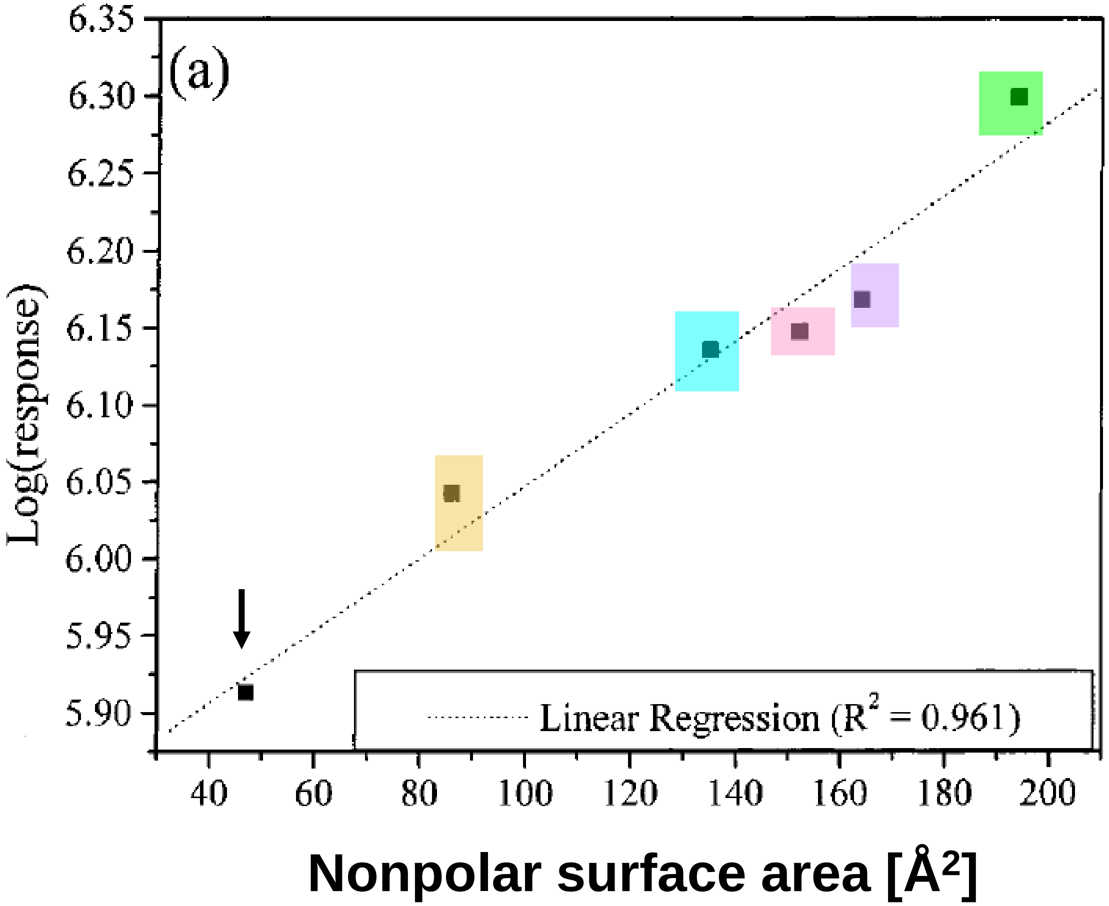
Saturation with analyte means,
25 to 50 quasimolecular ions of such
size are spread over the surface.

But which analytes may better occupy the surface and evaporate as ions?

Effect of polarity of analytes on the efficiency of ionization

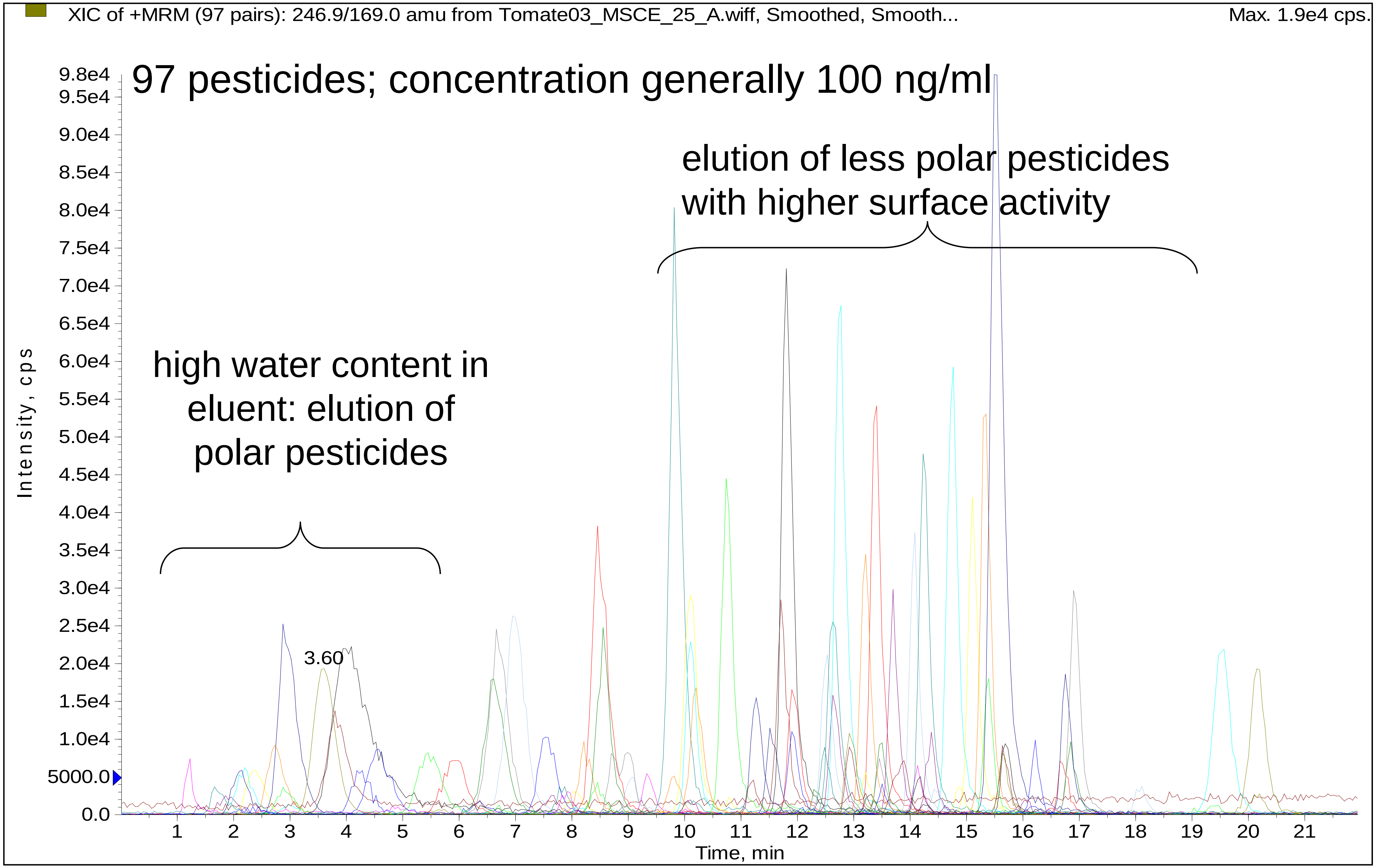
Relation between response and ”nonpolar surface area” of six similar tripetides





Reference:
N.B. Cech and Ch. G. Enke; Anal. Chem. 72 (2000) 2717-2723

Effect of polarity of analytes on the efficiency of ionization



97 pesticides; concentration generally 100 ng/ml

elution of less polar pesticides with higher surface activity

high water content in eluent: elution of polar pesticides

Second summary of important aspects of electrospray ionization

1. The electrospray ion source permanently produces a fixed number of ions (about 3×10^{-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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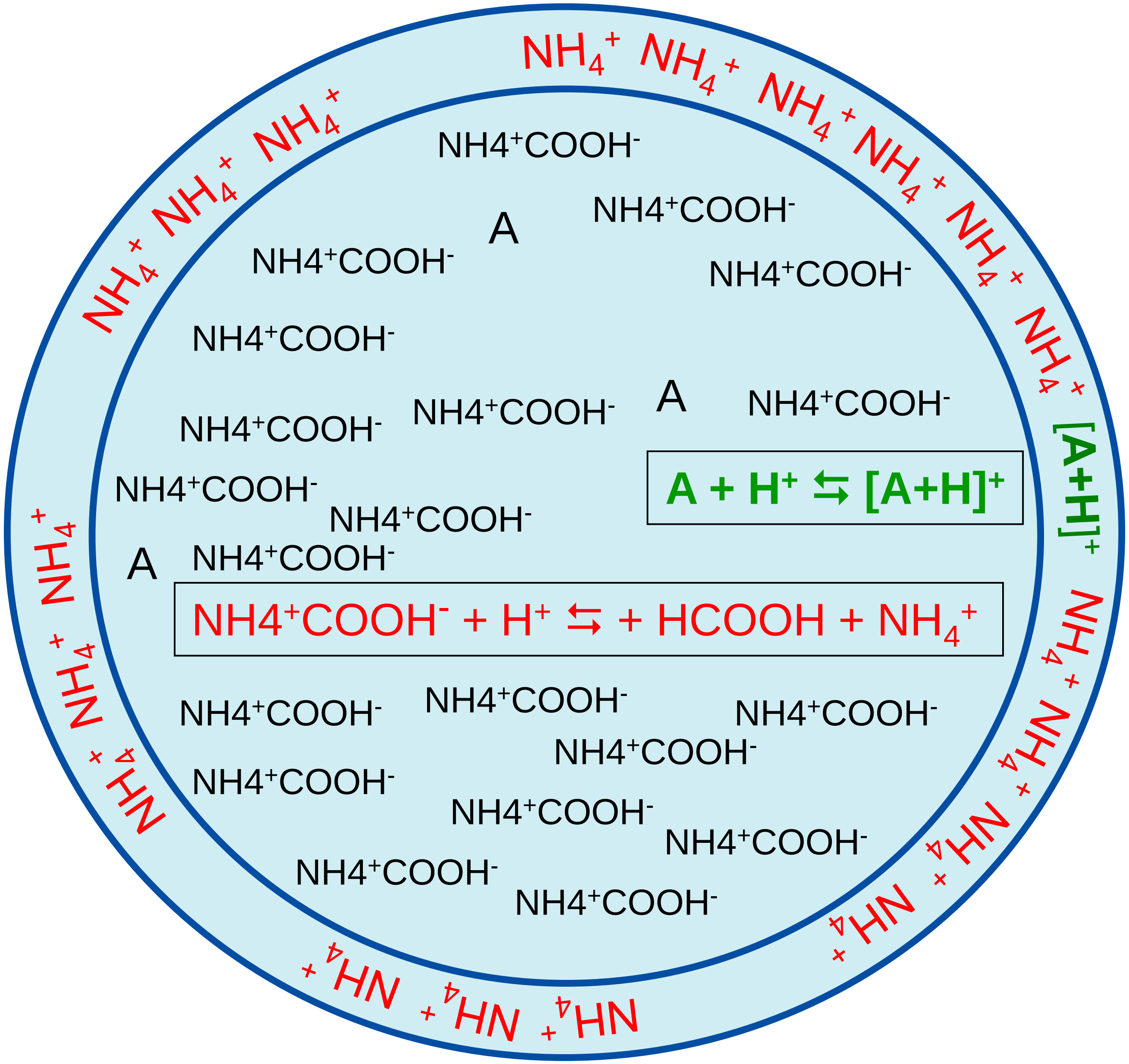
The theoretical model (without co-eluting matrix)



