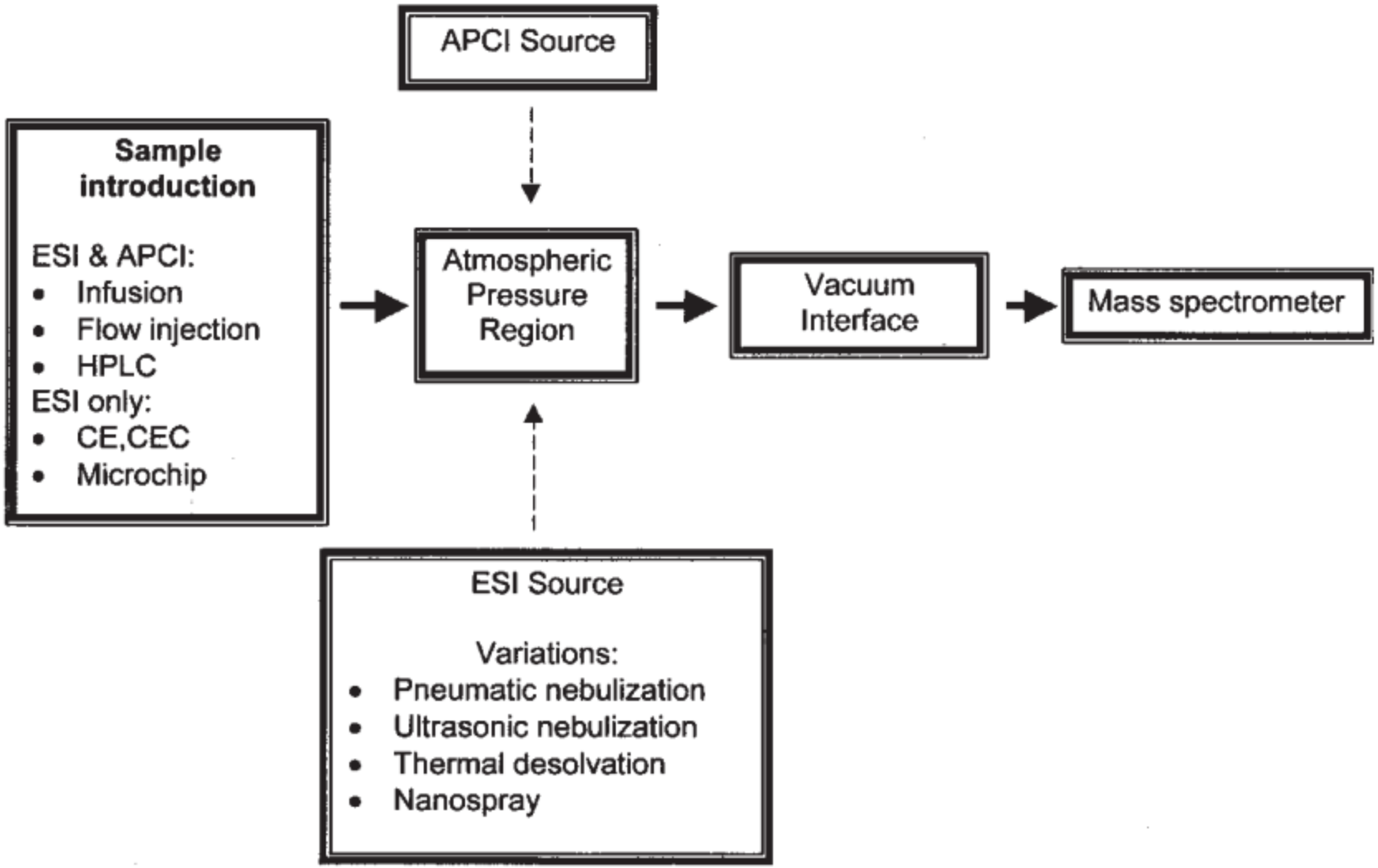
Electrospray and Nanospray



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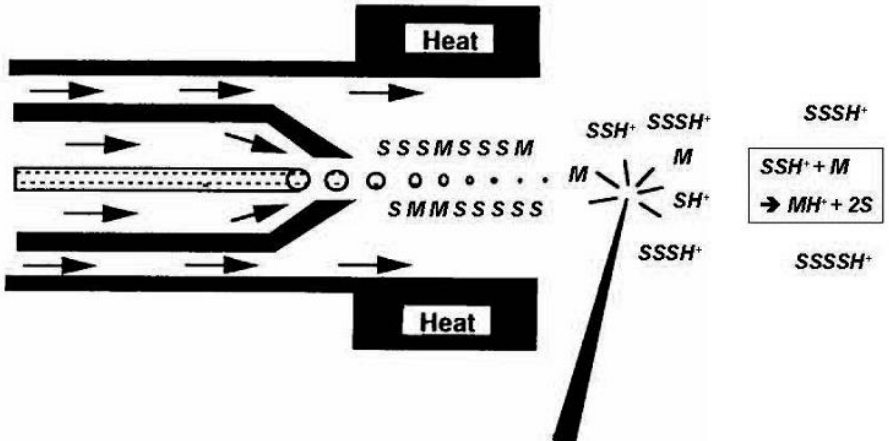
ESI:

- An electrospray needle at high electric potential
- A means for establishing an electric potential difference between the
- Spray needle and the orifice into the high vacuum region
- An optional desolvation apparatus (usually thermal or pneumatic)

APCI:

- A spray needle
- A thermal desolvation chamber
- A corona discharge needle interact with both the nitrogen nebulizing gas and the vaporized HPLC solvent to produce a corona of ions formed through direct ionization. Though nitrogen is the principal gas in the APCI chamber, nitrogen ions feed into a cascade of ion/molecule reactions in which water molecules (usually part of the mobile phase) capture a proton and react with additional water molecules to form a high flux of protonated clusters of water molecules.

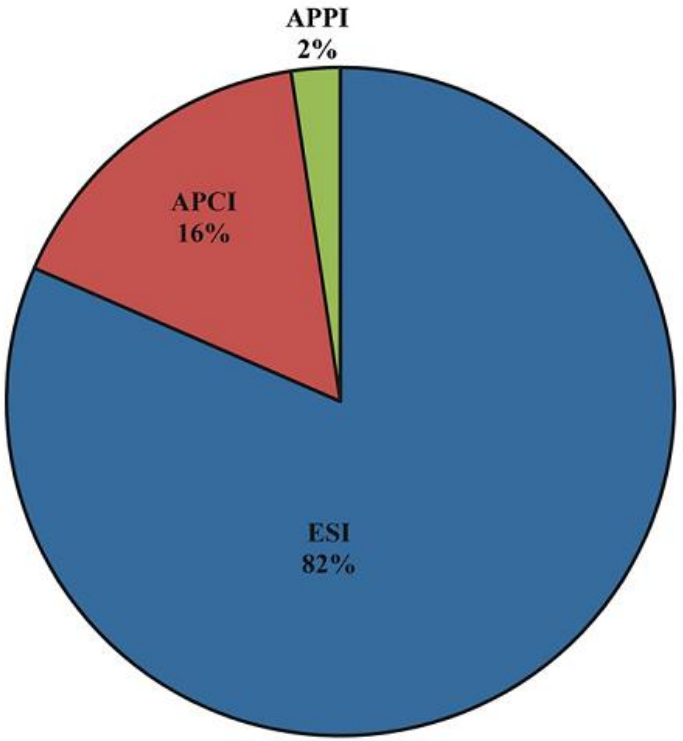


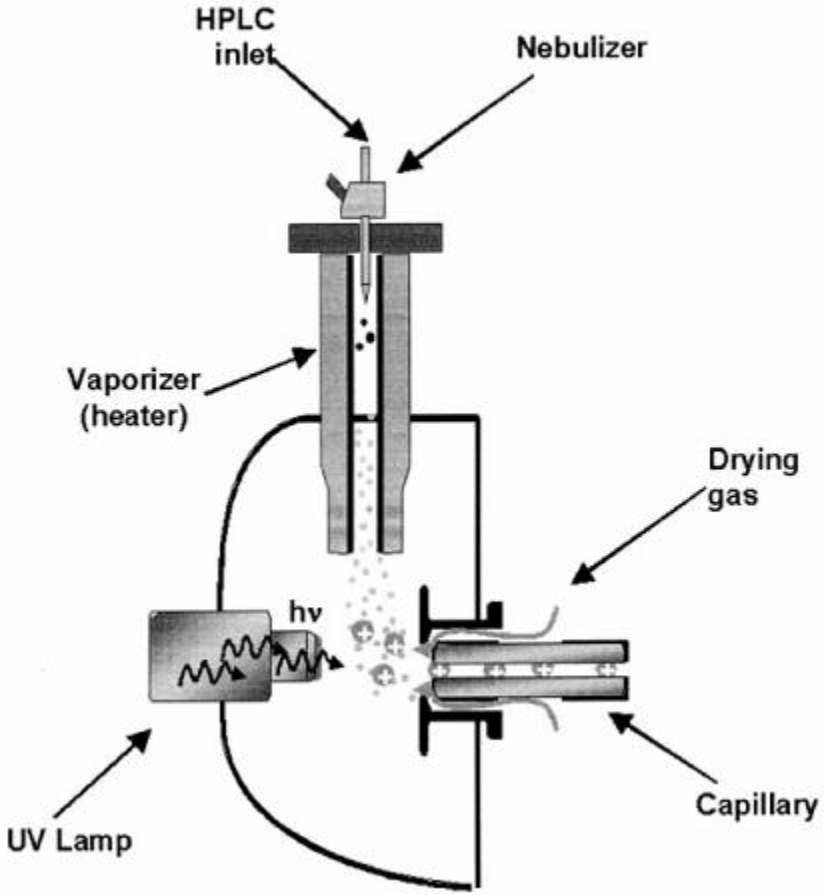
Atmospheric pressure chemical ionization is usually the first choice for smaller, less polar compounds such as steroids or carotenoids, Normal-phase liquid chromatography is more easily interfaced with an APCI source than an ESI source.

In negative-ion operation of API, air is recommended as the nebulizing gas because oxygen is necessary to start the ionization process (e.g., $O2^-$), in the accumulation of hydroxylated water clusters as the primary reagent ions; pure nitrogen is not appropriate as a nebulizing gas.

Operating Mechanics for APPI (Atmospheric Pressure Photoionization)

The high concentration of solvent vapor and entrained droplets in the APPI source absorbs most of the radiation before it reaches the center of the sample chamber; therefore, little radiation is available for photoionization in the center of the sprayer plume, which is highly sampled by the orifice leading to the mass spectrometer.





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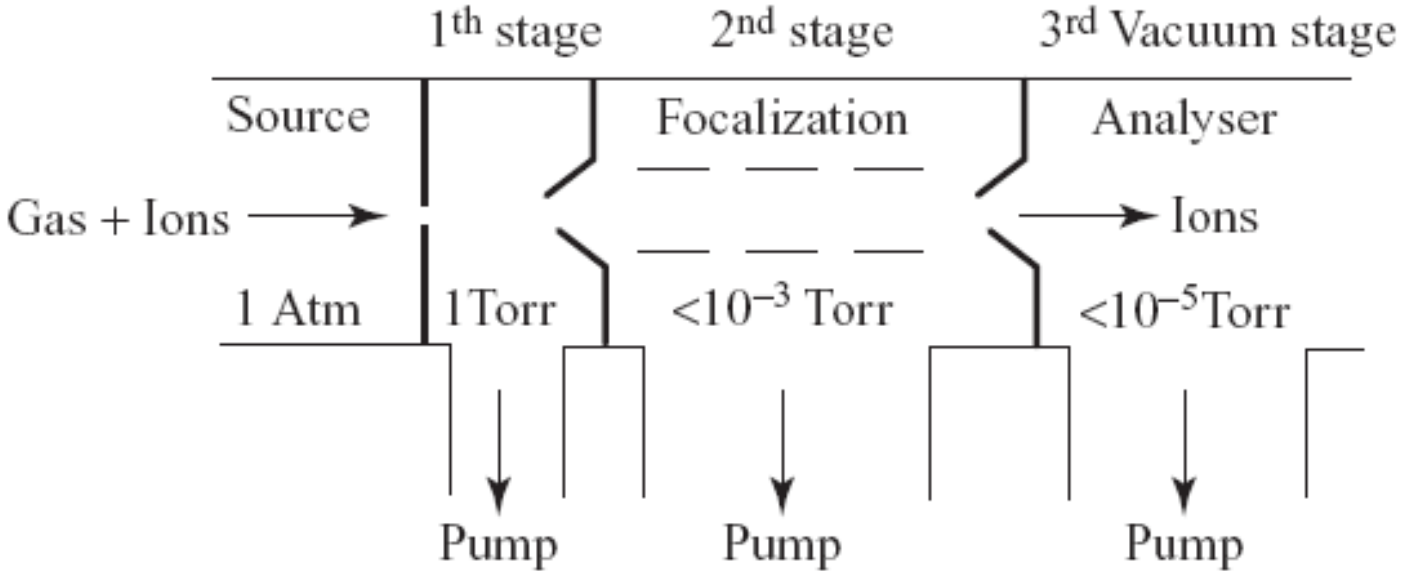
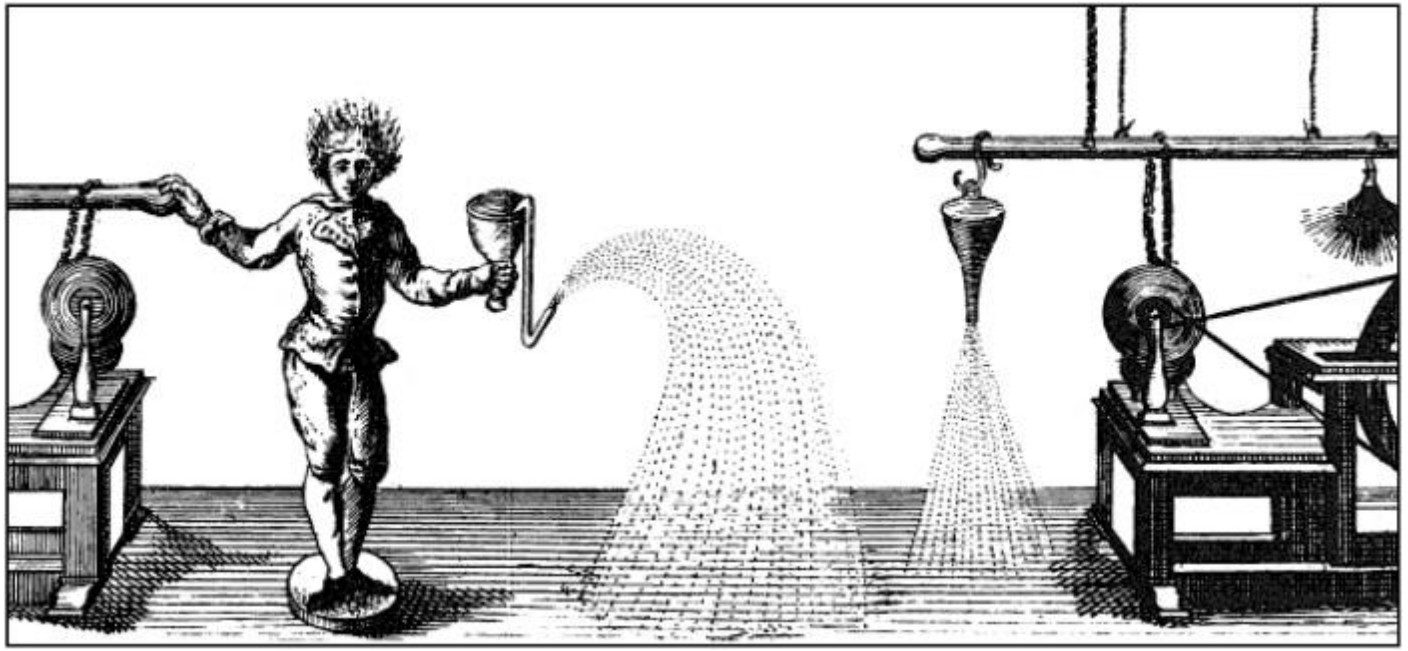


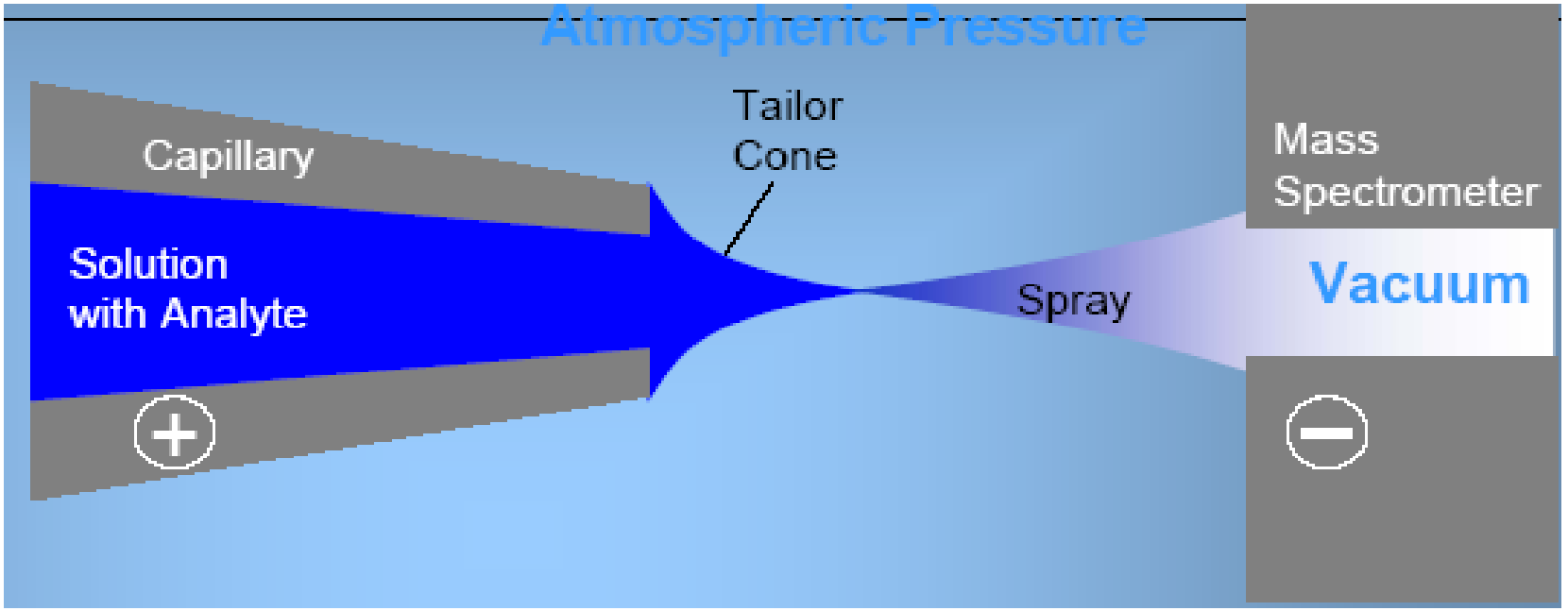
- Electrospray ionization is often referred to as a *desolvation* process, in contrast to "*desorption*" methods such as FAB, SIMS, and MALDI.
- The ions observed in ESI mass spectra are not the same as the ions that exist in the solution phase. ESI belongs to the "field desorption" category.