$$A + H^+ \leftrightarrows [A+H]^+$$

$$NH4^+COOH^- + H^+ \leftrightarrows + HCOOH + NH_4^+$$

Reference: Enke Ch. G., Anal. Chem. 69 (1997) 4885-4893

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

$[NH_4^+COOH^-] + [NH_4^+] =$ concentration of buffer (C_B)

$$K_E = \frac{[NH4^+]}{[NH_4^+COOH^-] \times [\ H^+]}$$

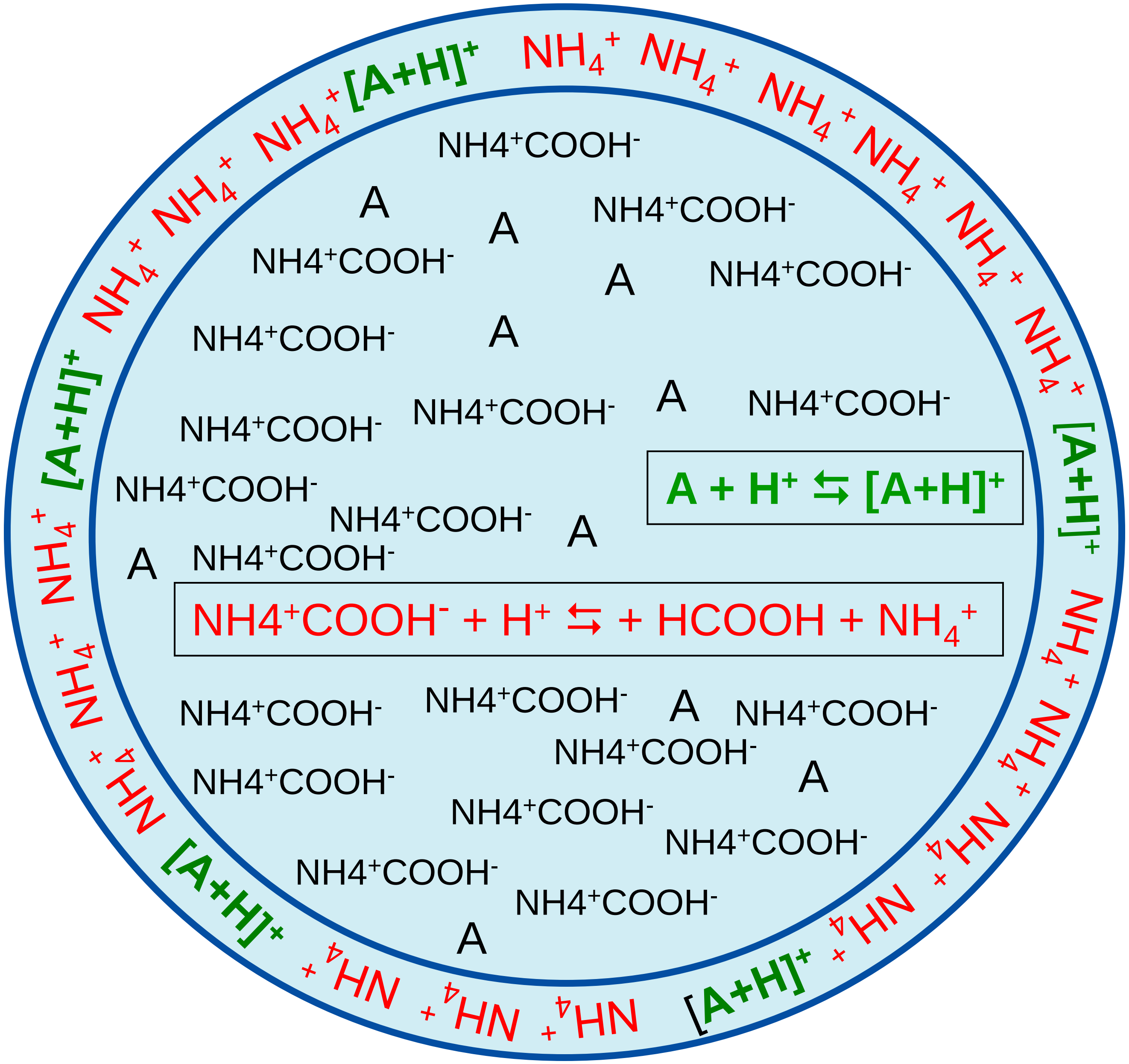
A = Analyte

[A+H]+ = quasimolecular ion of A

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

$[A+H]^+ + [NH_4^+] =$ concentration of excess charges (Q)

The theoretical model (without co-eluting matrix)



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$[A+H]^+ + [NH_4^+] =$
concentration of excess charges (Q)

The theoretical model (without co-eluting matrix)

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

$$\boxed{a \times [A+H]^{+2} + b \times [A+H]^{+} + c = 0}$$

$a = K_A/K_E - 1$

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

$c = C_A \times Q \times (K_A/K_E)$

$[A+H]^+$	concentration of the qauasimolecular 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 … 10^{-6} mol/L
$C_B = [NH_4{+}COOH^-] + [NH_4^+]$	concentration of buffer (ammonium formiate); 10^{-4} … 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

Reference with incorrect equation: Enke Ch. G., Anal. Chem. 69 (1997) 4885-4893

Application of the theoretical model

The influence of buffer concentration on sensitivity and linearity

rescaled (×10)

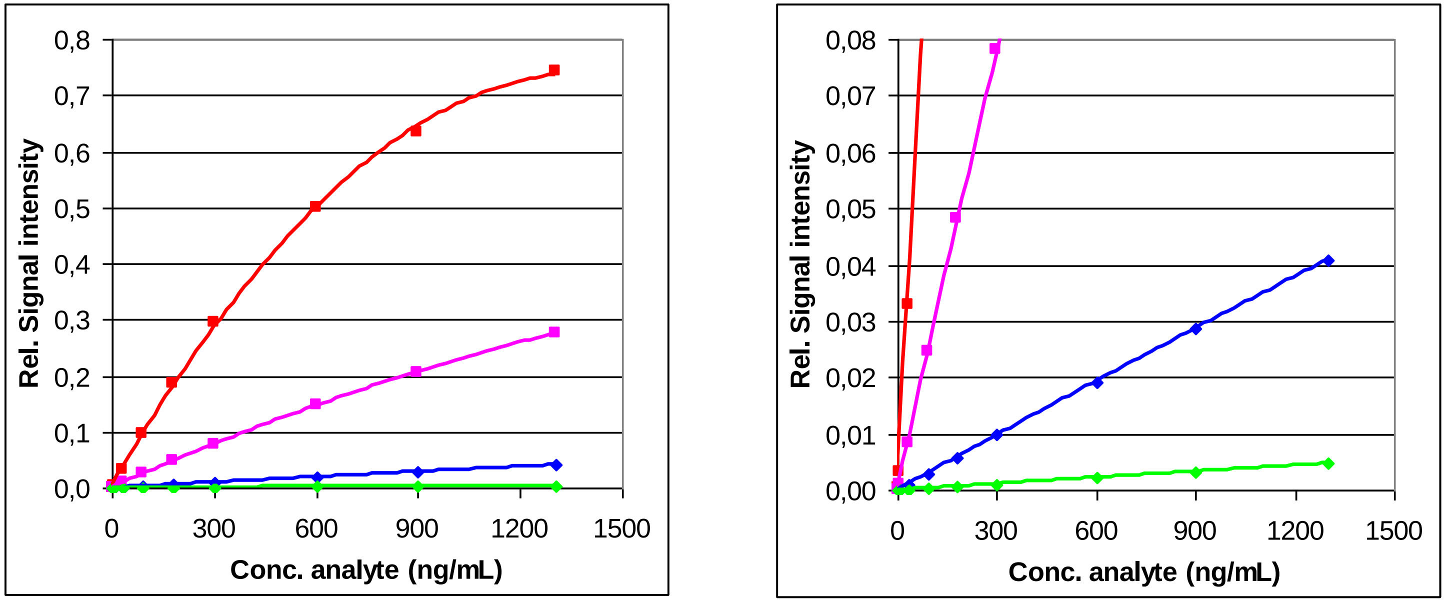
Buffer concentration:
10^{-3} mol/L

Red calibration line:
analyte with best ability to stay on surface (K_A/K_E = 1000)

analyte with K_A/K_E = 100

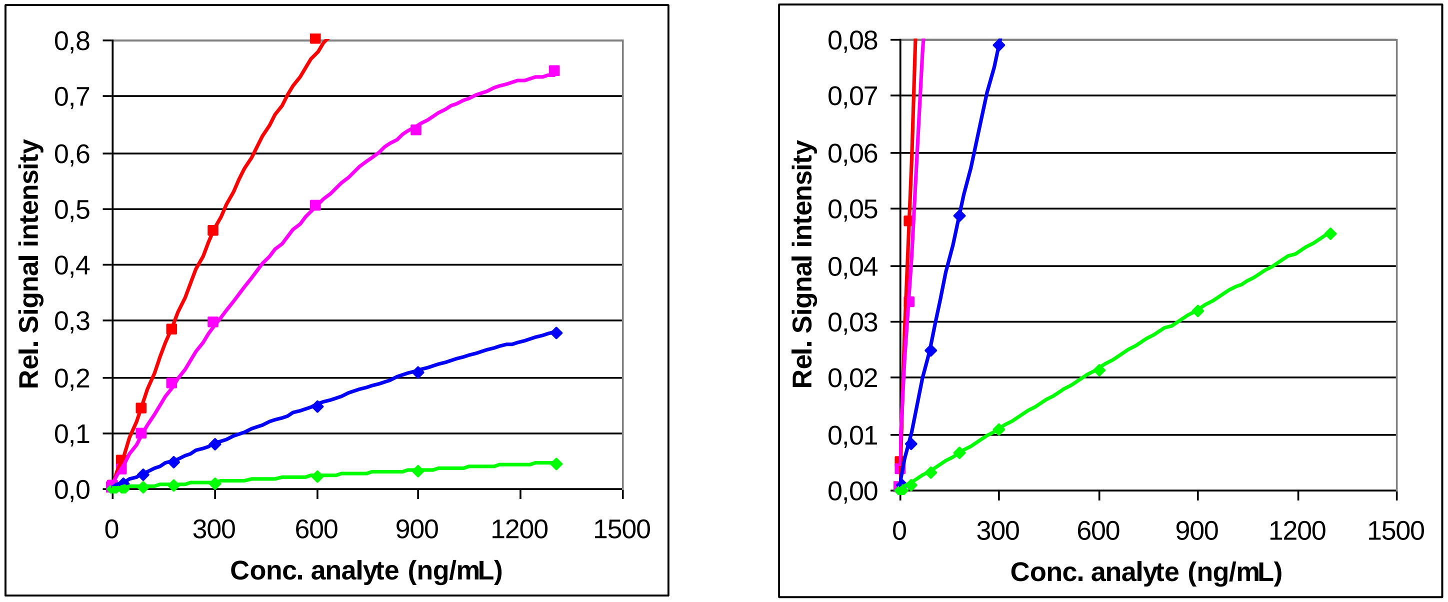
analyte with K_A/K_E = 10

analyte with K_A/K_E = 1

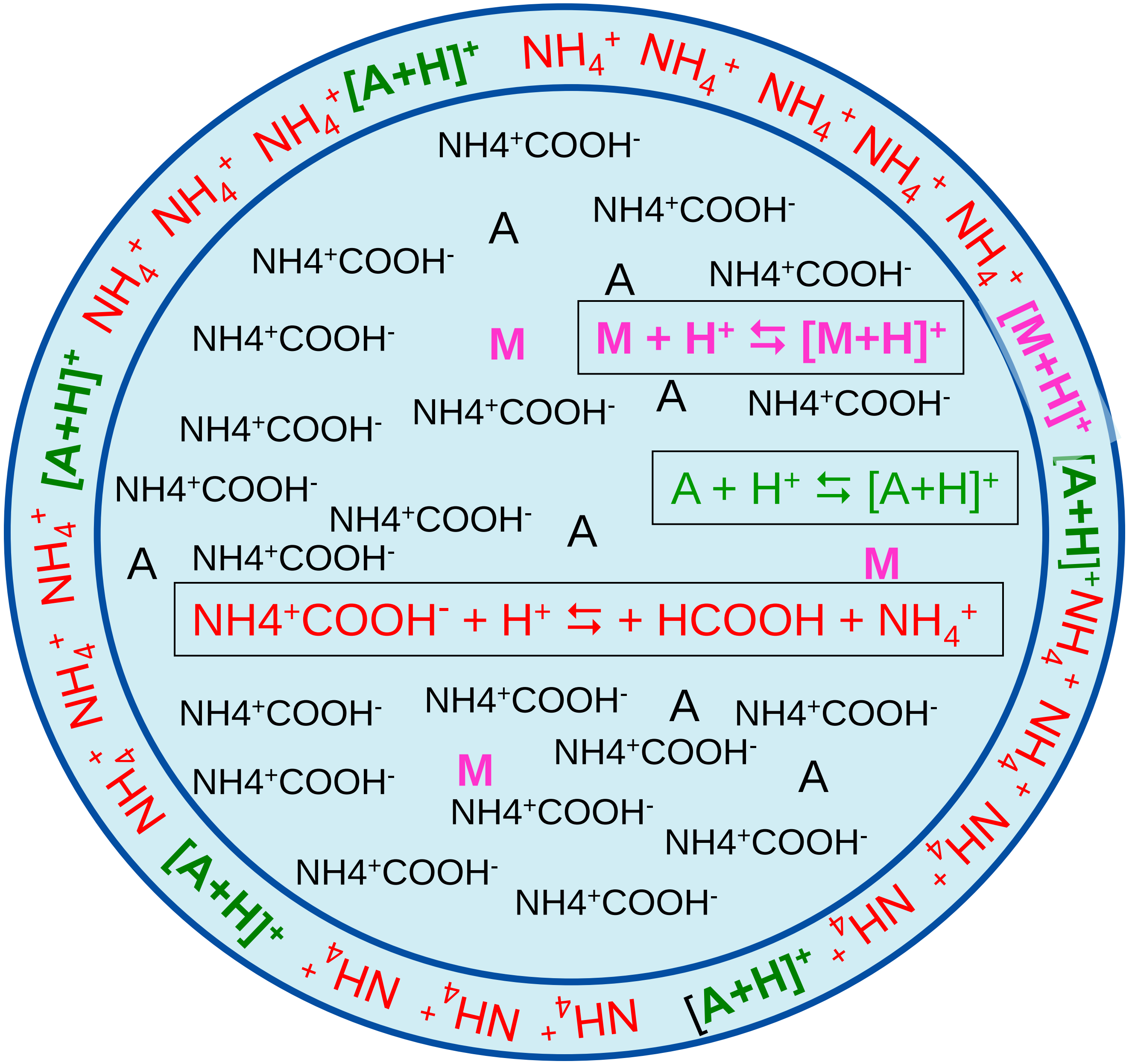


Buffer concentration:
10^{-4} mol/L

Reduction of buffer concentration will enhance sensitivity, but simultaneously this reduction will lower linearity!



The theoretical model (with co-eluting matrix)



$$\boxed{M + H^+ \leftrightharpoons [M+H]^+}$$

$$\boxed{A + H^+ \leftrightharpoons [A+H]^+}$$

$$\boxed{NH4^+COOH^- + H^+ \leftrightharpoons + HCOOH + NH_4^+}$$

Additional parameter and equations:

M = (uncharged) matrix

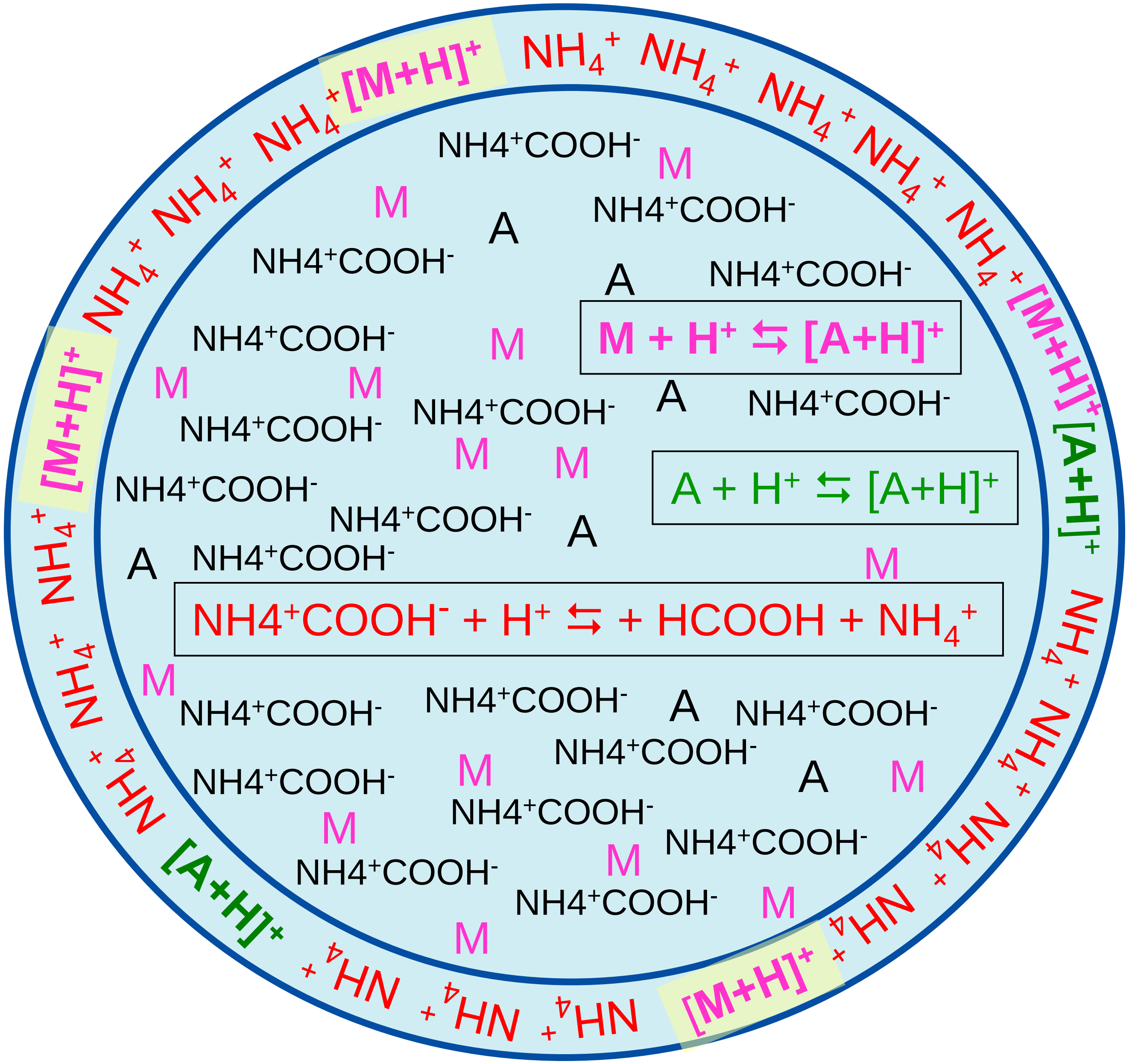
$[M+H]^+$ =
quasimolecular ion of matrix