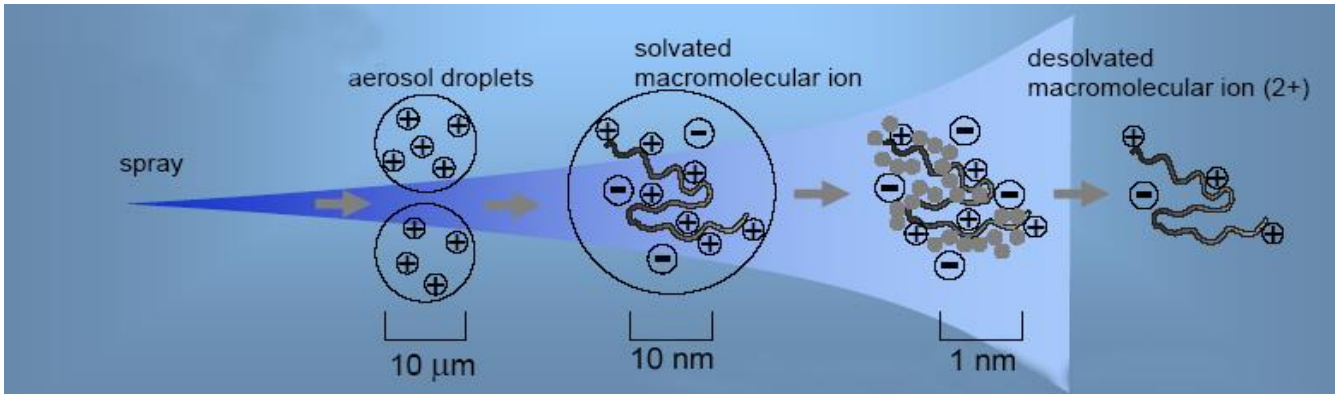


First experiments reported in 1775 that demonstrate the influence of voltage on a jet of water.

Book: Sport drug, Wiley 2010, Page 52.

The positive charges at the surface repel each other, and the liquid surface expands away from the needle tip. When the electrostatic force and the surface tension are balanced, the cone-shaped liquid surface has a half-angle of 49.3° at the apex. This is referred to as a “Taylor cone” following the work of G. Taylor. As the droplet grows small, the potential increases, the excess positive charge overcomes the surface tension, and droplet formation occurs from the tip of the Taylor cone.





- Another problem lies in the cooling caused by the sample and the **solvent adiabatic expansion** that favours the appearance of ion clusters.
- Efficient desolvation is provided by the introduction of

1. a heated metallized transfer tube (about 200°C)
2. by applying a counter-current flow of heated dry gas also called curtain gas.

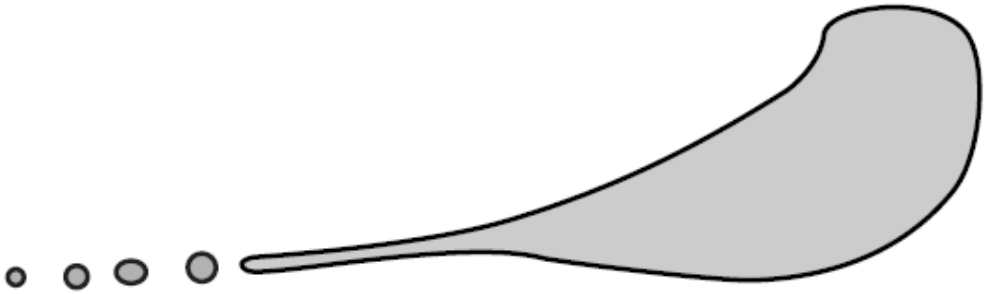
The ions collide with residual gas molecules:

1. increase their internal energy, which induces their final desolvation
2. disappear the ion clusters

But it give enough energy to induce ion fragmentation (in-source fragmentation).

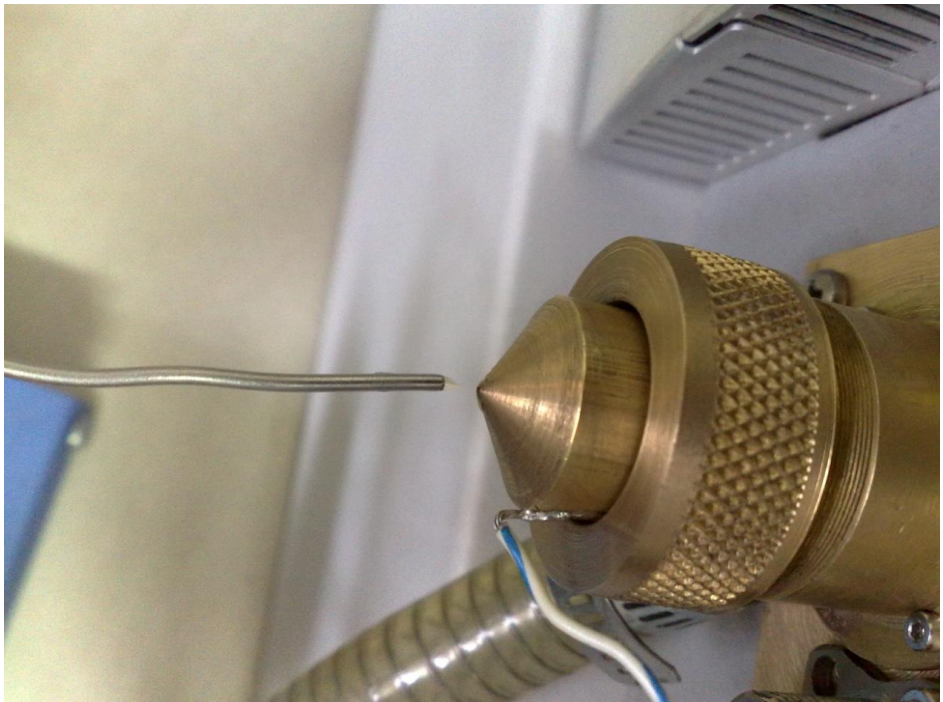
ESI device (voltage apply)

The point at which the charge repulsion is equal to the surface tension is the Rayleigh stability limit .
Where q is the droplet charge, R is the droplet radius, ε_0 is the permittivity of
vacuum, and is the surface tension.



Rayleigh: $q^2 = 8\pi^2 \varepsilon_0 \gamma D^3$

Electrospray ionization tends to produce ions with relatively low internal energies.



D.P.H. Smith equation: $$V_{on} \approx 0.2 \sqrt{r\gamma} \ln\left(\frac{4000\,d}{r}\right)$$

The potential V_{on} (kV) required for the onset of electrospray is related to the radius r (μm) of the electrospray needle, the surface tension of the solvent, γ (N/m), and the distance d (mm), between the needle tip and the counter electrode (the vacuum orifice).

With methanol as the solvent (γ =0.0226 N/m), a spray needle radius of 50 μm, and a needle–counter electrode distance of 5 mm, the onset potential is 1.27 kV. Changing the solvent to water (γ =0.073 N/m) increases the onset potential to 2.29 kV.

Separated by 0.3–2 cm (needle tip to counter electrode), producing electric fields of the order of 10^6 V m^{-1}.

Axial configuration of ESI

1. The orifice is no longer saturated by solvent. Instead, only ions are directed towards the inlet thus orifices can be larger than in the axial configuration.
2. The combination of larger orifices and noise reduction largely compensates for transmission losses due to the orthogonal geometry, giving a large gain in sensitivity.
3. The flow rates can be increased.
4. This configuration gives better protection of the orifice against contamination or clogging, giving a gain in robustness.
5. The orthogonal configuration with indirect trajectory of analyte ions also introduces unwanted discrimination based on mass or charge.

Optimization for normal ESI (cone-jet spray) can be *difficult with gradient chromatography* because mobile phase characteristics such as surface tension, viscosity and flow rate change. One set of ESI tuning conditions is unlikely to yield a stable spray mode throughout an LC gradient.

The resulting spray instabilities can shift charge state distribution and *increase the relative standard deviation* of ion current.

Multiply Charged Ions

Consider a positive ion with charge z_1 *whose mass-to-charge ratio is measured as being* m_1 *Th, issued from* ***a*** *molecular ion with mass MDa to which* z_1 *protons have been added.*

m=M/z

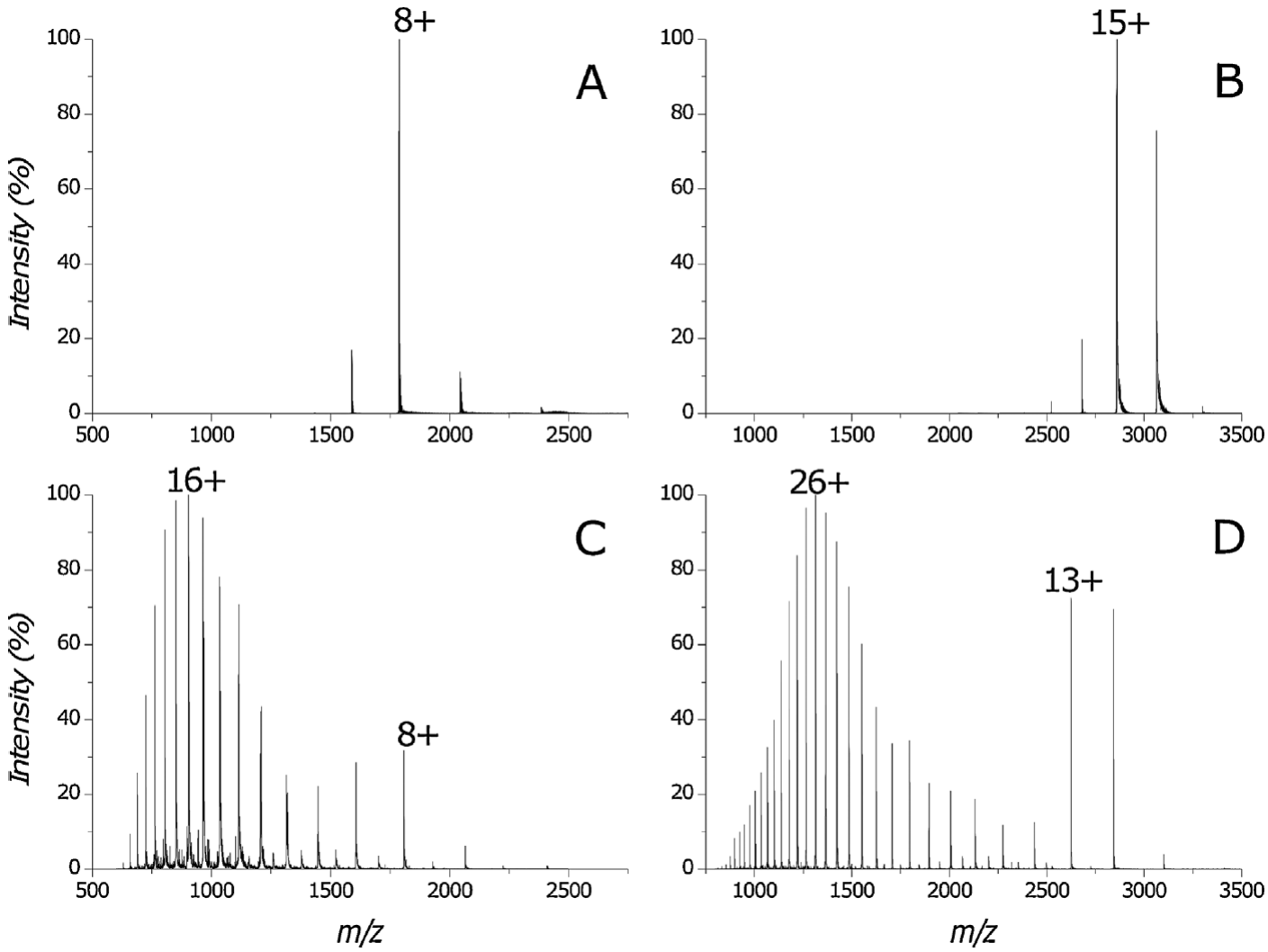
Really, we can see m_1 *in mass spectrum which equal to* $M/z_1 + m_p$ *thus*

$$z_1 m_1 = M + z_1 m_\mathrm{p}$$

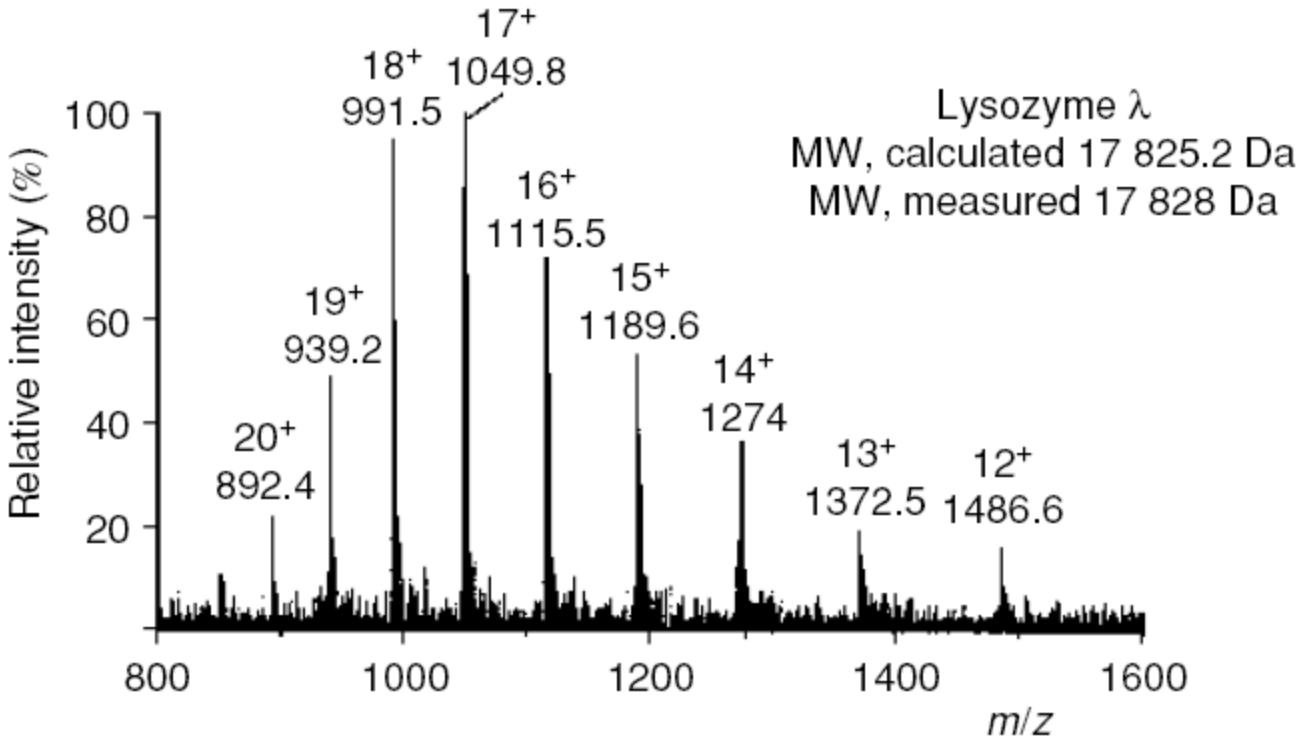
An ion separated from the first one by *(j−1) peaks, in increasing order of mass-to-charge* ratio, has a measured ratio of m_2 *Th and a number of charges* $z_1 - j$, *so that*

$$m_2(z_1 - j) = M + (z_1 - j)m_\mathrm{p}$$

$$z_1 = \frac{j(m_2 - m_\mathrm{p})}{(m_2 - m_1)} \quad \text{and} \quad M = z_1(m_1 - m_\mathrm{p})$$

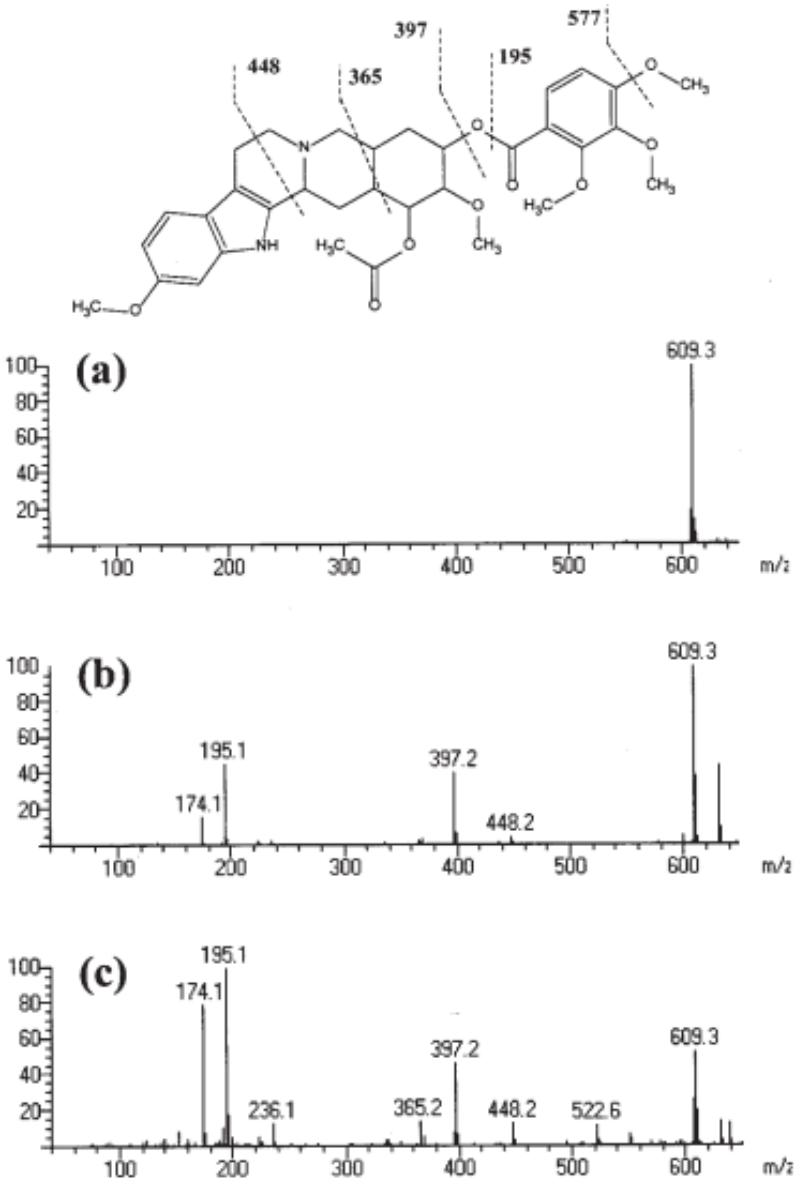


Charge state distribution (CSD) of globular, folded proteins (A, B) and IDPs (C, D). Nano-ESI-MS spectra in 10 mM ammonium acetate pH 7 of (A) chicken lysozyme, (B) maltose-binding protein, (C) human alpha-synuclein, and (D) fragment 1-291 of human ataxin-3. The most intense peak of each component is labeled by the corresponding charge state.