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

$[A+H]^+ + [NH_4^+] + [M+H]^+$ =
concentration of excess charges (Q)

Reference with incorrect equation: Constantopolus et al., Anal. Chimica Acta 406 (2000) 37-52

The theoretical model (with co-eluting matrix)



$M + H^+ \leftrightharpoons [A+H]^+$

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$NH4^+COOH^- + H^+ \leftrightharpoons + HCOOH + NH_4^+$

Additional parameter and equations:

M = (uncharged) matrix

$[M+H]^+$ = quasimolecular ion of matrix

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

$[A+H]^+ + [NH_4^+] + [M+H]^+$ = 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 …

Reference with incorrect equation: Constantopolus et al., Anal. Chimica Acta 406 (2000) 37-52

The theoretical model (with co-eluting matrix)

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

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

$a = K_M - K_A + K_E\,(1 - K_M/K_A)$

$b = C_A\,(2K_A - K_M - K_E) + C_B(K_M - K_E(K_M/K_A) + C_E K_E(1 - K_M/K_A) + Q(K_A - K_M – K_E\,(1 - K_M/K_A))$

$c = -C_A\,(Q(2K_A - K_M - K_E) + C_B K_M + C_A K_A + C_E K_E)$

$d = C_A{}^2 \times Q \times K_A$

$[A+H]^+$	concentration of the qauasimolecular 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 … 10^{-6} mol/L
$C_B = [NH_4{+}COOH^-] + [NH_4{}^+]$	concentration of buffer (ammonium formiate); 10^{-4} … 10^{-2} mol/L
$C_M = [M+H]^+ + [M]$	total concentration of the matrix substance; 0 … 10^{-4} 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
$K_M = [\,M+H^+] / [M] \times [\,H^+]$	equilibrium constant of matrix substance

Reference with incorrect equation: Constantopolus et al., Anal. Chimica Acta 406 (2000) 37-52

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:

$K_A = 1000$

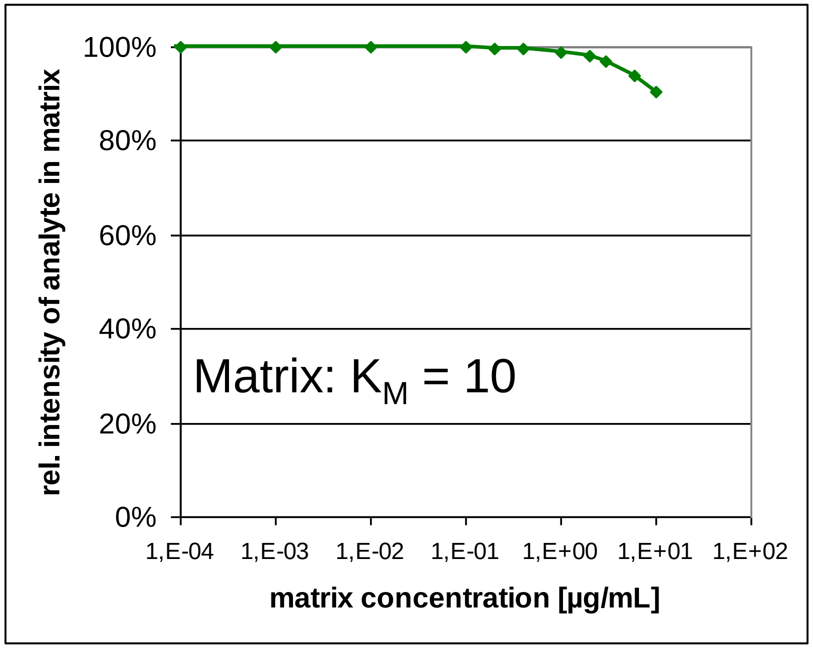
$C_A = 300$ ng/ml

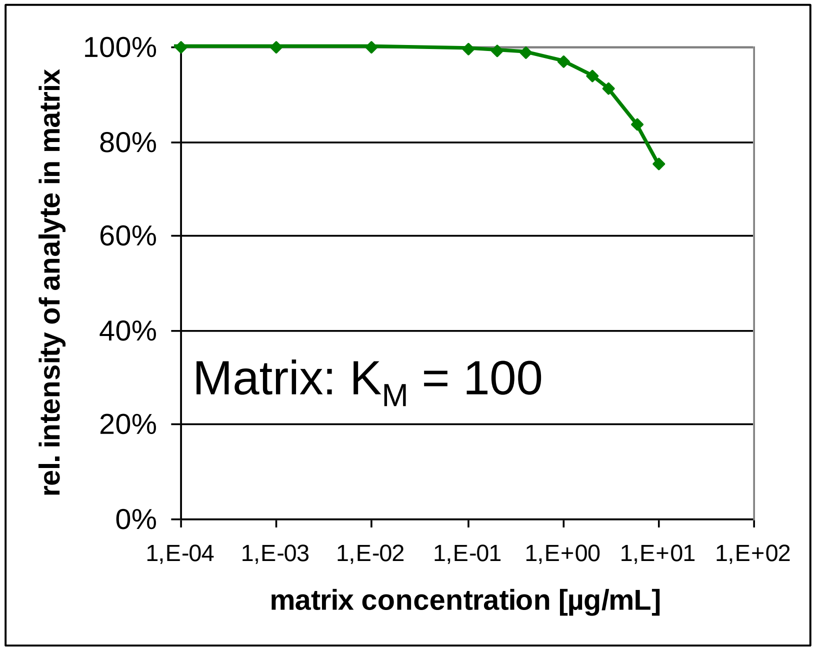
matrix:

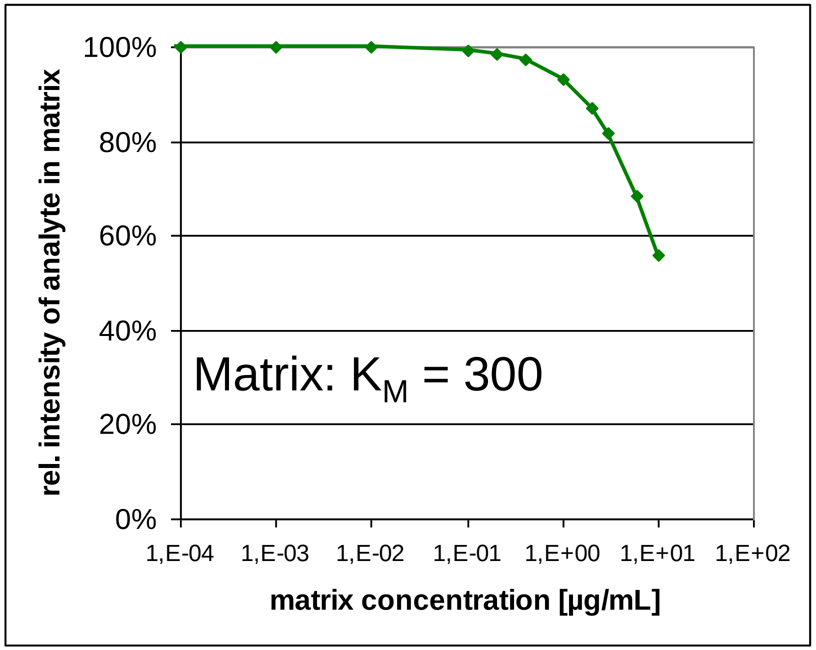
$K_M = 10 \dots 1000$

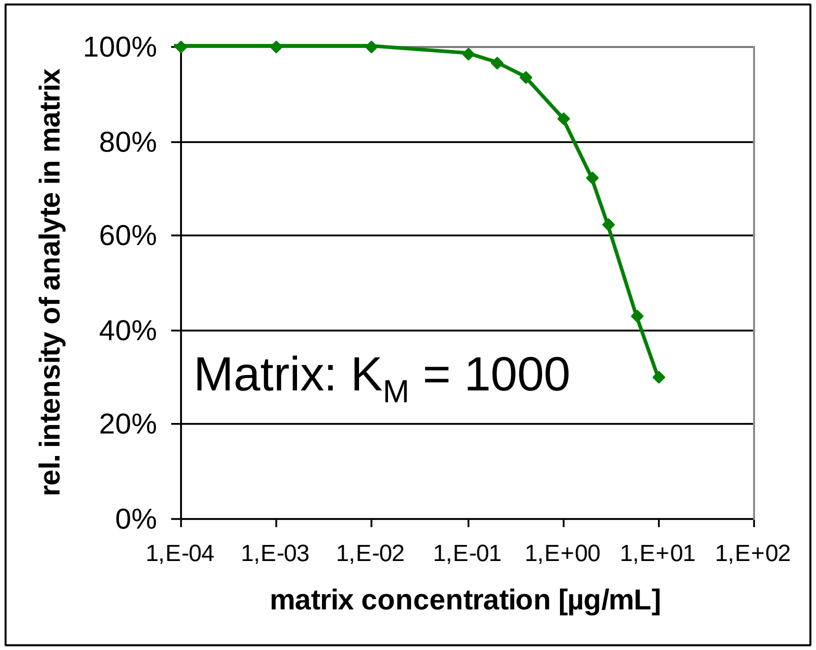
$C_M =$ 0,0001…10 µg/mL

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)

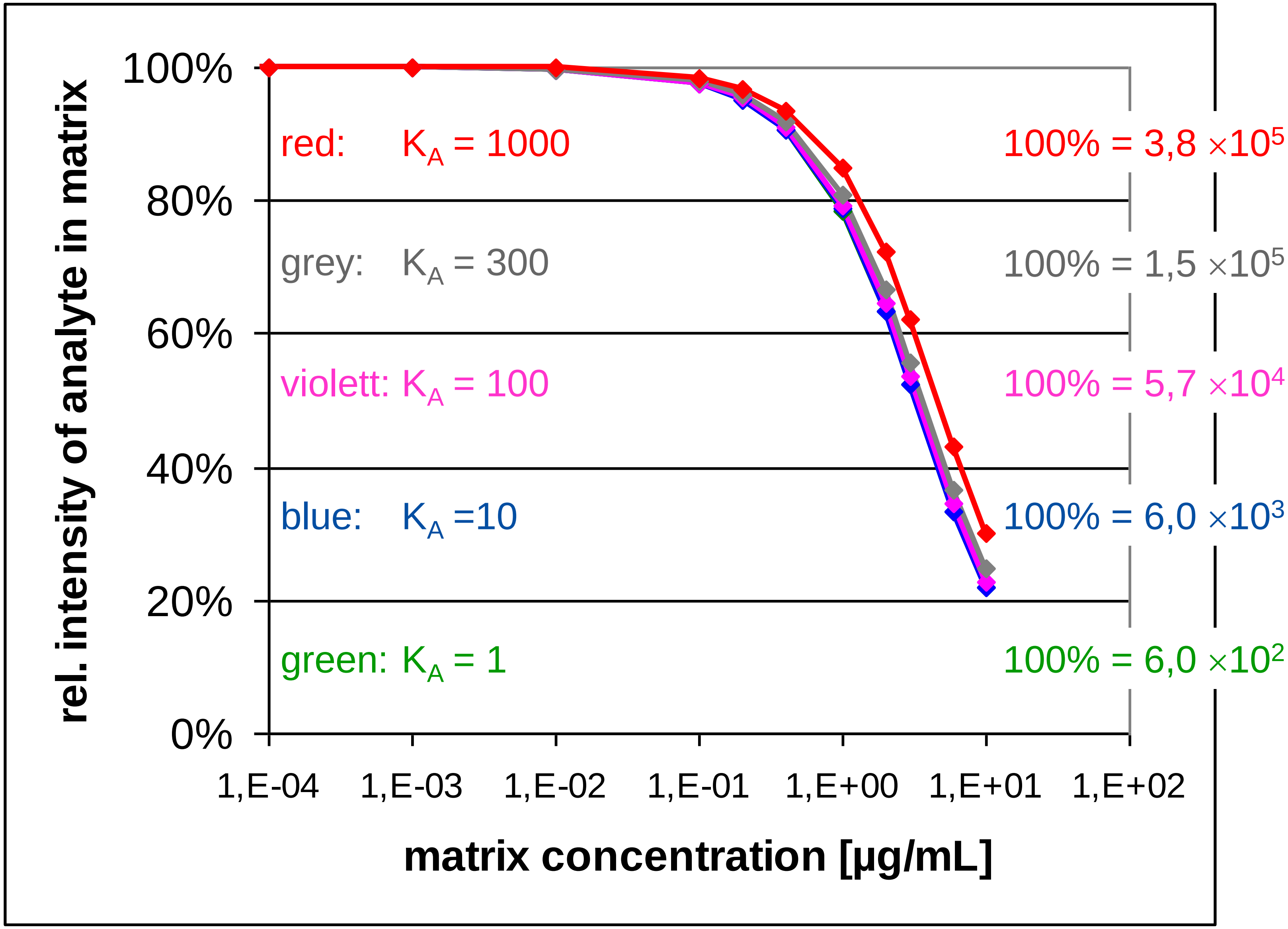
The influence of analyte’s ability to occupy the surface

electrolyte:
$K_E = 1$
$C_M = 5$ mmol/L

analyte:
$K_A = 1 \ldots 1000$
$C_A = 300$ ng/ml

matrix:
$K_M = 1000$
$C_M =$ 0,0001…10 µg/mL

Prediction of theory: Analyt’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)

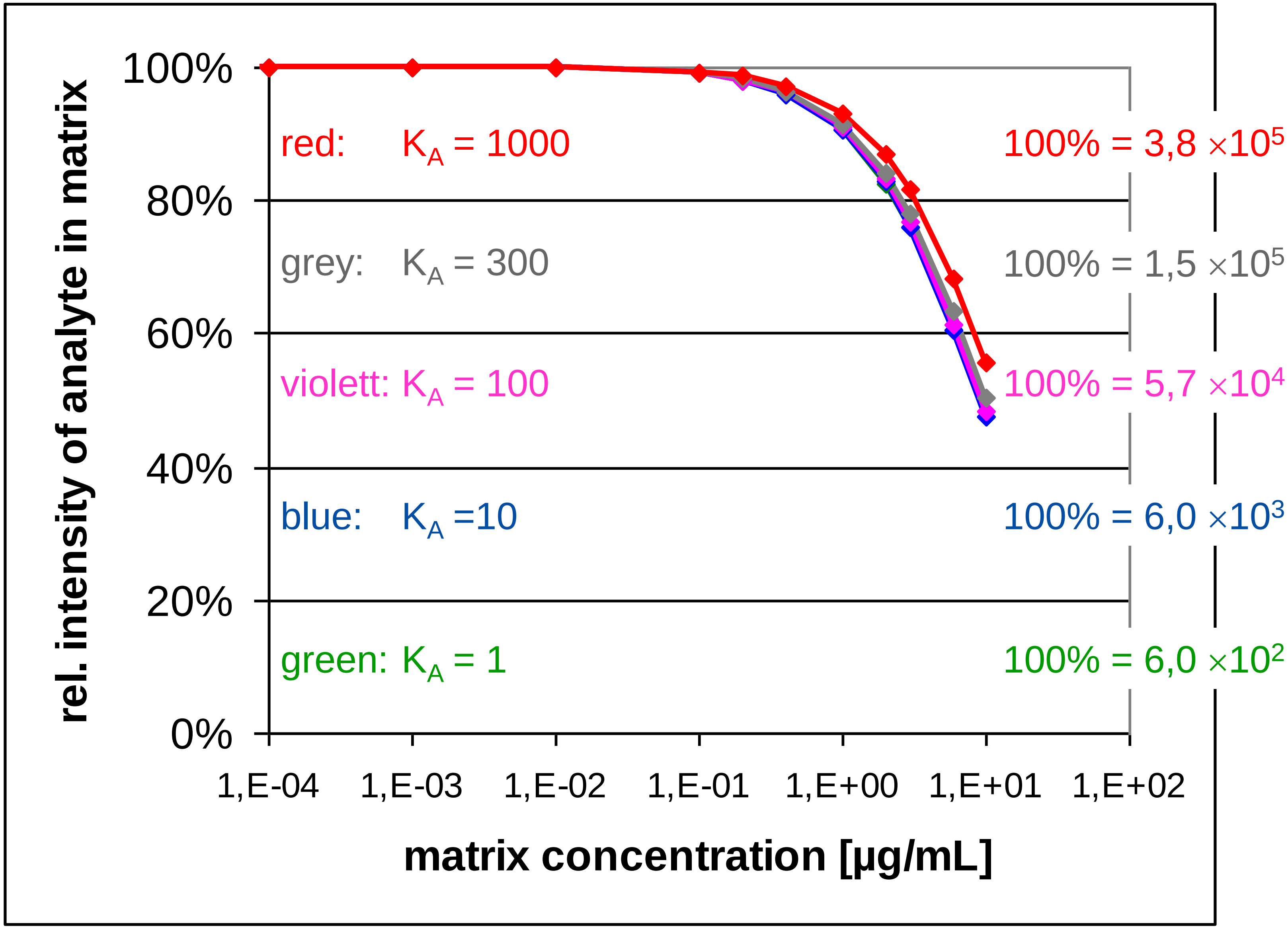
The influence of analyte's ability to occupy the surface

electrolyte:
$K_E = 1$
$C_M = 5$ mmol/L

analyte:
$K_A = 1 \ldots 1000$
$C_A = 300$ ng/ml

matrix:
$\mathbf{K_M = 300}$
$C_M =$
0,0001…10 µg/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:

$K_E = 1$

$C_M = 5$ mmol/L

analyte:

$K_A = 1 \ldots 1000$

$C_A =$

0.003 … 300 ng/ml

matrix:

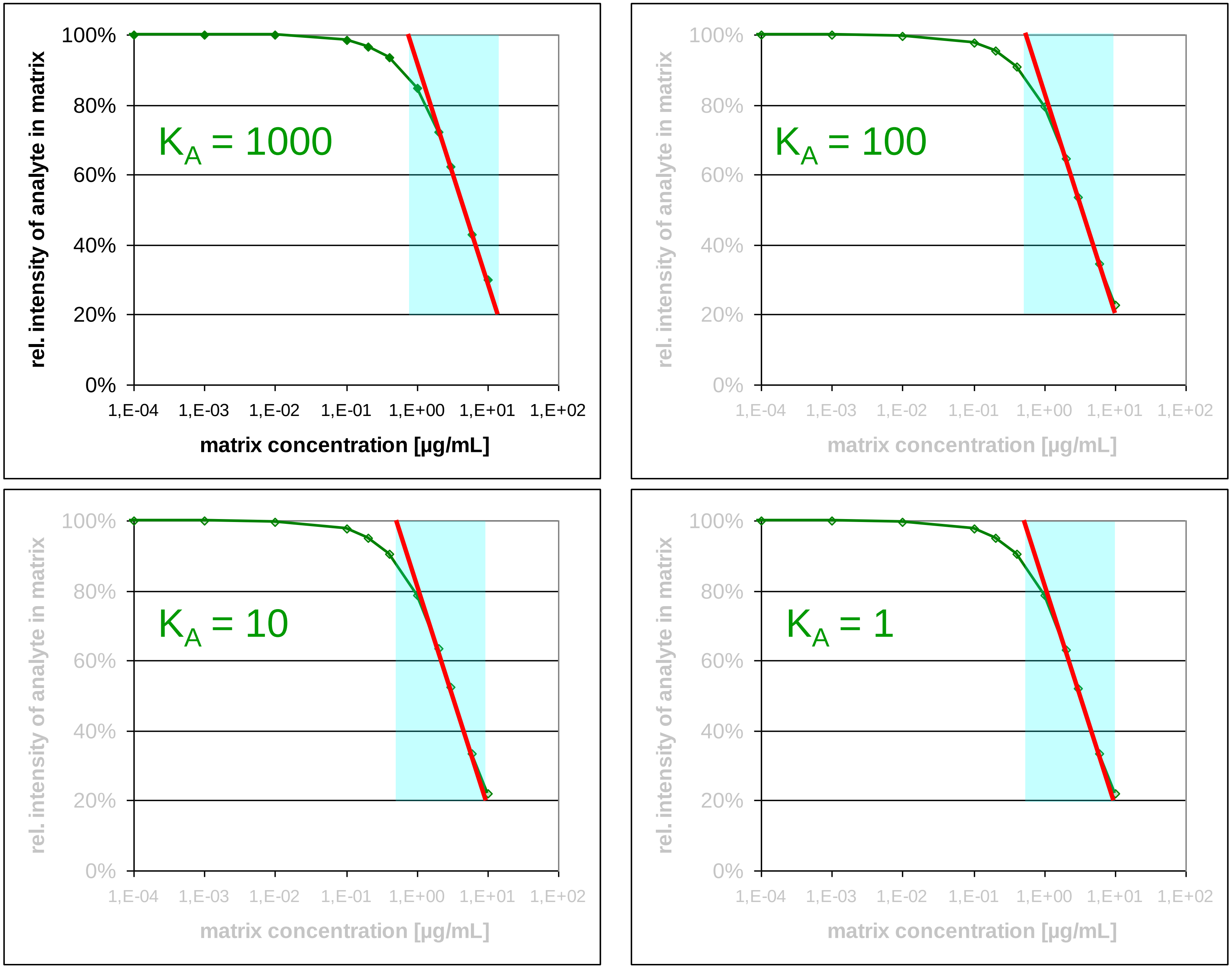
$K_M = 300$

$C_M =$

0,0001…10 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:

$K_E = 1$

$C_M = 1$ mmol/L

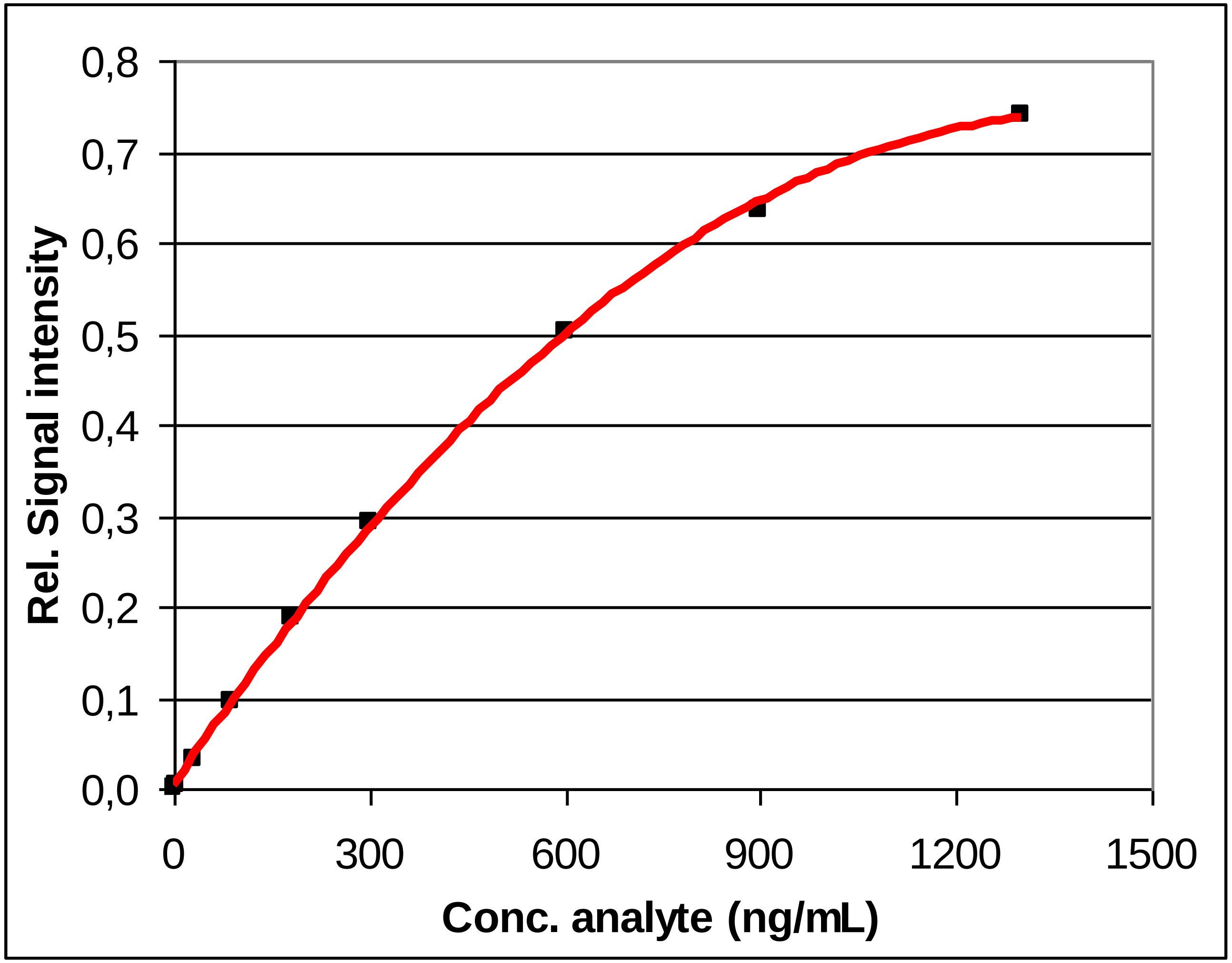
analyte:

$K_A = 1000$

$C_A = 0.3 \ldots 1300$ ng/ml

Do you remember slide 31?

This slide had shown a non-linear calibration line in the absence of matrix

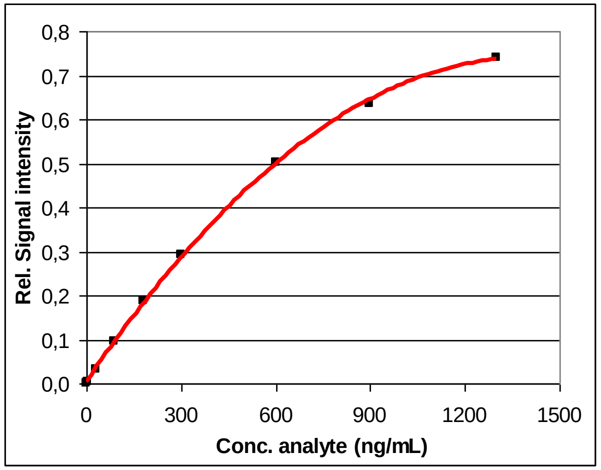


The prediction of the theoretical model for the influence of matrix on linearity

electrolyte:
$K_E = 1$
$C_M = 1$ mmol/L

analyte:
$K_A = 1000$
$C_A = 0.3 \ldots 1300$ ng/ml

matrix:
$K_M = 10$ or 100
$C_M = 10$ µg/mL

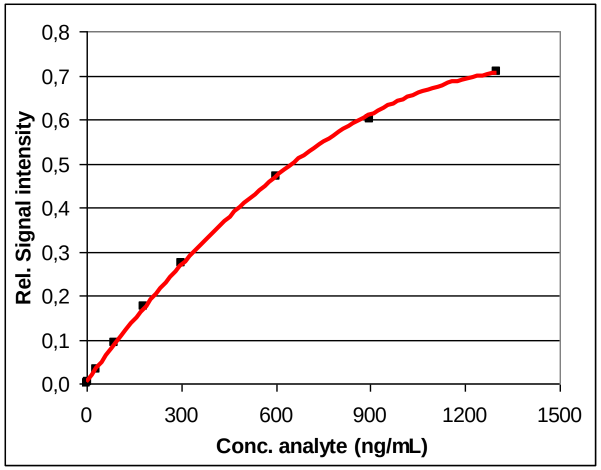


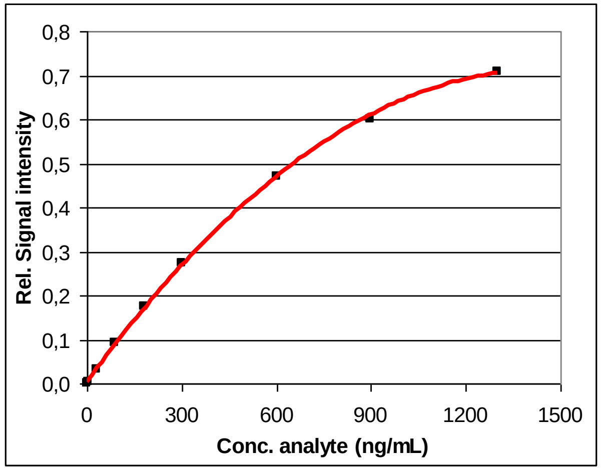
non-linear calibration line in the absence of matrix