In this example, using the peaks at *m/z 939.2 and 1372.5 (j=6),* obtain *z1 =6(1372.5−1.0073)/(1372.5−939.2)=19*

In-source CID. Effect of changing vacuum interface ***potentials*** on the ESI mass spectrum of reserpine. The potential difference between orifice 1 and the ring lens is increased from (a) to (b) to (c).



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Heating

If the temperature of the desolvating chamber is kept to a reasonable value (e.g., 200°C), the heat achieves desolvation without inducing decomposition of the analyte.

Therefore, many “fragile” species such as thermally labile analytes and noncovalently bound complexes can readily be analyzed with an ESI source that makes use of thermal desolvation.

Species that cannot tolerate high temperatures, such as some unstable organometallic complexes, can be analyzed by the “cold ESI” technique developed

Solvents and Buffers

However, this solvent mixture caused protein denaturation and was of limited use for many other compound classes also.

However, in its normal mode of operation, ESI efficiency is limited by the presence of salts–buffers in the milieu, which are often prevalent in samples derived from biological fluids.

Though it is less amenable to coupling with separations techniques,

MALDI is more tolerant than ESI to the presence of salts–buffers, and furthermore shows impressive detection limits for species obtained from biological fluids.

Solvents that are commonly used for reverse-phase HPLC, such as water, methanol, and acetonitrile, are compatible with ESI.

Other useful solvents include dichloromethane and dichloromethane–methanol mixtures, dimethyl sulfoxide (DMSO), higher alcohols such as isopropanol and butanol, tetrahydrofuran (THF), acetone, and dimethyl formamide (DMF).

Solvents that do not work well for ESI are hydrocarbons such as hexane, aromatics such a benzene, and other nonpolar solvents such as carbon tetrachloride.

Toluene is not particularly well suited for use as an ESI solvent, but it has been used in some fullerene studies.

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Solvent Purity and Sample Contaminants

- ESI-MS is very sensitive to the presence of contaminants, and solvents should be free of salts and compounds that might contribute to the chemical background or suppress the analyte.

- The sodium is probably introduced to the solvents through contact with glassware or glass containers or transfer lines to the ESI source and produce detectable ions such as $[M+Na]^+$.

Tplasticizer bis(ethylhexyl) phthalate (also called dioctyl phthalate), which appears as an $[M+H]^+$ species at m/z 391 or the $[M+Na]^+$ species at m/z 413 as contaminate.

The $[M+H]^+$ species or cation attachment to produce $[M+Na]^+$, $[M+K]^+$, or $[M+NH_4]^+$.

Preformed ions such as quaternary ammonium salts are observed as M. Negative ion mass spectra show $[M-H]^-$ species for compounds that can lose a proton.

The tendency to form dimers such as $[2M+H]^+$ or $[2M+Na]^+$ increases with analyte concentration.

Steffanson et al. showed that the tendency to produce multimers could be reduced by adding primary amines.

Multiply charged ions such as: $[M+nH]^{n+}$, $[M+nNa]^+$, or $[M-nH]^{n-}$.

Buffers and Additives

The phosphate or sulfate buffers should be avoided for ESI-MS..

Volatile buffers and additives such as acetic acid, formic acid, ammonium acetate, ammonium hydroxide, and trifluoroacetic acid (TFA) are commonly used to control the pH for LC/MS analyses. Typical concentrations are in the 0.1–1% range.

The strong ion-pairing agent trifluoroacetic acid is known to reduce the analyte signal in ESI because analyte cations pair wit trifluoroacetate anions, resulting in charge neutralization.

A postcolumn addition of 50% propanoic acid in isopropanol is effective in improving sensitivity for separations performed with a solvent containing 0.1% TFA.

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The special ***advantages*** for ESI include:

(1) High reproducibility: no crystallization process is involved.

(2) High flexibility to attach to different types of mass spectrometer: due to the preference of multiply charged ions, the electrospray ionization source with lower m/z due to the multiple charges of each biomolecule can be fitted to ion-trap, quadrupole, Fourier-transform ion cyclotron resonance (FT-ICR) and TOF mass spectrometer.

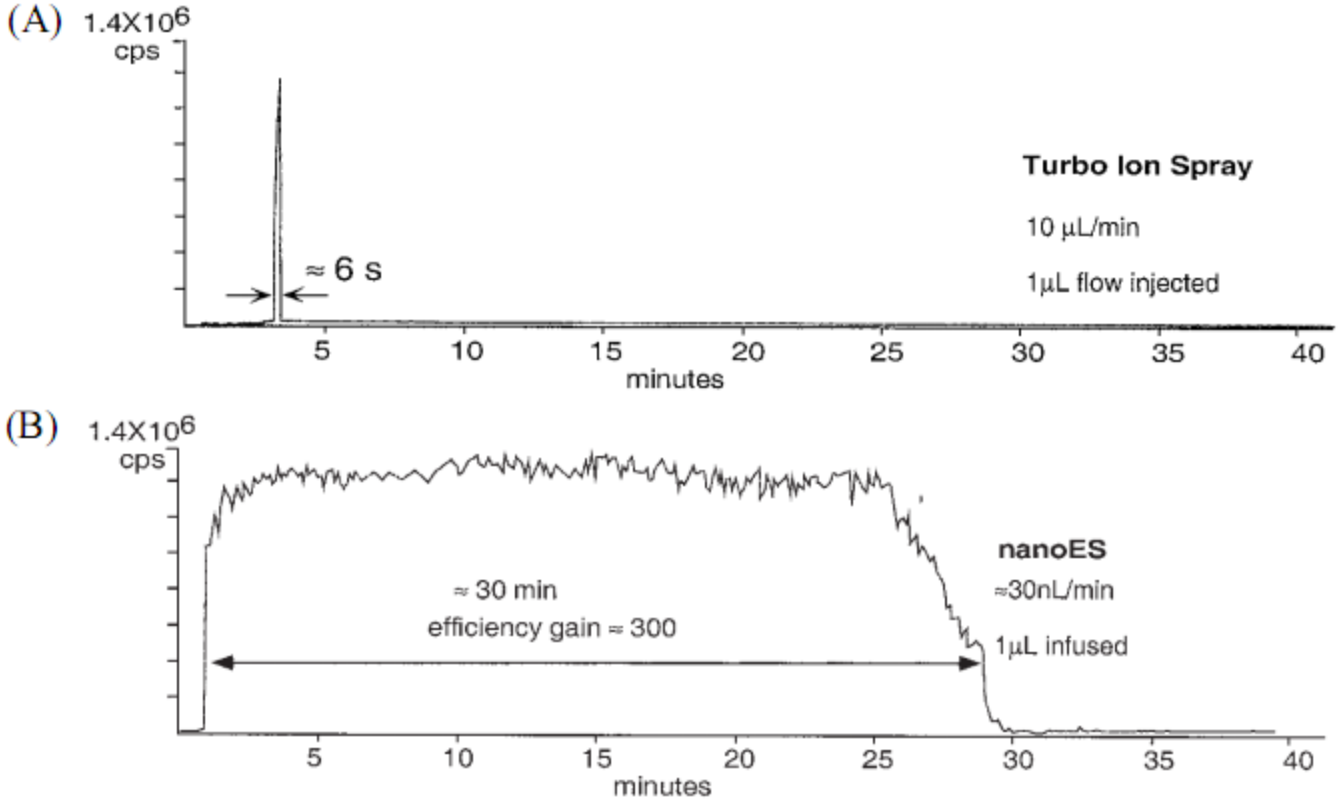
The major ***disadvantages*** are

(1) Complex spectra due to peaks from multiple charged ions.

(2) Large sample quantity: this disadvantage more or less disappears after the introduction of nanospray. ESI cannot be used for molecular imaging. Desorption electrospray ionization (DESI) can be used for molecular imaging but the space resolution is still significantly worse than that obtained by MALDI.

Nanospray

A solution of glufibrinopeptide (0.5 pmol/µL) was used to monitor the doubly charged molecule (selected ion monitoring; SIM). (A) A 1 µL aliquot of solution was injected into a 10 µL/min flow of solvent and ionized with a Turbo IonSpray heated pneumatically assisted electrospray nebulizer with a 50 µm i.d. fused silica capillary emitter. (B) One microliter of the same sample as that used in (A) was deposited into a nanoES tube with a drawn tip of approximately 2 µm exit aperture. Gas pressure of 20 psi was applied, and the sample was electrosprayed at a rate of approximately 30 nL/min until the sample was consumed.



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In nanospray glass capillaries are used as spray capillaries which are drawn out at one end either by a mechanical or a laser puller to give orifices of only 1–10 mm in diameter. For sufficient conductivity the capillaries are sputter coated with conductive material, e.g. gold. The capillaries are loaded from the back with only 1–5 mL of sample solution.

Moreover, the nanospray needle can be located very close to the orifice of the MS, especially for ESI sources using the heated transfer capillary (and no curtain gas) for which only crude and uncritical adjustment is needed. Thus, despite a factor of ca. 30 to 100 lower analyte-solution liquid flow the ESI mass spectra recorded under identical mass spectrometric settings typically exhibit a factor of 2 to 3 more intense signals.

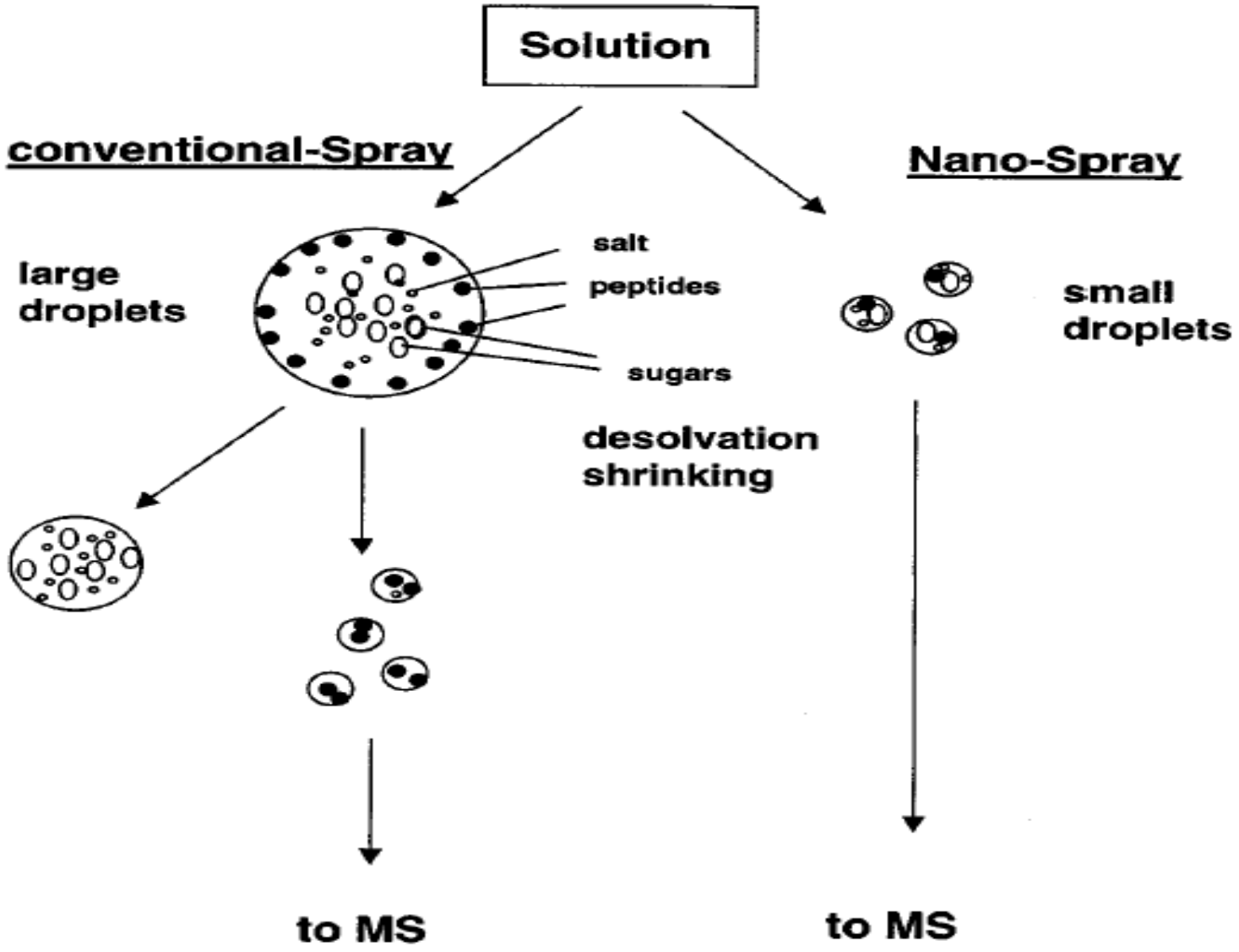
The condition whereby charge (q) becomes sufficiently large to overcome the surface tension of the liquid (γ) to produce droplets with radius (R) is described by the well-known Rayleigh equation, Eq. (1)

$$q^2 = 64\pi^2 \varepsilon_o \gamma R^3 \qquad (1)$$

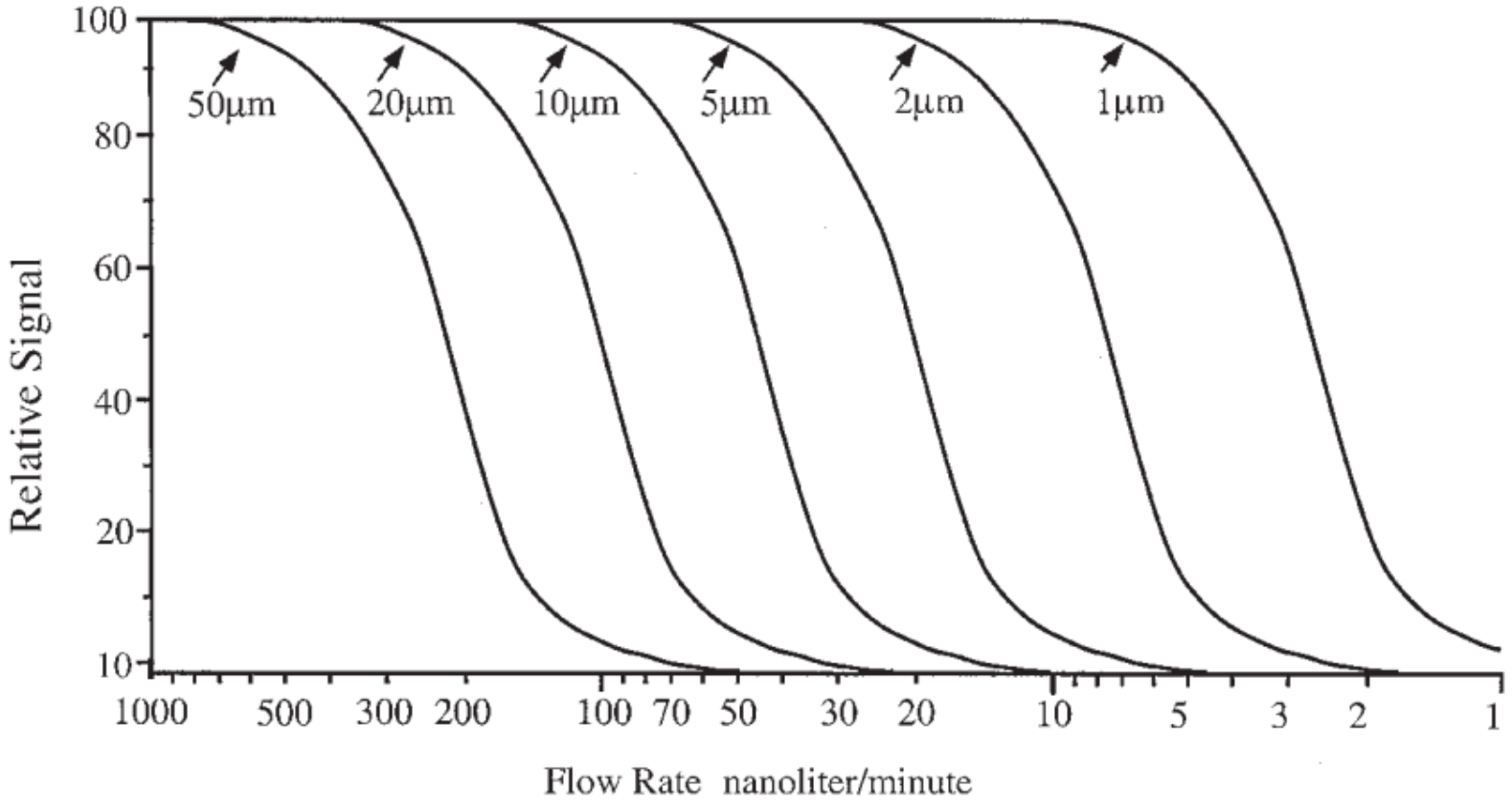
where (ε_0) is the permittivity of the vacuum. The radius of a droplet produced by electospray also depends upon the fluid flow rate (V_f), the permittivity of the solvent (ε), and solution conductivity (κ),

$$R \cong \left(V_f \frac{\varepsilon \varepsilon_o}{\kappa} \right)^{1/3} \qquad (2)$$

From this equation it is immediately obvious that smaller droplets are produced by using lower flow rates.