fixed y-axis

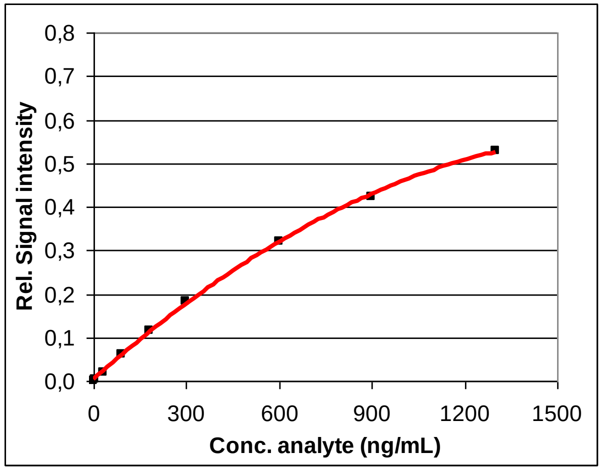
rescaled y-axis

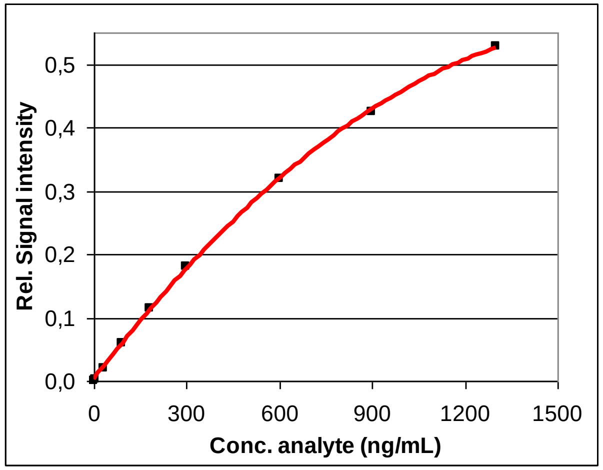
$K_M = 10$





$K_M = 100$





Matrix substances (or analytes) with weak tendency to occupy the surface have small influence.

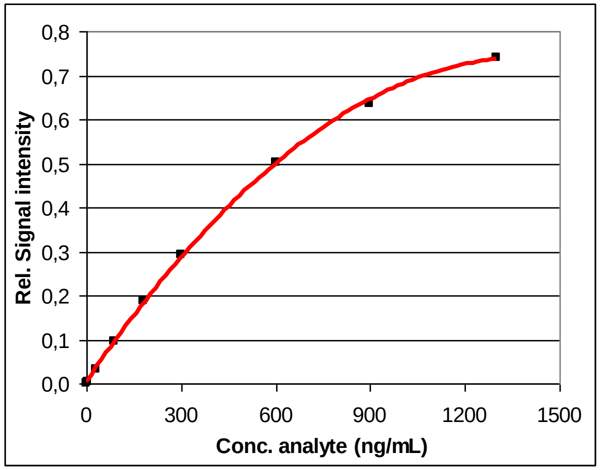
The prediction of the theoretical model for the influence of matrix on linearity

electrolyte:
$K_E = 1$
$C_M = 1$ mmol/L

analyte:
$K_A = 1000$
$C_A = 0.3 \ldots 1300$ ng/ml

matrix:
$K_M = 300$ or 1000
$C_M = 10$ µg/mL

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

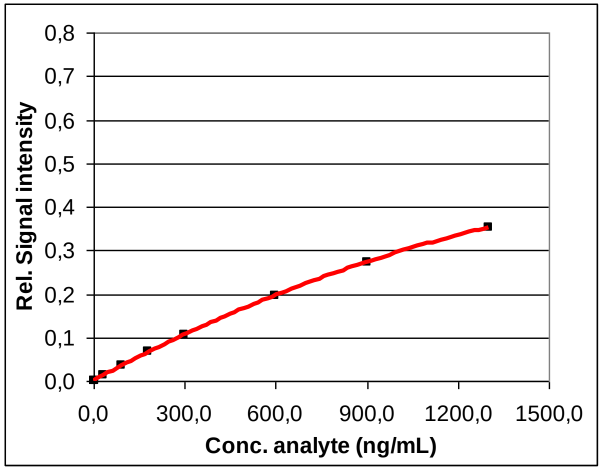


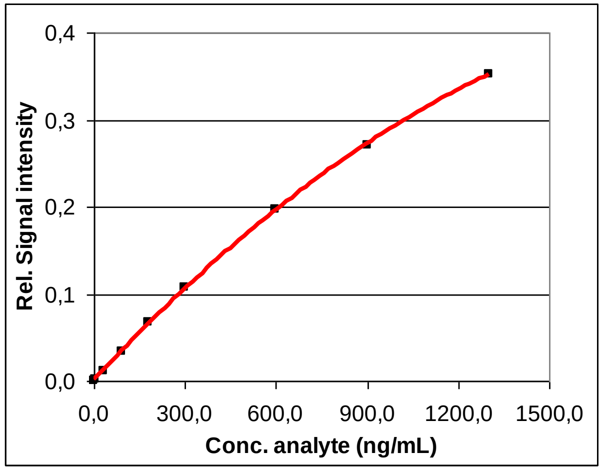
non-linear calibration line in the absence of matrix

fixed y-axis

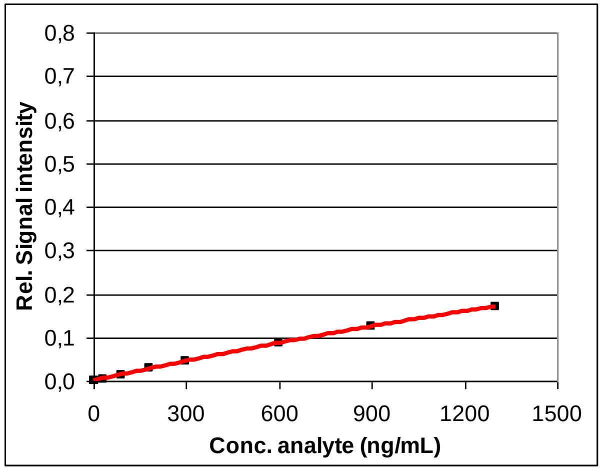
rescaled y-axis

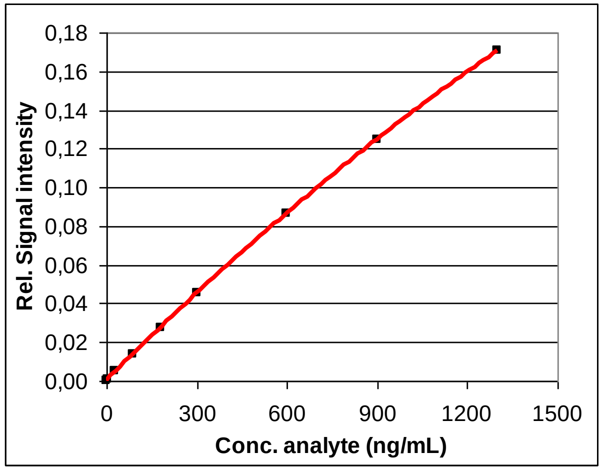
$K_M = 300$





$K_M = 1000$





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 3×10^{-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).

Risiken erkennen – Gesundheit schützen

Many thanks for listening!

Lutz Alder

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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

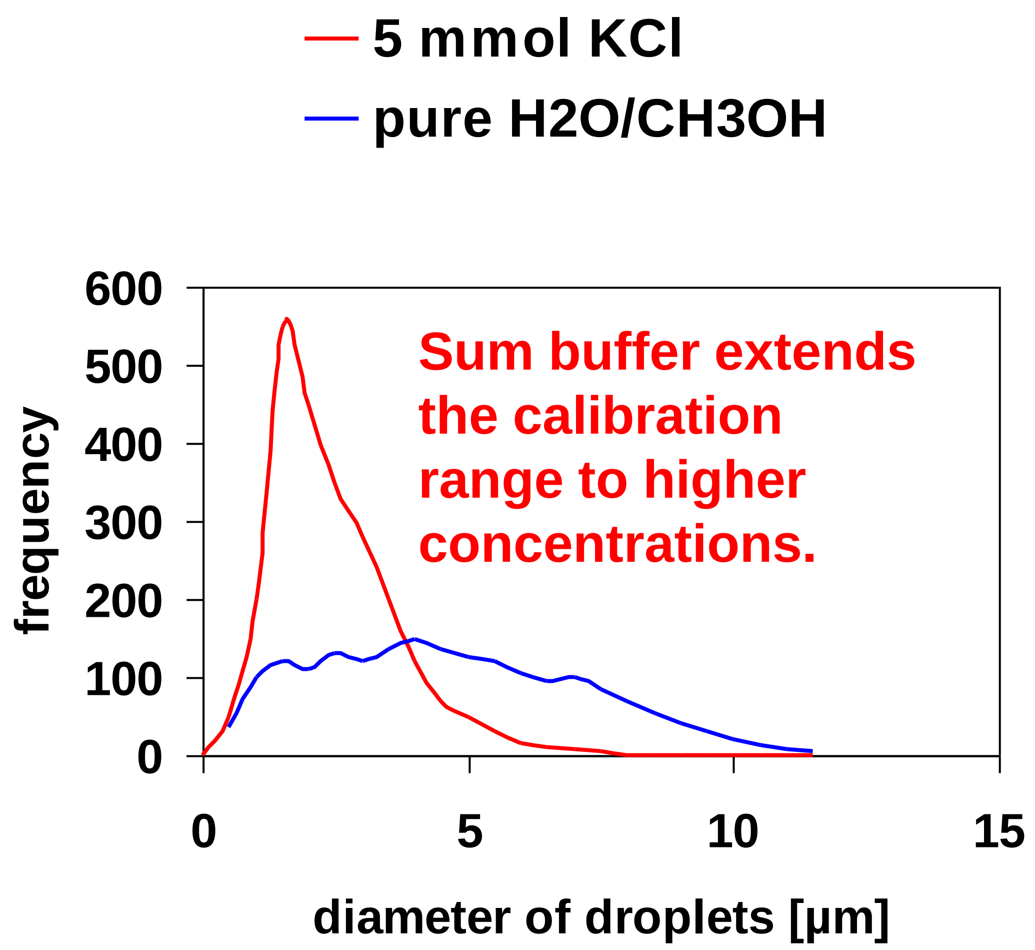
V_f: flow rate (µl/min)

ε : permittivity

σ: conductivity of the eluent

Smaller droplets at higher conductivity!

Smaller droplets means smaller number of excess charges per droplet but **higher number of charges per mL**!

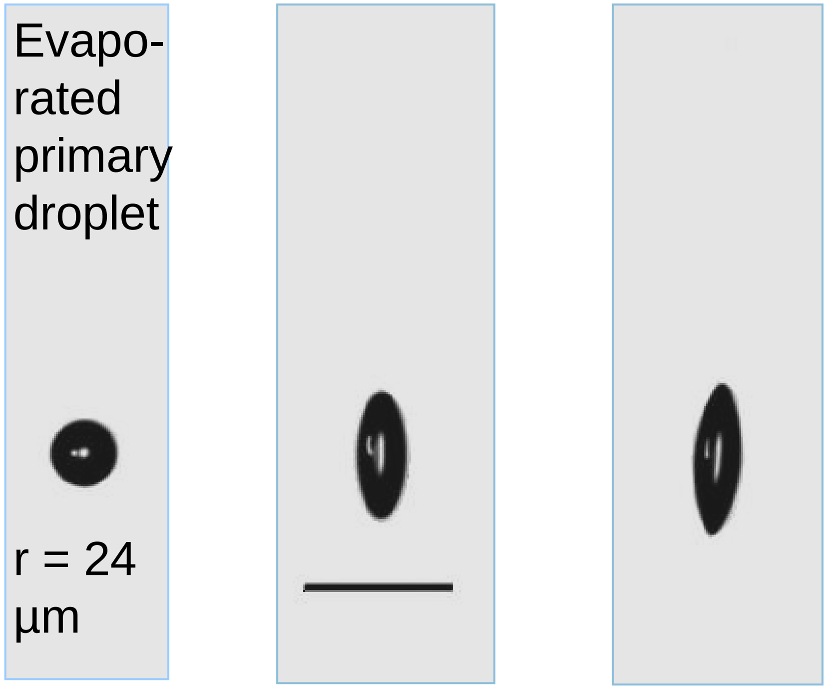


Sum buffer extends the calibration range to higher concentrations.

Diagramme from: Z Olumee, J.H. Callahan and A. Vertes; J. Phys. Chem. 102 (1998) 9154

Process of Coulomb explosion – Development of Rayleigh Jets

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



$\Delta t = 140\ \mu s$ $\Delta t = 150\ \mu s$

Reference:

D. Duft, T. Achtzehn, R. Müller, B.A. Huber and T. Leisner: Nature 42 (2003) 128

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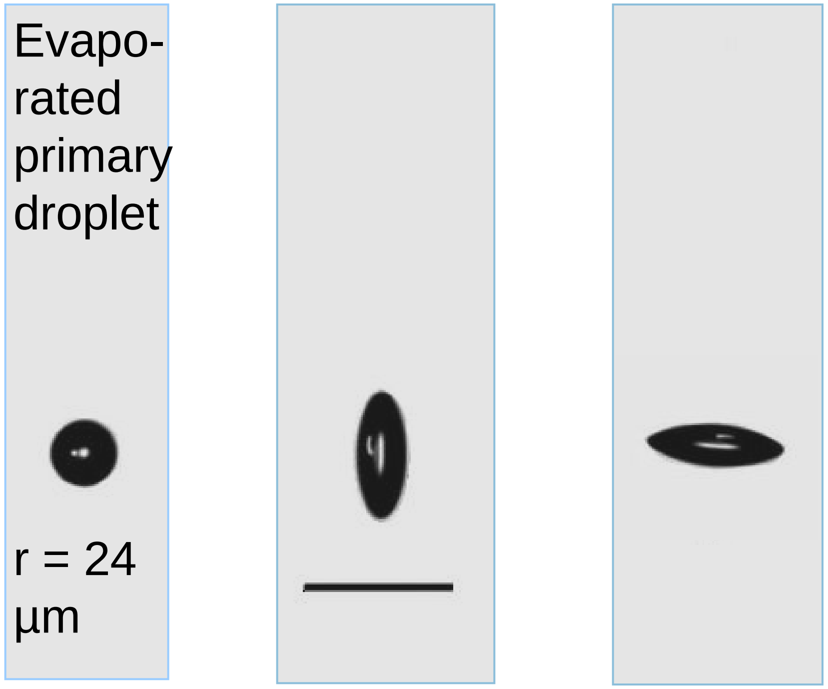

∆ t = 140 µs ∆ t = 150 µs

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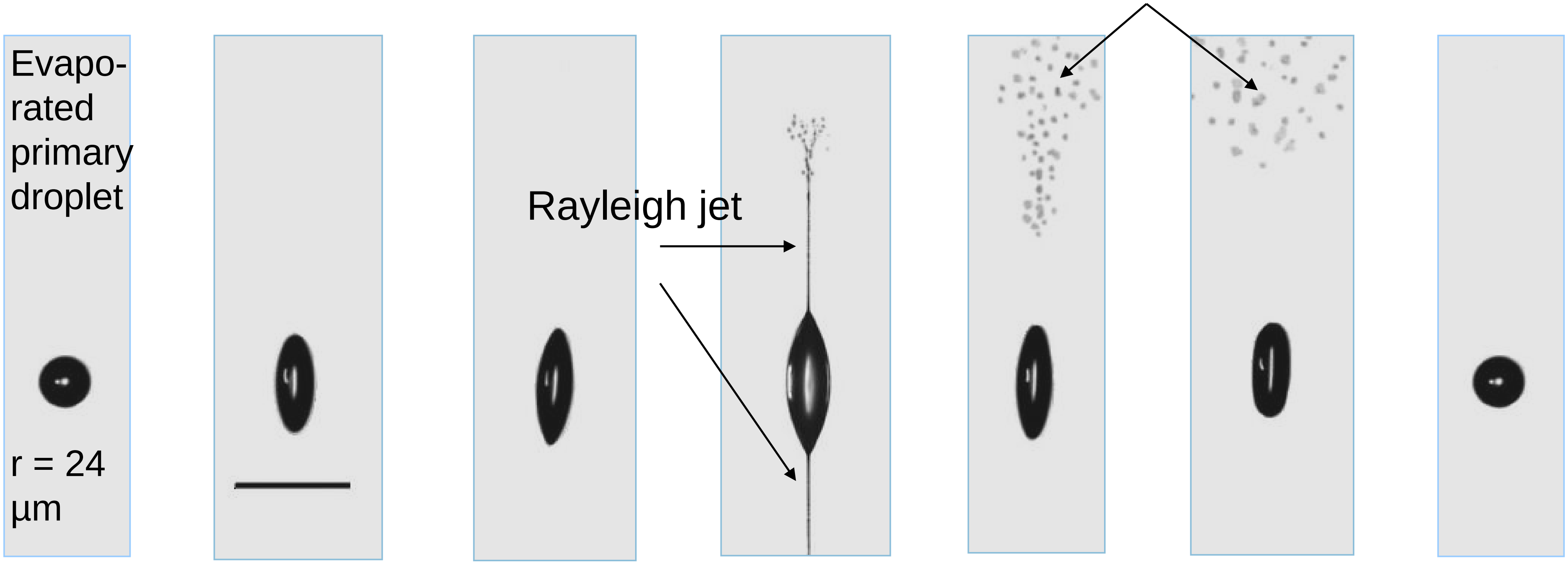

Offspring droplets

Evapo-rated primary droplet

r = 24 µm

Rayleigh jet

Δ t = 140 µs

Δ t = 150 µs

Δ t = 155 µs

Δ t = 160 µs

Δ t = 180 µs

Δ t = 210 µs

Reference:

D. Duft, T. Achtzehn, R. Müller, B.A. Huber and T. Leisner: Nature 42 (2003) 128