Solution
conventional-Spray
Nano-Spray
large
droplets
salt
peptides
sugars
small
droplets
desolvation
shrinking
to MS
to MS

Some results from experiments showing the relationship between tip inner diameter and lowest sustainable flow.



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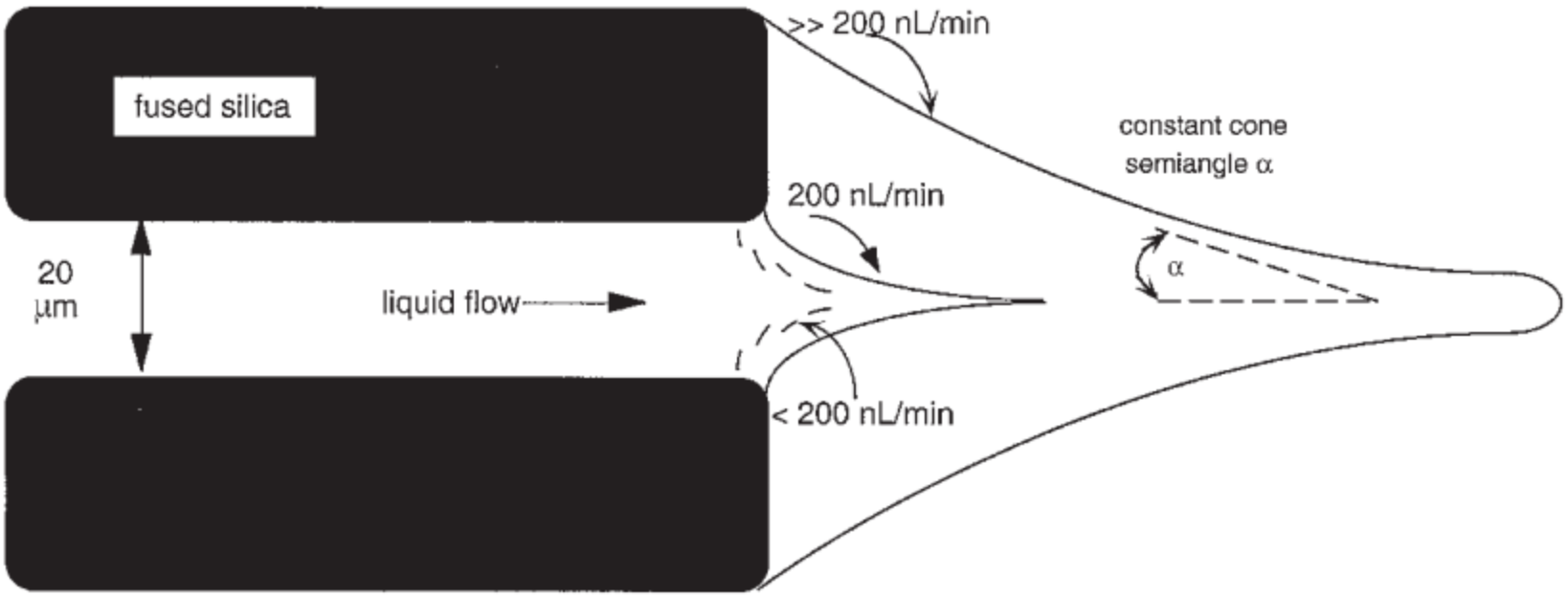
The diameter of the droplets emitted from the tip will increase with increasing flow, which, if the cone semiangle were to remain constant, could occur only if the cone were to increase in volume by broadening at the base and lengthening.



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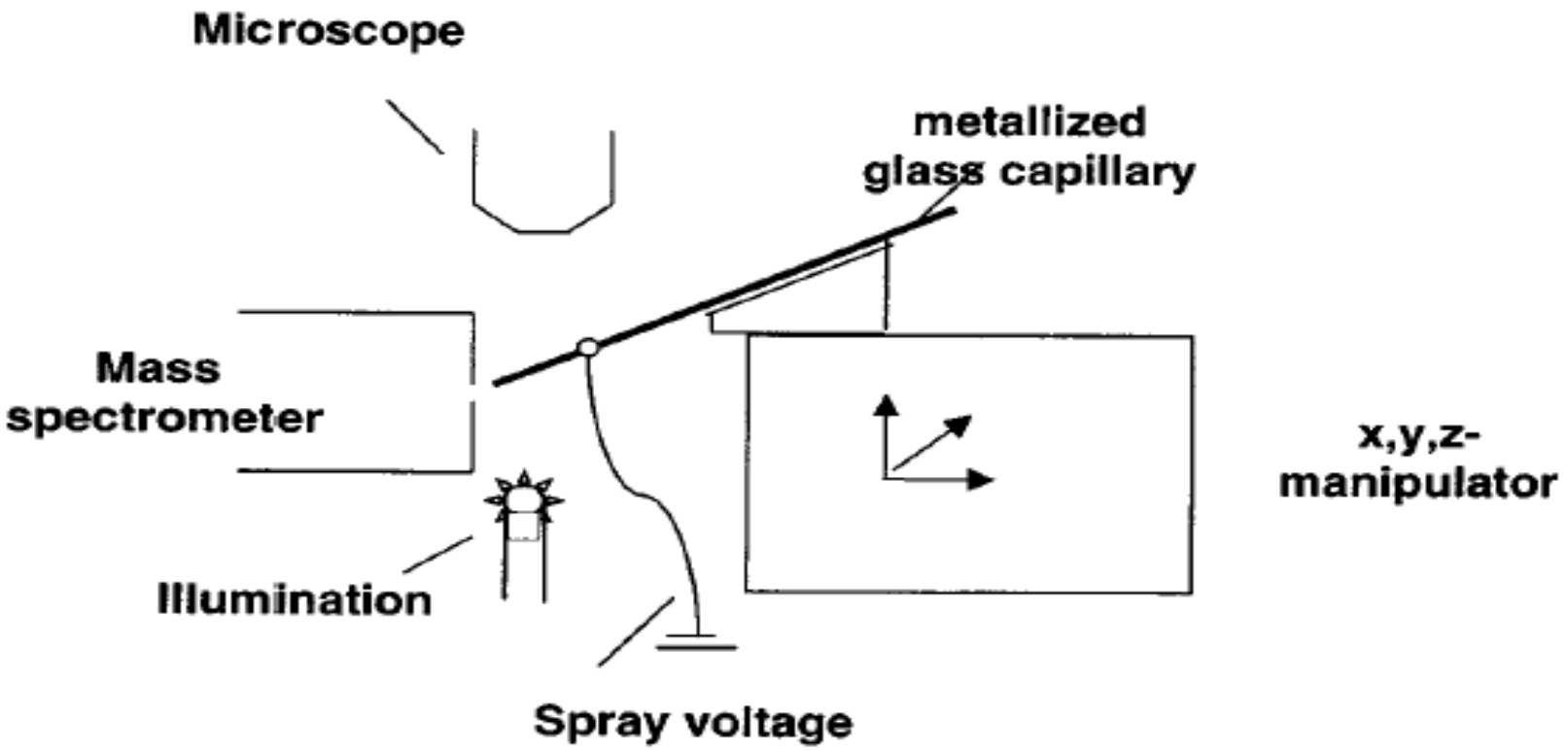
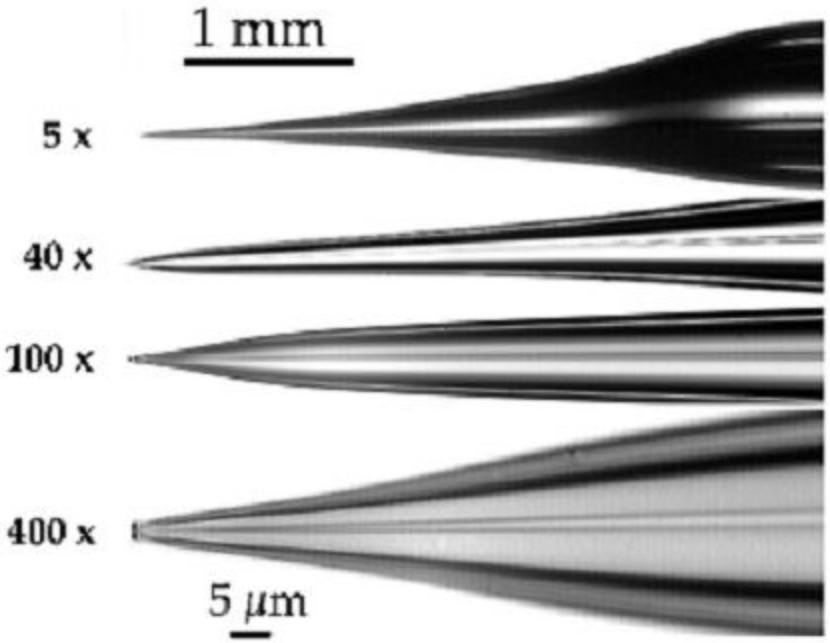
Schematic drawing of a nano-ESI device: the metallized glass capillary is adjusted using a micromanipulator under microscopic control 0.5–2 mm in front of the mass spectrometer orifice. A stable spray is obtained from some mL sample solution in the capillary by applying a voltage of 500–1000 V



Four photographs at different magnifications of the type of electrode drawn from a borosilicate glass tube.



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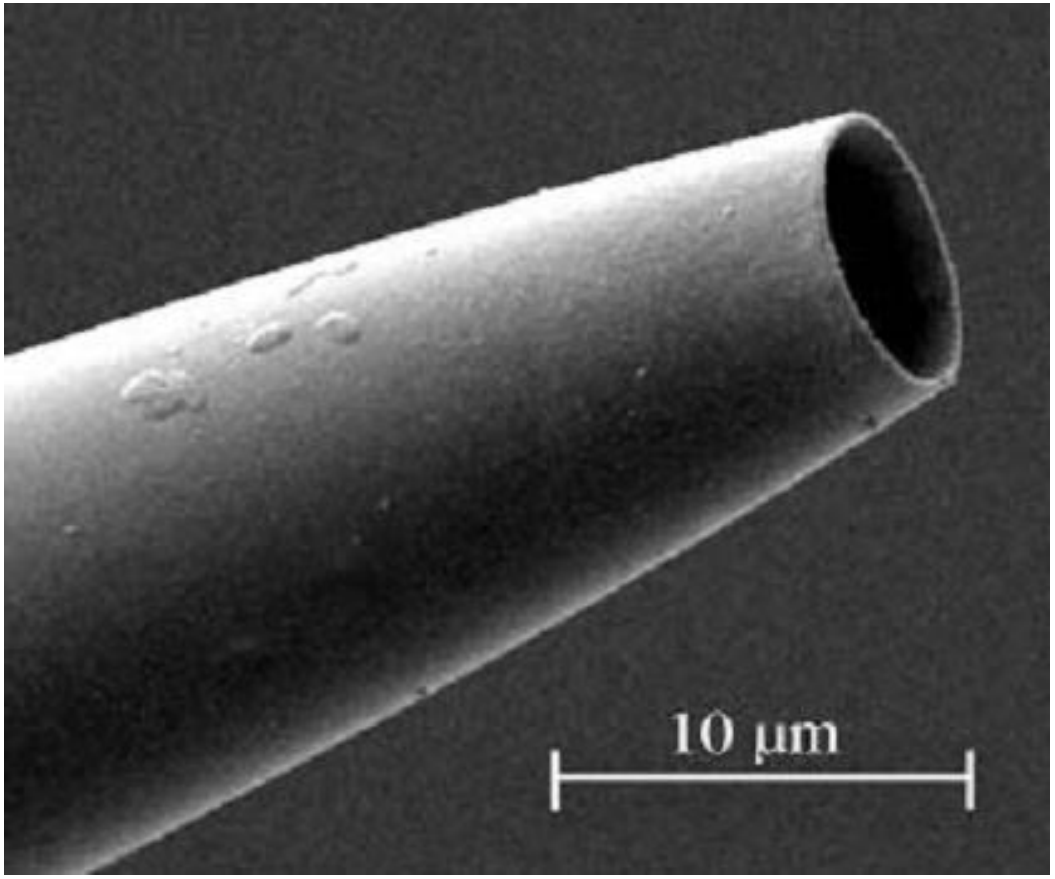
An electron micrograph of a 5 m tip from a nanoES electrode etched with HF.



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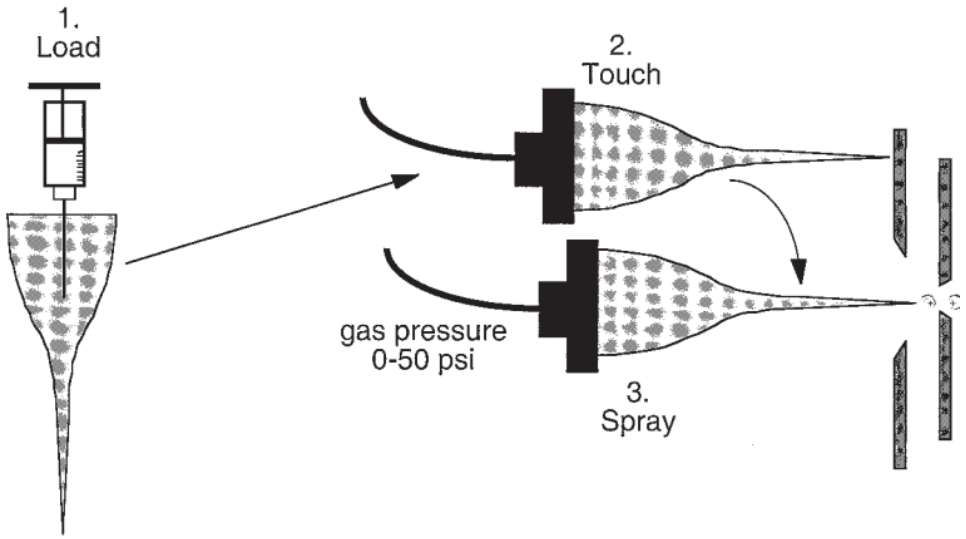
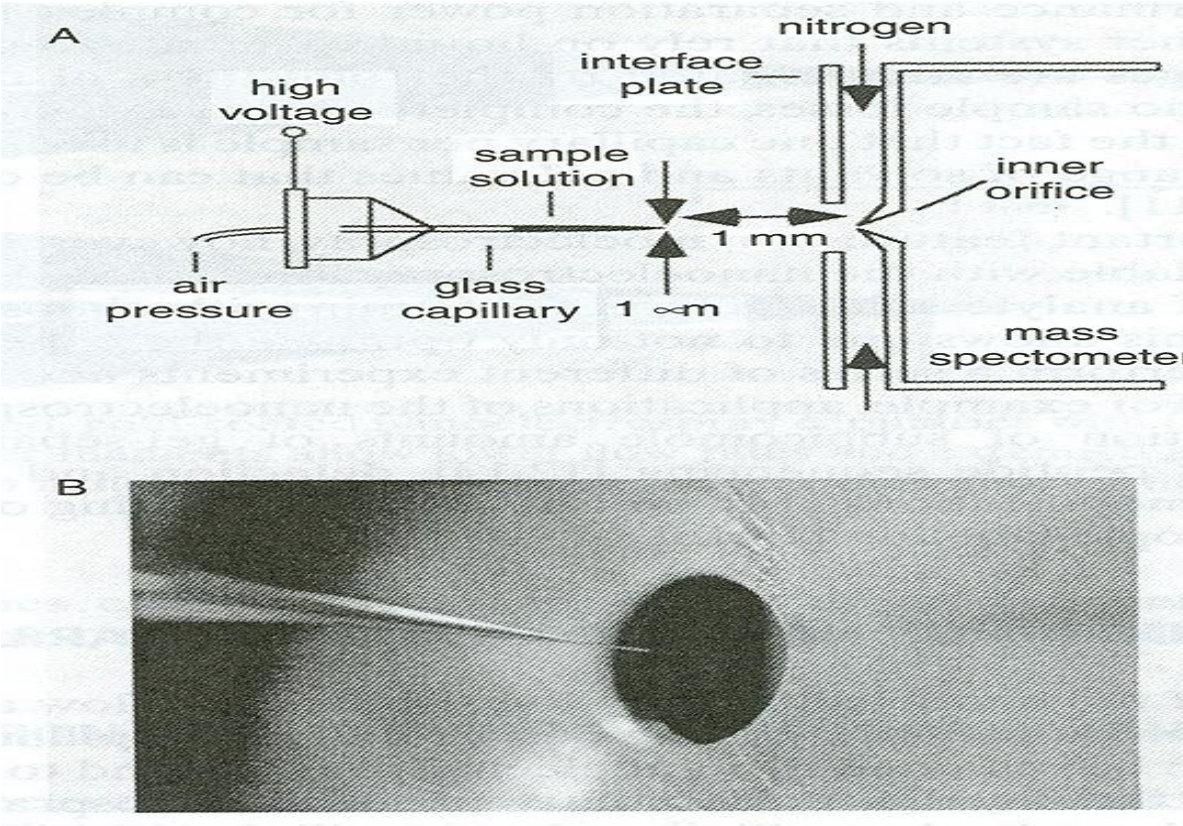
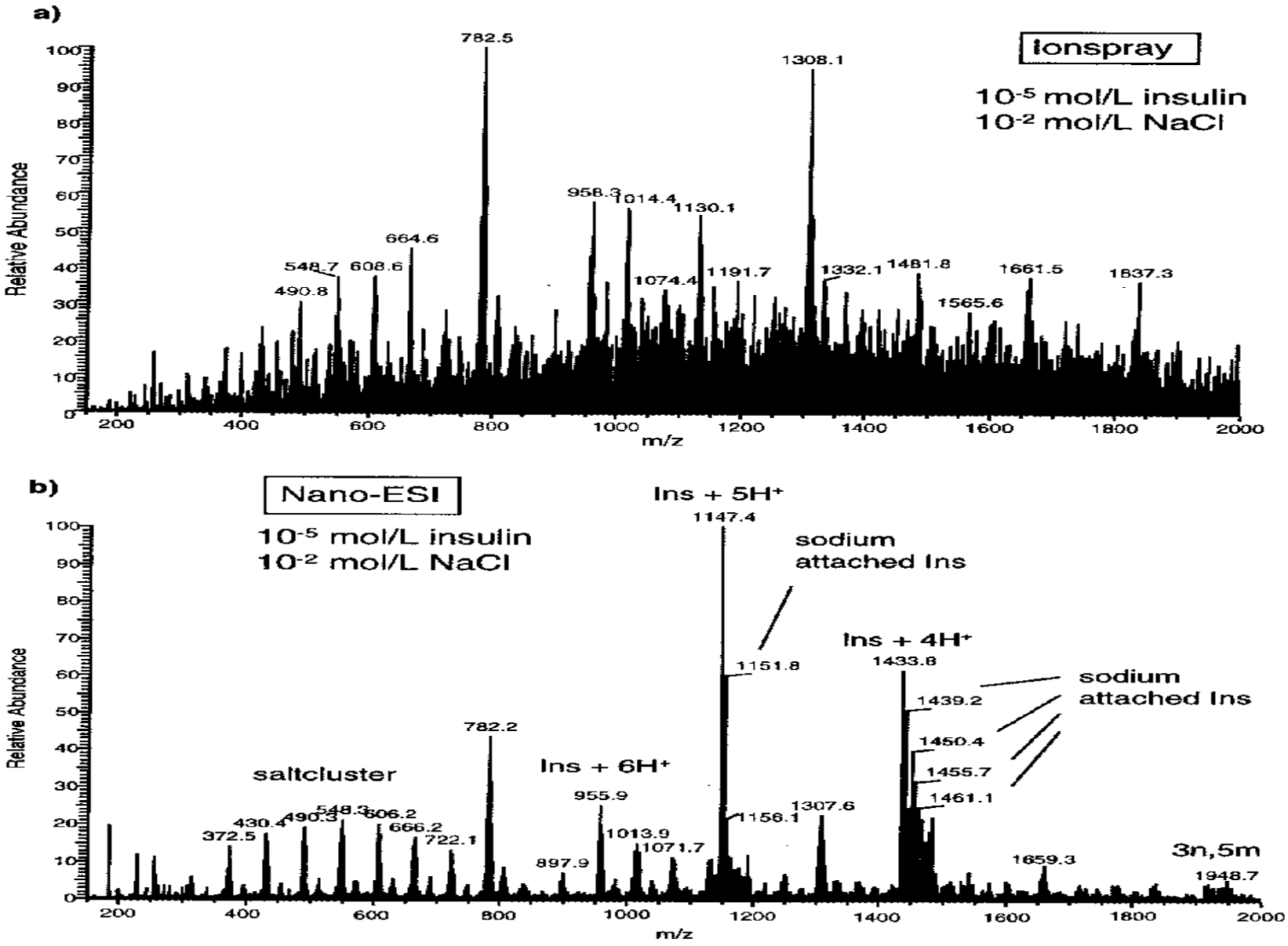


General sequence for nanoES operation. 1, Sample is put into the capillary with gel-loading Eppendorf tips. 2, If the tips are sealed, a touch/fracture step with the aid of microscopic inspection is required to initiate flow. 3, Slight gas pressure is used to tune the flow.




a)
Ionspray
10-5 mol/L insulin
10-2 mol/L NaCl
Relative Abundance
m/z
782.5
1308.1
958.3
1014.4
1130.1
664.6
548.7
608.6
490.8
1074.4
1191.7
1332.1
1481.8
1661.5
1837.3
1565.6
b)
Nano-ESI
10-5 mol/L insulin
10-2 mol/L NaCl
Ins + 5H+
1147.4
sodium
attached Ins
Ins + 4H+
1433.8
1151.8
sodium
attached Ins
1439.2
1450.4
1455.7
1461.1
782.2
saltcluster
Ins + 6H+
955.9
1307.6
1156.1
1013.9
1071.7
897.9
372.5
430.4
490.3
548.3
606.2
666.2
722.1
1659.3
3n,5m
1948.7

Thermo Scientific – LCQ MS

atmospheric pressure interface

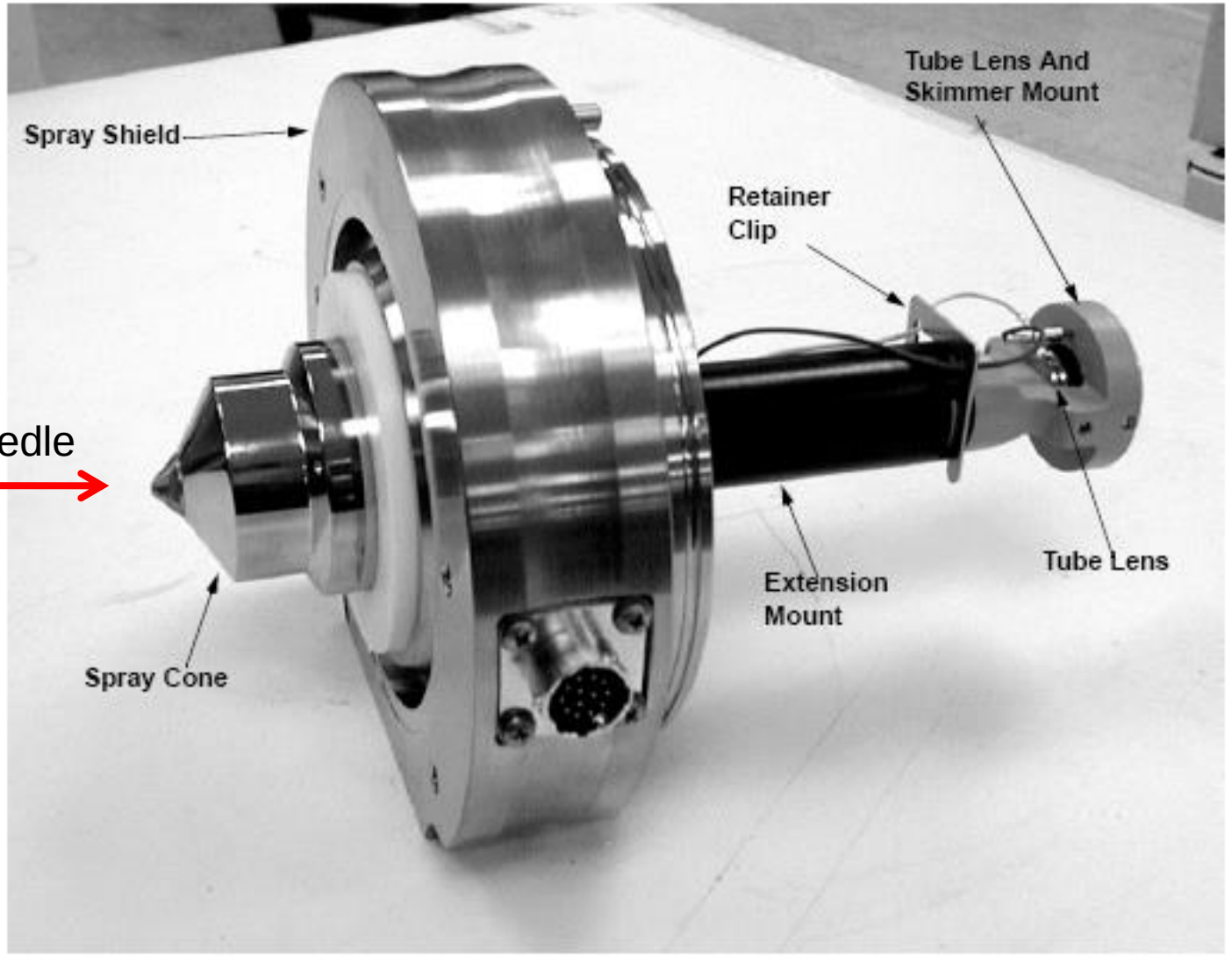


Figure 4-1. API stack

[Manual, Thermo Scientific]

Thermo Scientific – LCQ MS

ESI probe and source interface

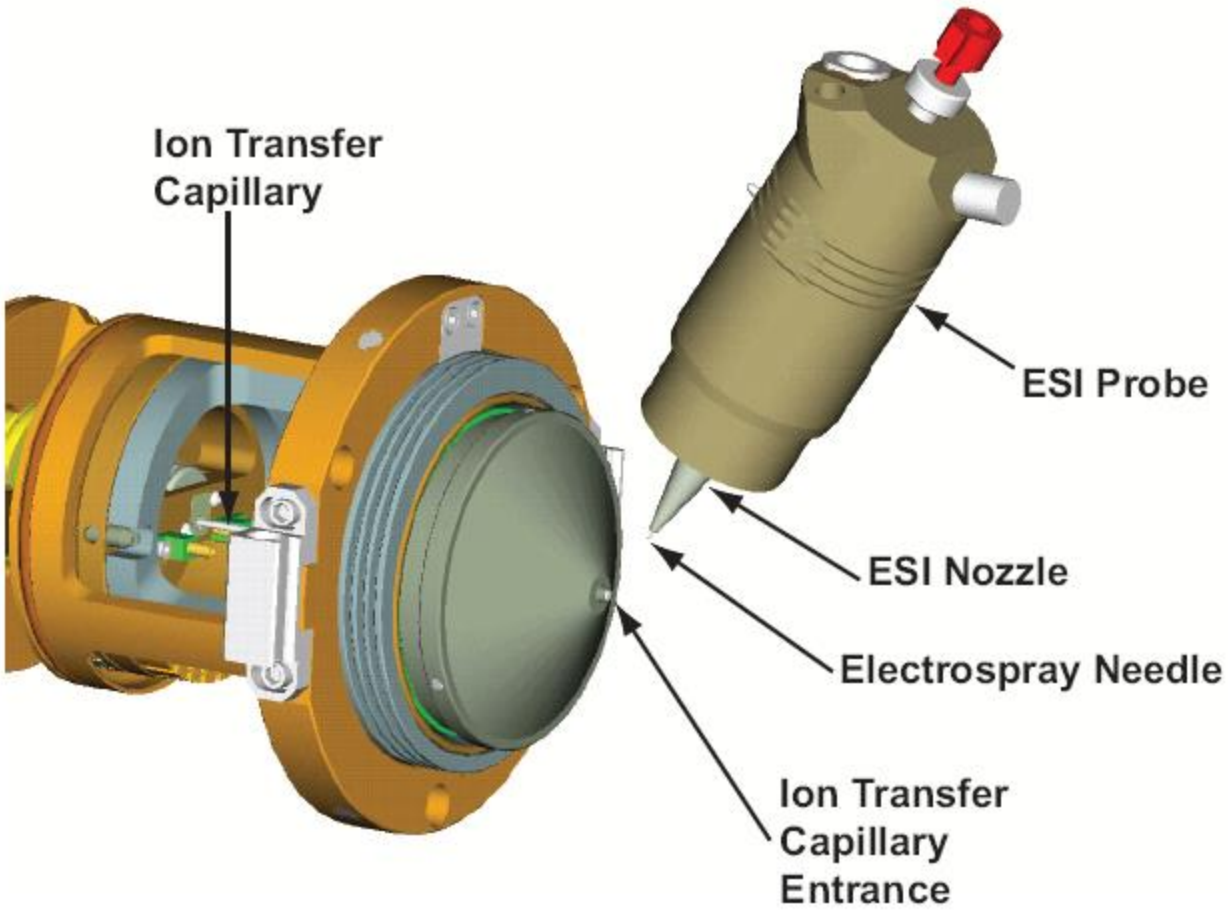


Figure 3. ESI probe and ion source interface

[Manual, Thermo Scientific]

Thermo Scientific – LCQ MS

atmospheric pressure interface

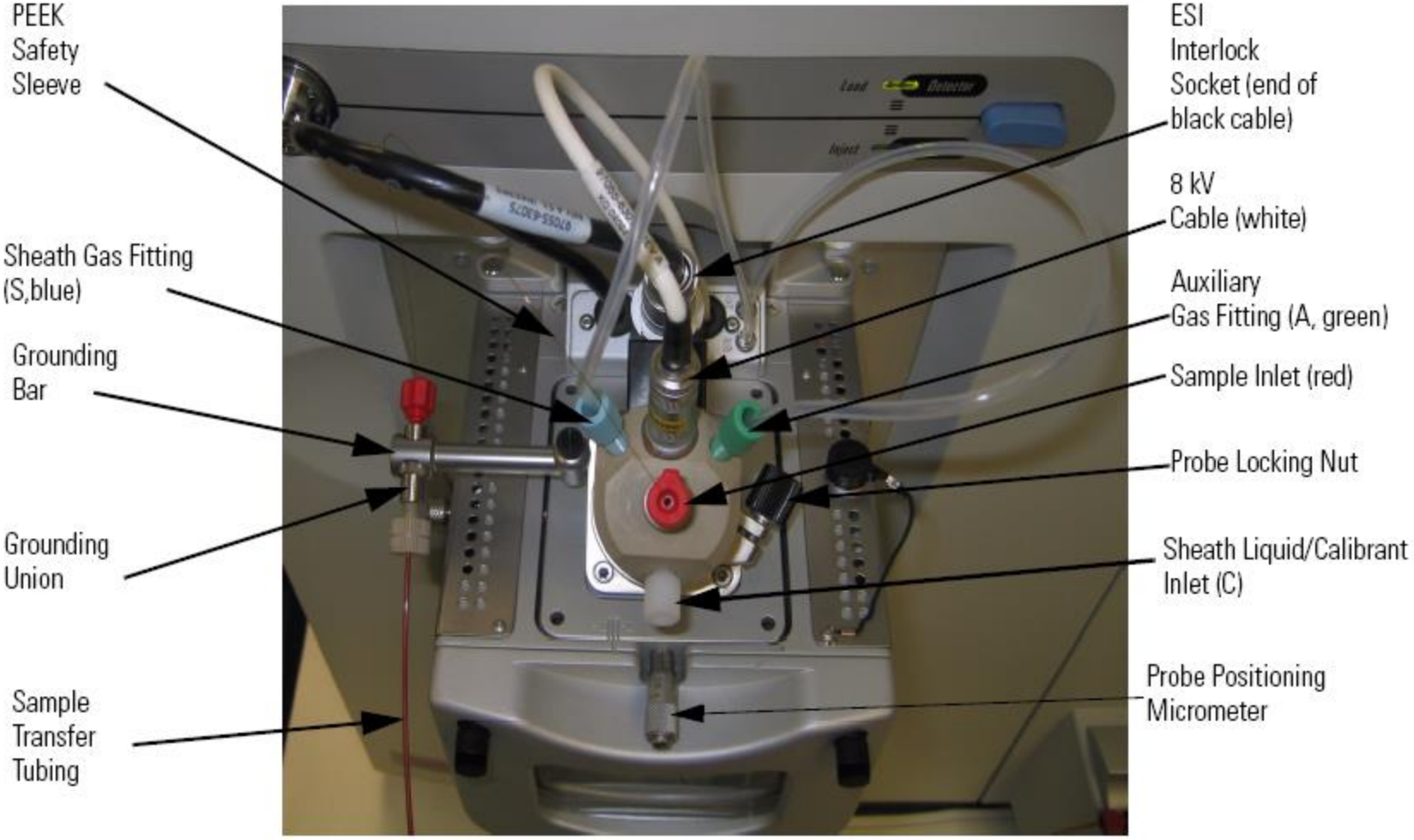


Figure 5. Ion Max ion source housing with ESI probe installed

[Manual, Thermo Scientific]

Thermo Scientific – LCQ MS

atmospheric pressure interface

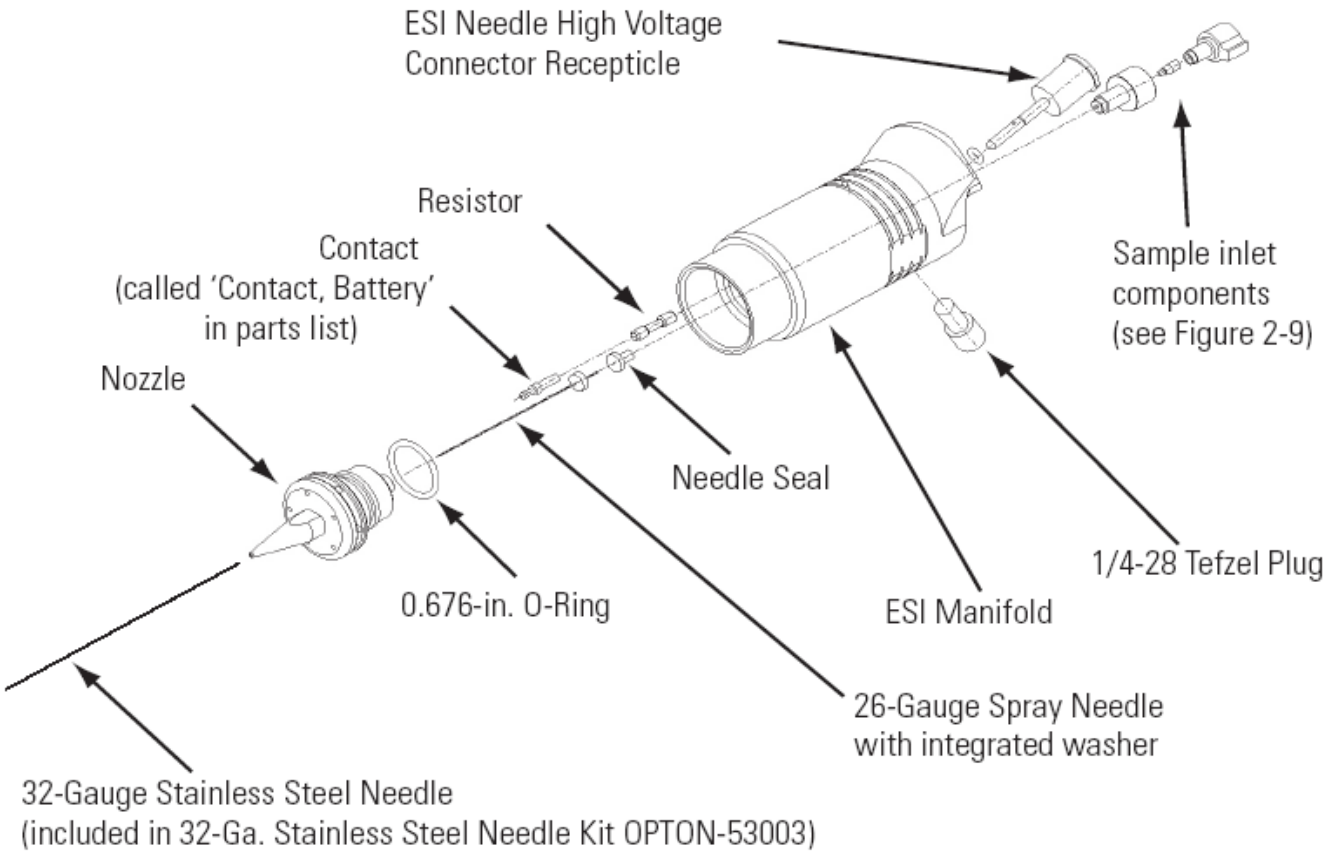


Figure 9. Exploded view of the ESI probe and parts (part numbers are given in Figure 24)

[Manual, Thermo Scientific]

Thermo Scientific – LCQ MS

atmospheric pressure interface

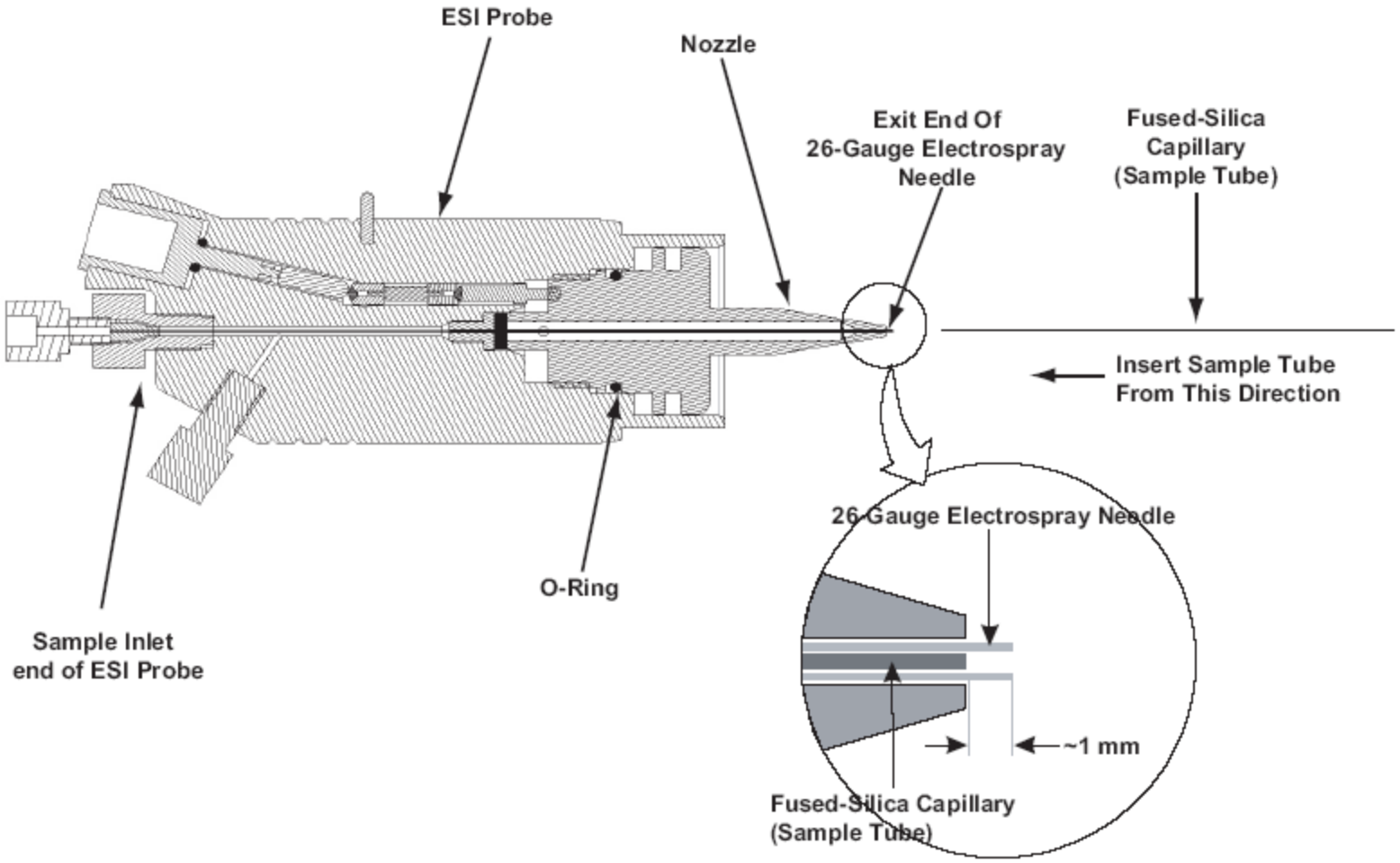


Figure 13. Installing the ESI fused-silica sample tube

[Manual, Thermo Scientific]

Thermo Scientific – LCQ MS

nanospray source

Figure 3-3. Camera mounted on the Finnigan LCQ Series MS detector

[Manual, Thermo Scientific]

Thermo Scientific – LTQ MS

nanospray source

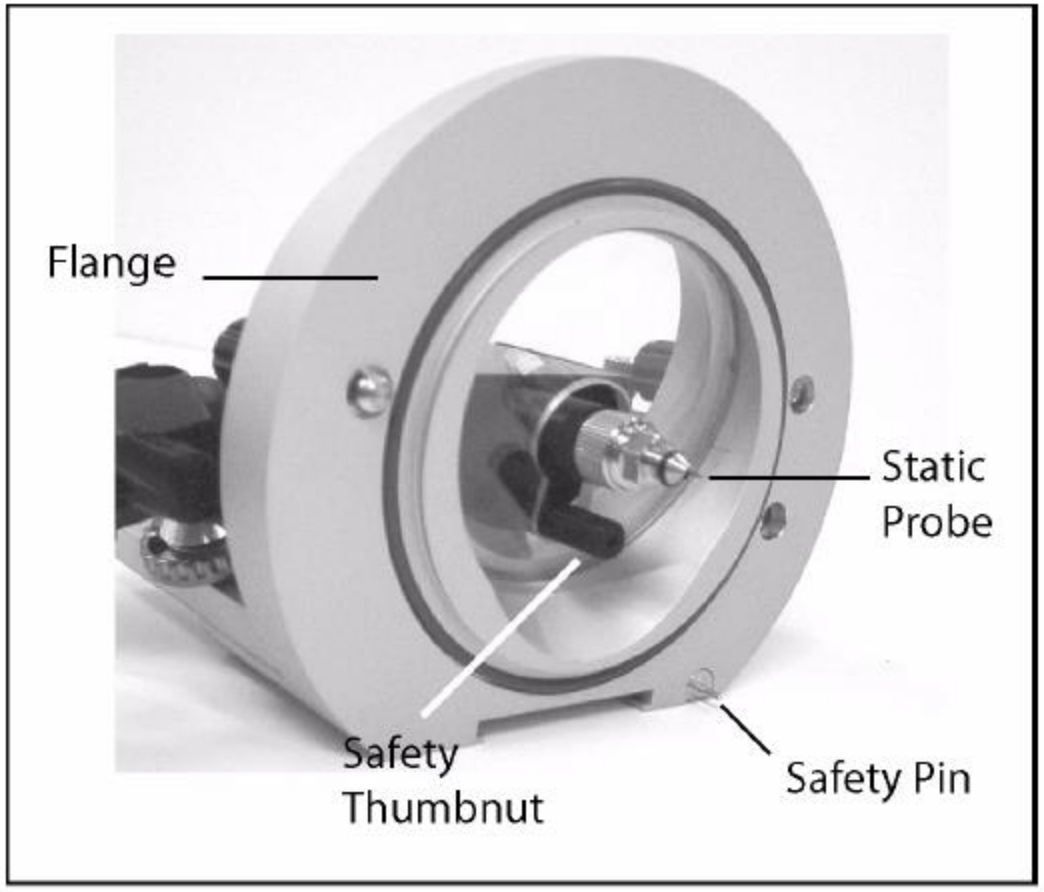


Figure 4-2. Static NSI source (Finnigan LCQ Series MS detector face)

[Manual, Thermo Scientific]

Thermo Scientific – LTQ MS
nanospray source

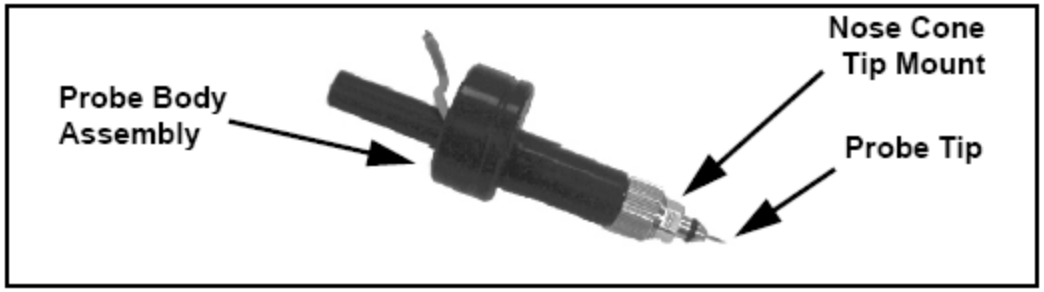


Figure 4-1. Static NSI probe assembly

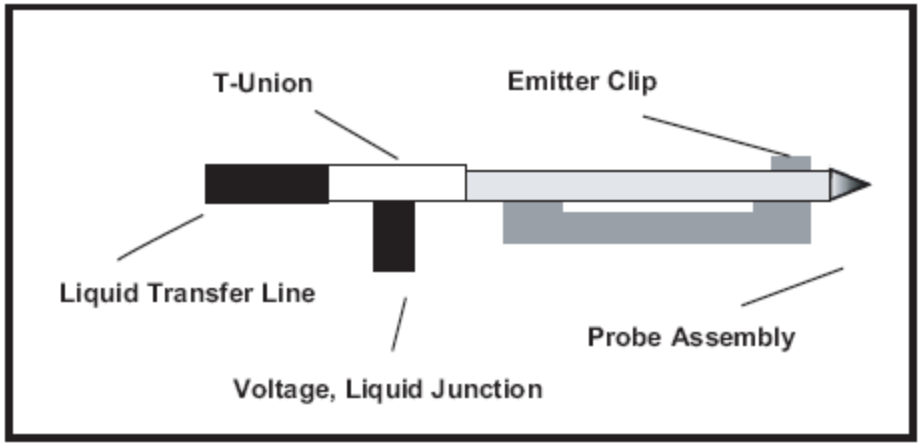


Figure 1-4. Illustration of the principal dynamic nanospray ion source components

[Manual, Thermo Scientific]

Thermo Scientific – LTQ MS
nanospray source

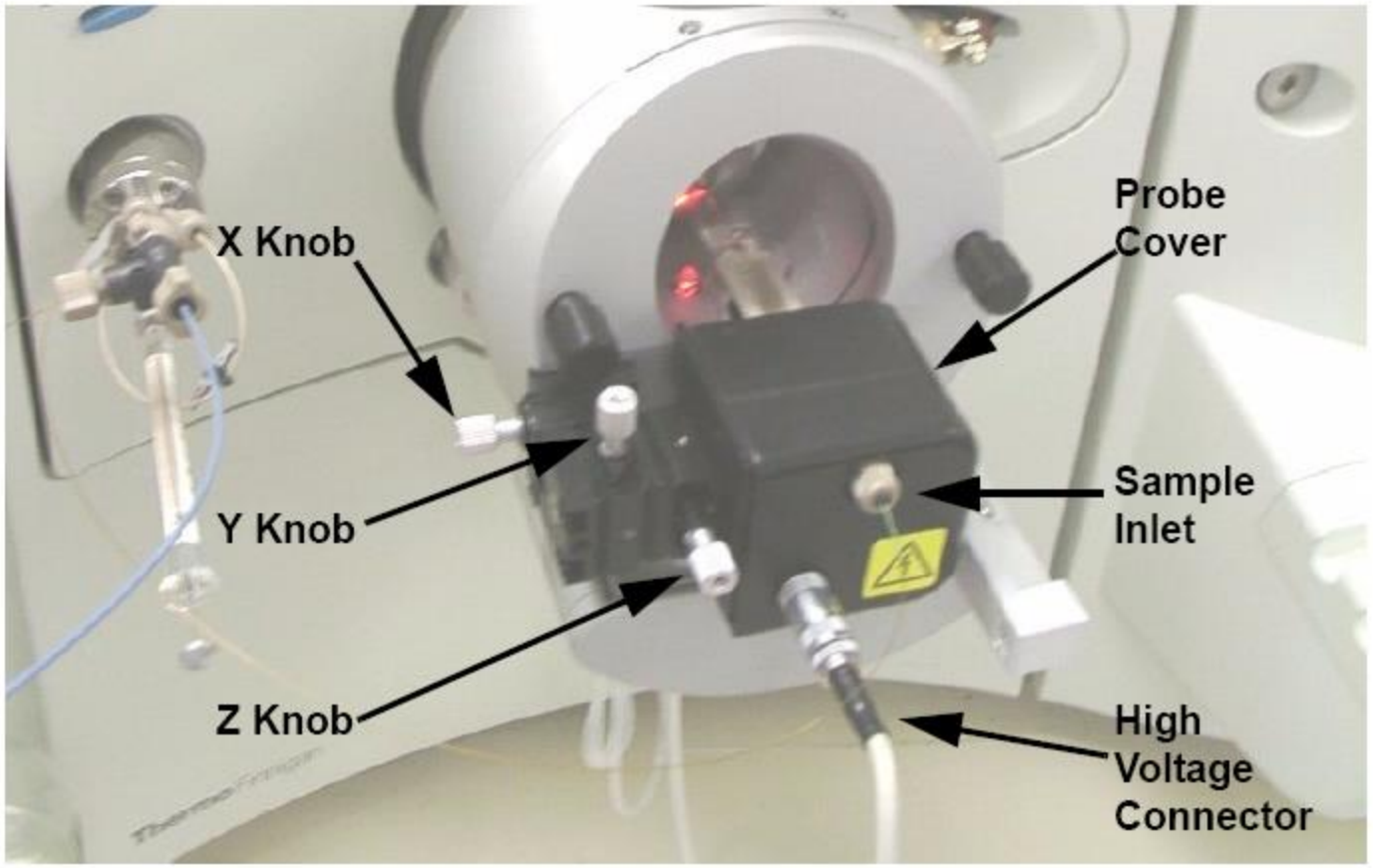
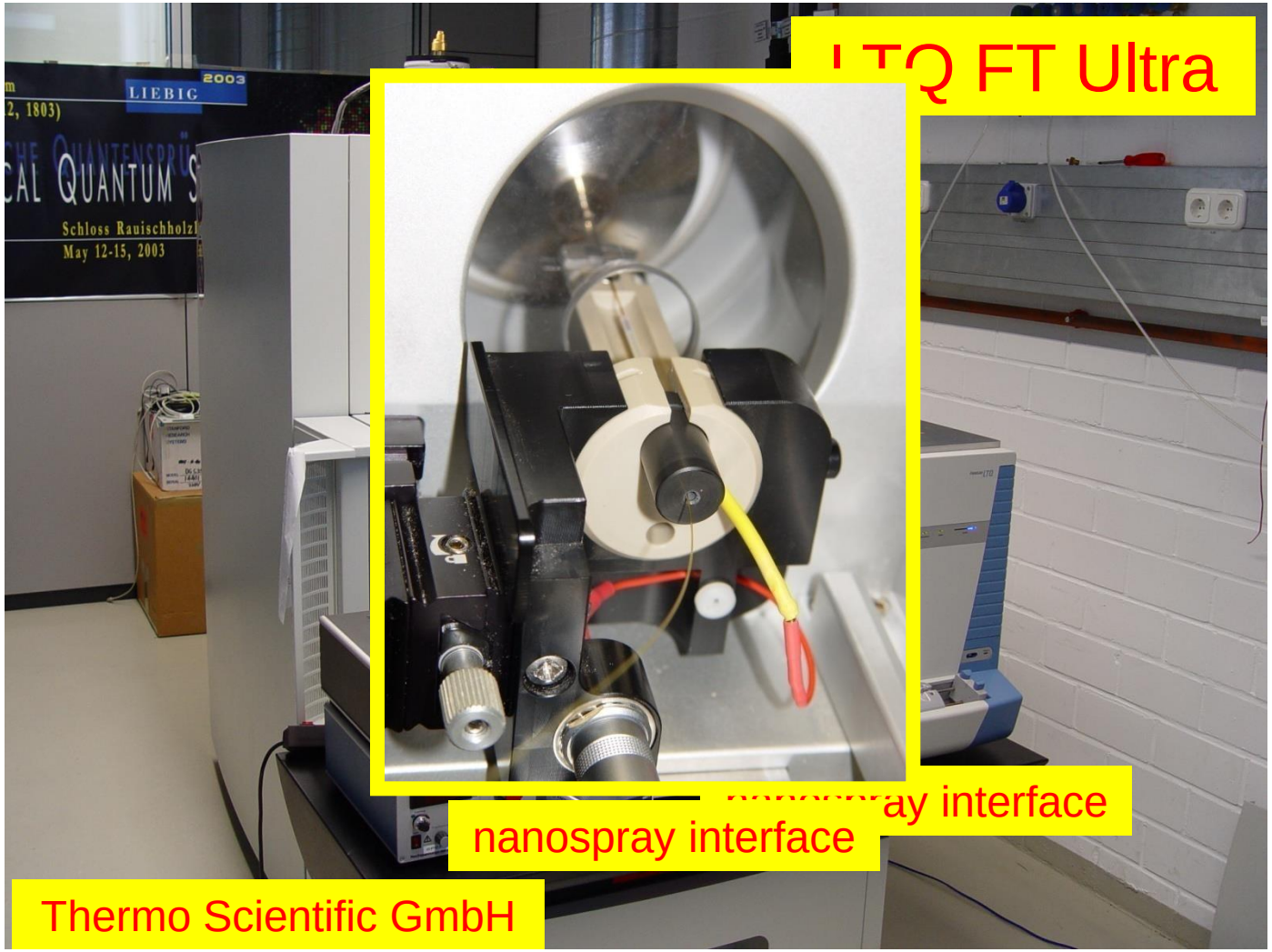


Figure 4-3. Static NSI source on a Finnigan LCQ Deca XP

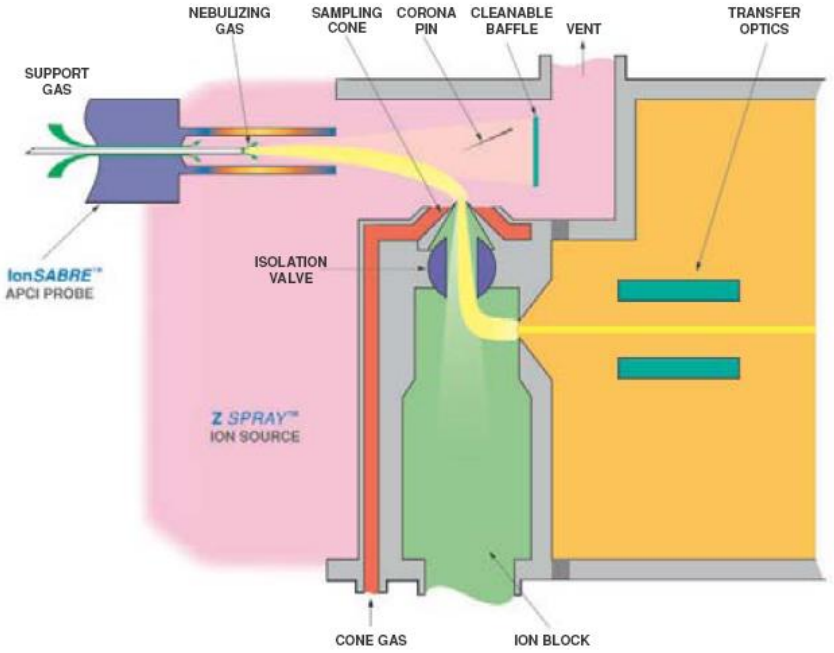
[Manual, Thermo Scientific]

ESI-FTMS


LIEBIG
2003
Schloss Rauischholz
May 12-15, 2003
LTQ FT Ultra
nanospray interface
Thermo Scientific GmbH

Zspray source

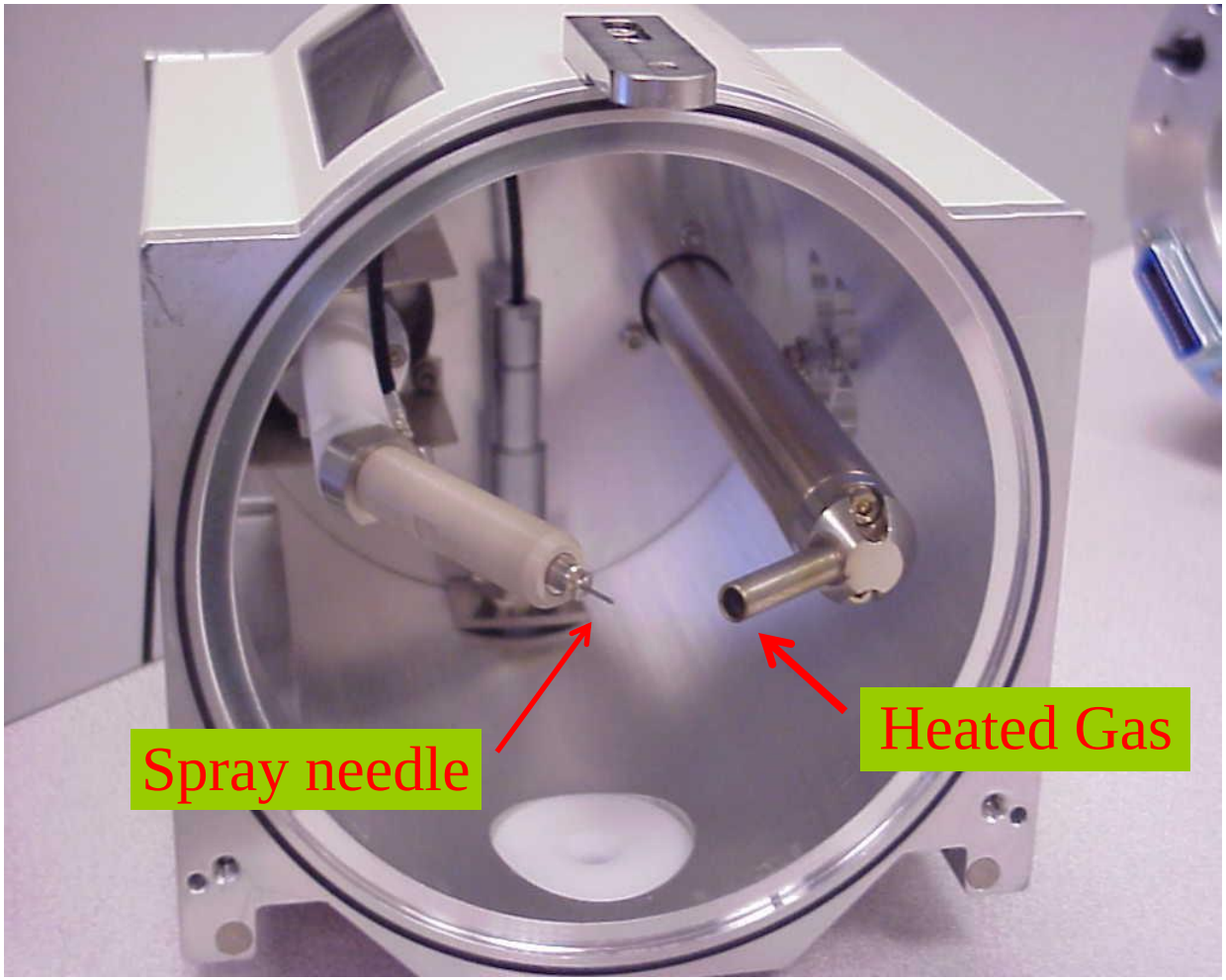
Micromass / Waters



[www.Waters.com]

Turboionspray Source – Applied Biosystems

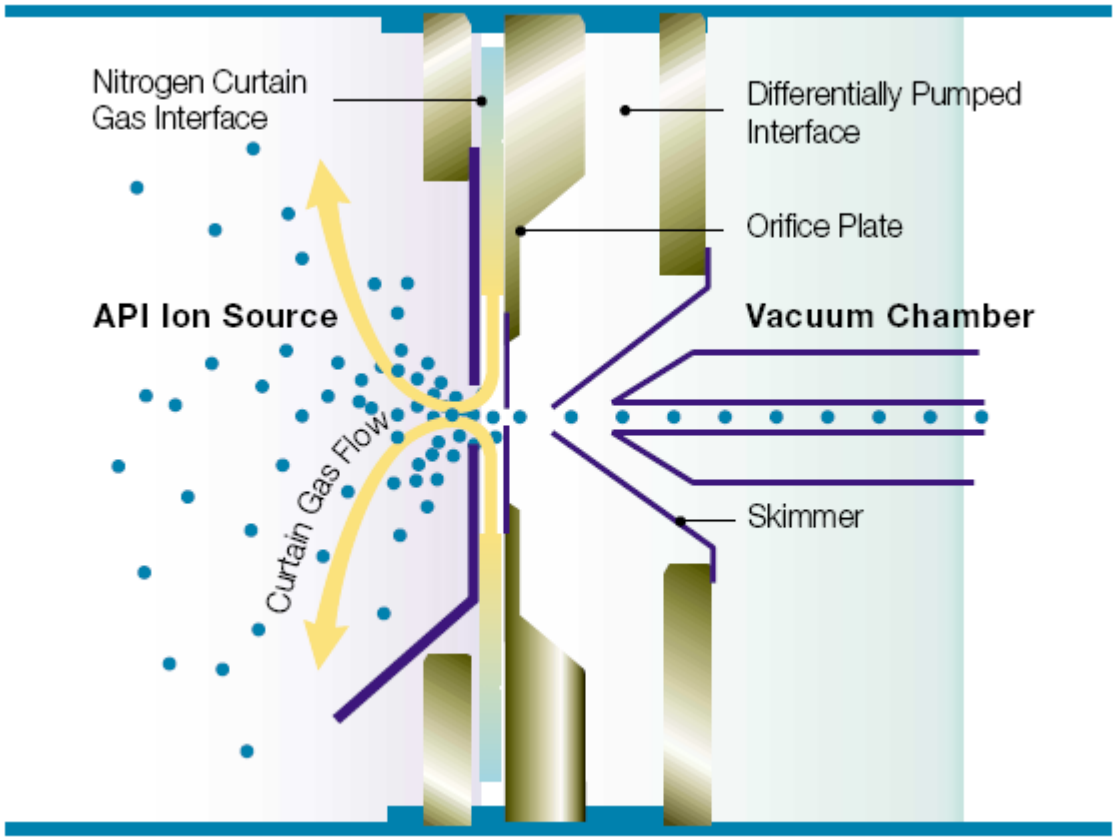
Flow Rates: 2 to 1000 µL/min



Curtain Gas

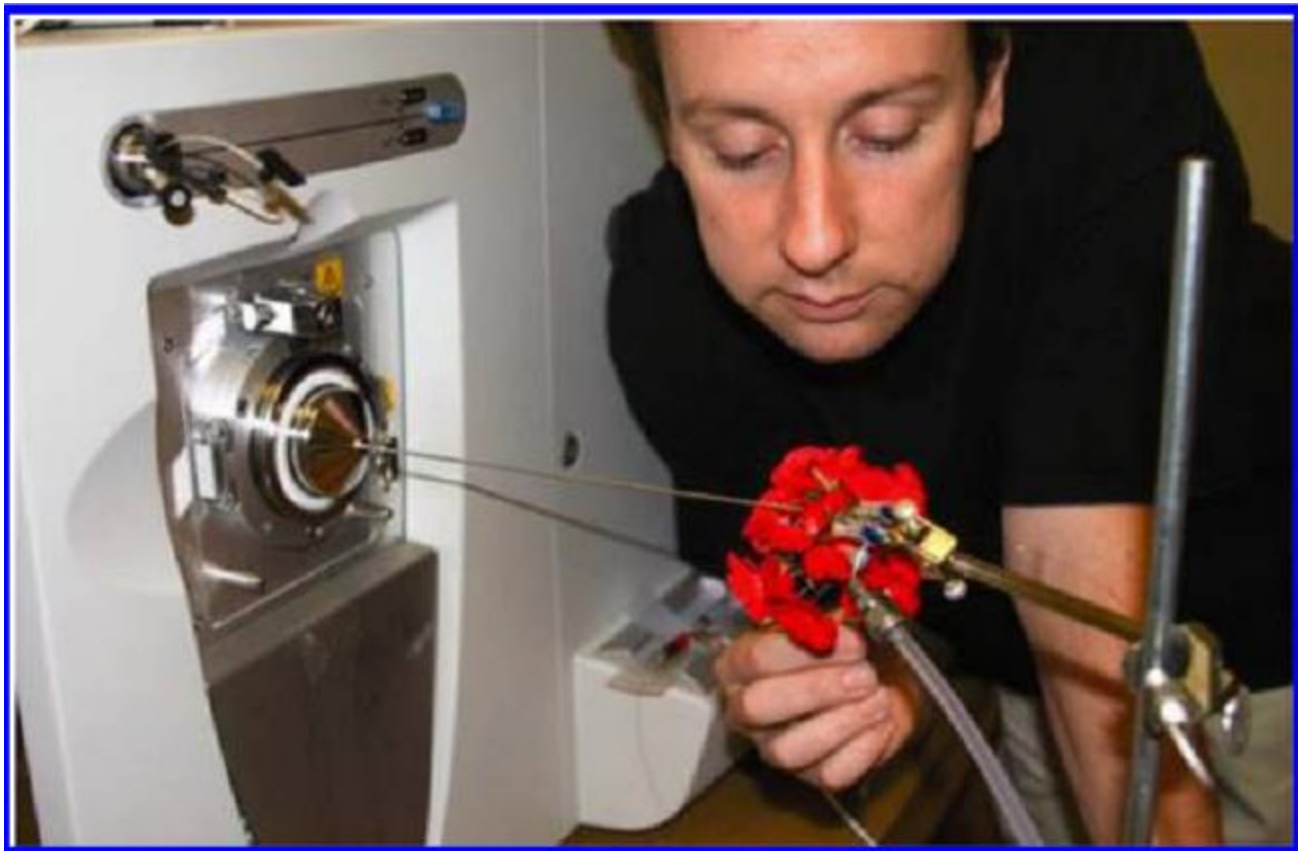
Applied Biosystems / MDS SCIEX

Spray needle

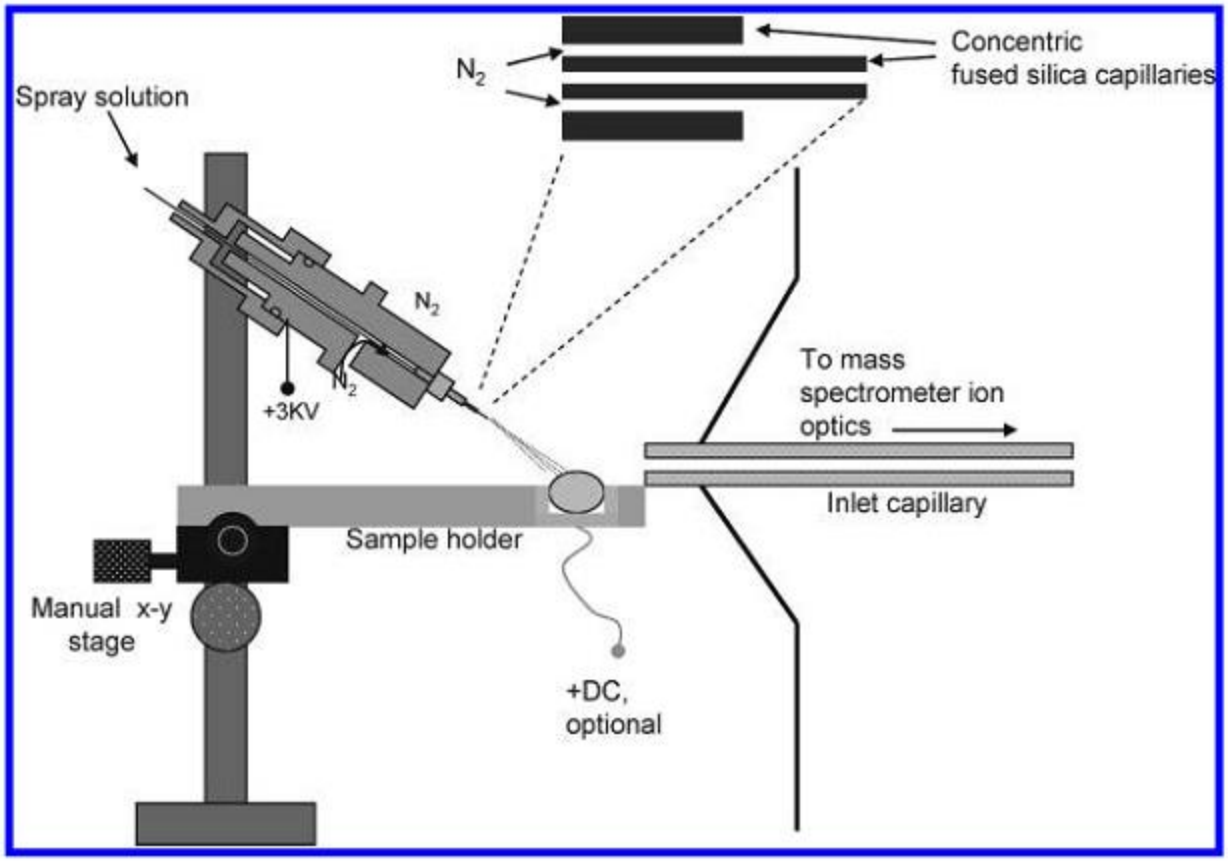


[Applied Biosystems, 2001]

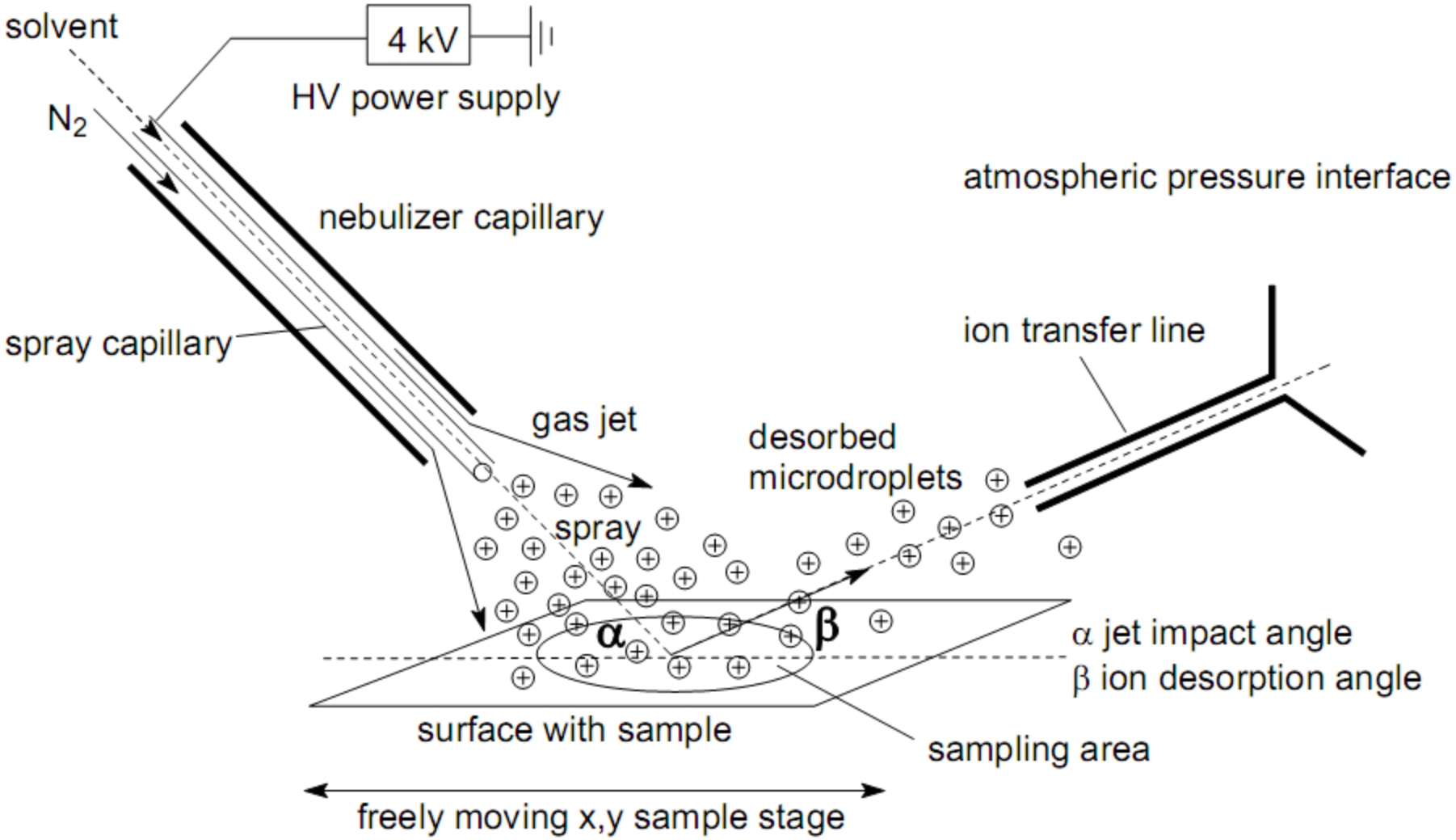
Direct Electrospray Ionization (DESI)



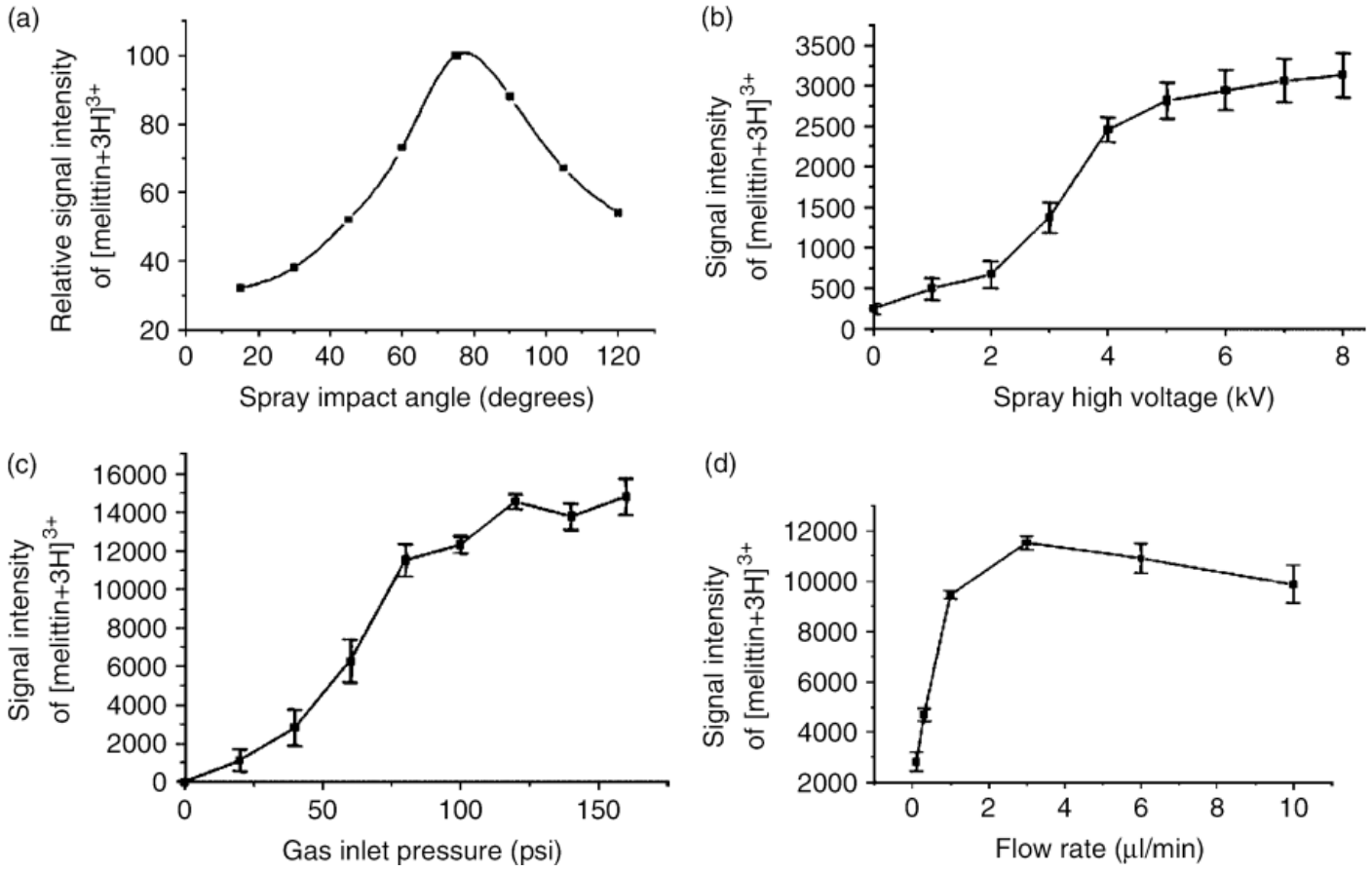
DESI analysis of a geranium flower using a methanol/water spray mixture. This experiment allowed interrogating the surface of this fragile sample in a spatially resolved fashion. In the background we see a linear ion trap mass spectrometer whose inlet was modified to allow remote sampling.



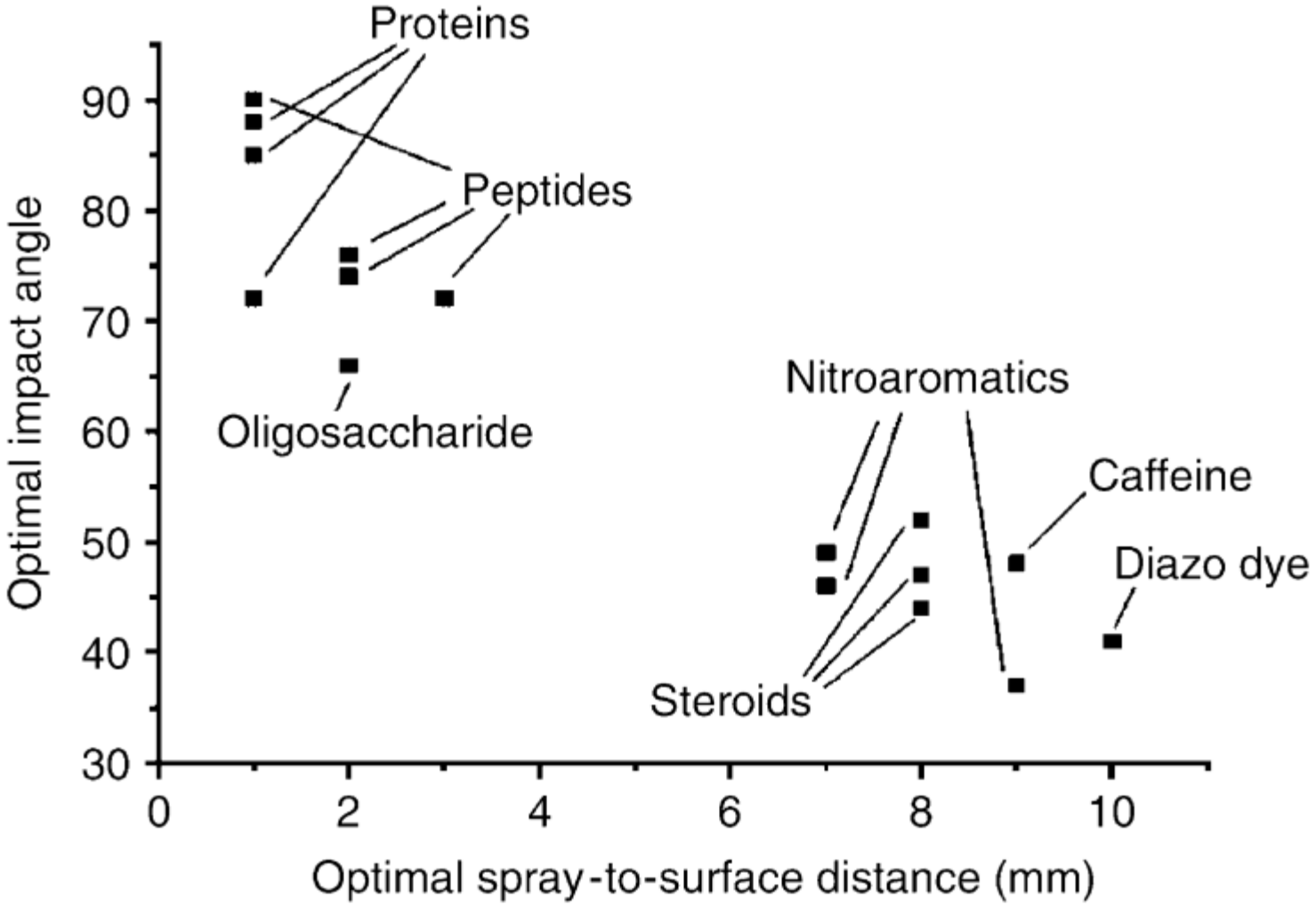
Home-built DESI ion source.



Schematic of a DESI interface. A jet of gas and charged microdroplets is created by means of a standard pneumatic ESI sprayer and directed onto a sample surface at angle α. As a result, charged microdroplets containing ions of the surface material are created and transported away due to the action of the reflected gas stream and electric repulsion at angle β. A portion of the "secondary ESI spray" may be taken up by the atmospheric pressure interface of the mass spectrometer. Although at the expense of optimum sensitivity, an extended ion transfer line is normally employed to bridge the gap from surface to interface sampling orifice

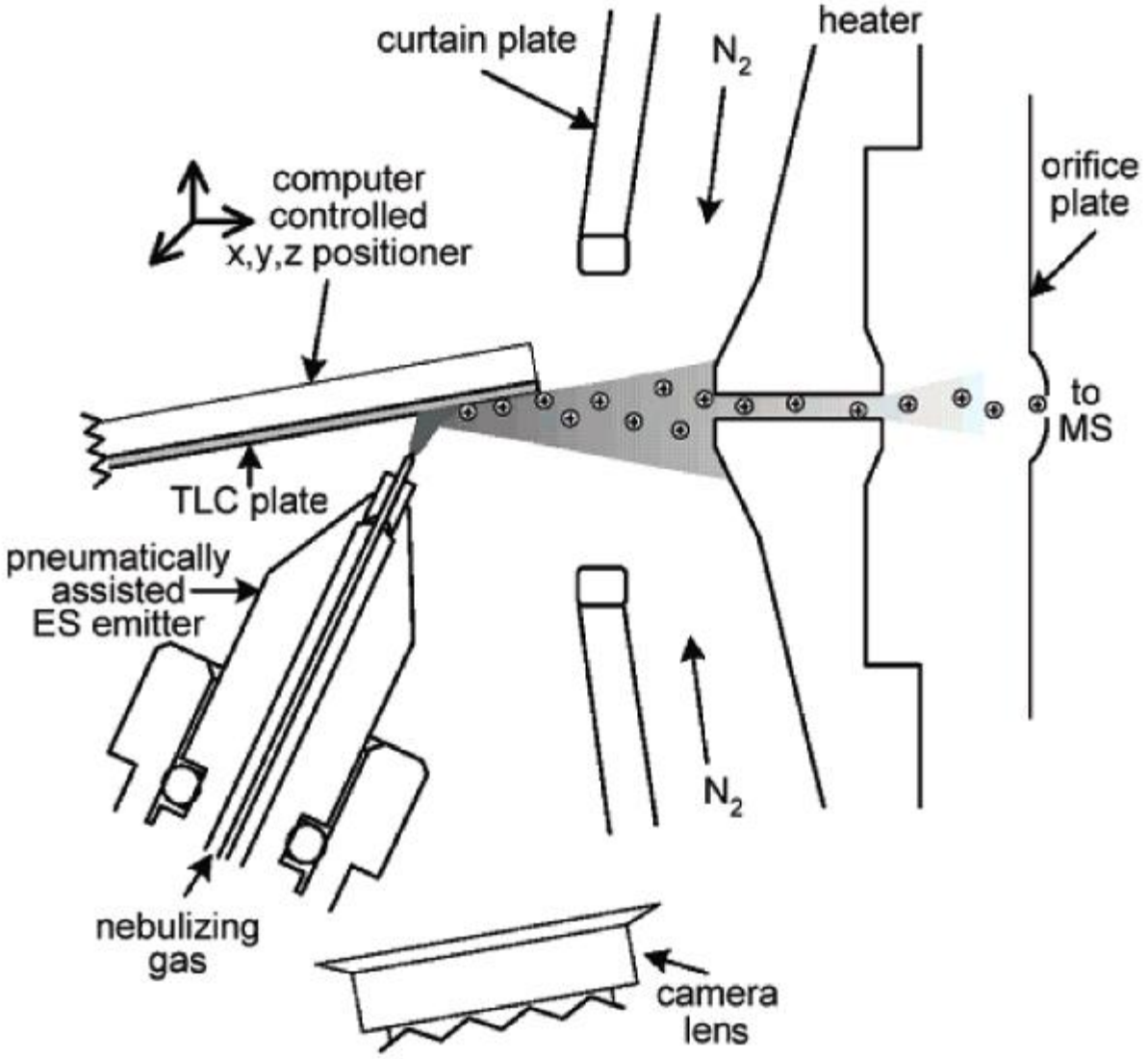


Influence of experimental parameters on the intensity of the $[M+3H]^{3+}$ ion of melittin: (a) spray impact angle, (b) spray high voltage, (c) nebulizing gas pressure (14.5 psi ≈ 1 bar), (d) solvent flow rate.



Compound class-specific optima of spray impact angle and spray needle distance to surface. The upper left group of analytes would preferably be analyzed by ESI
whereas the lower right group would rather demand for APCI

A commercial DESI ion source. This one here is the Prosolia Omni Spray 2D source coupled to a Thermo Fisher LTQ mass spectrometer. The surface shown is a 96-spot Omni Slide HC having the standard microtiter plate dimensions. Note the extended desolvation capillary and the adjustable sprayer.



Fully computer-controlled TLC-DESI unit attached to an ESI interface using the curtain gas design.