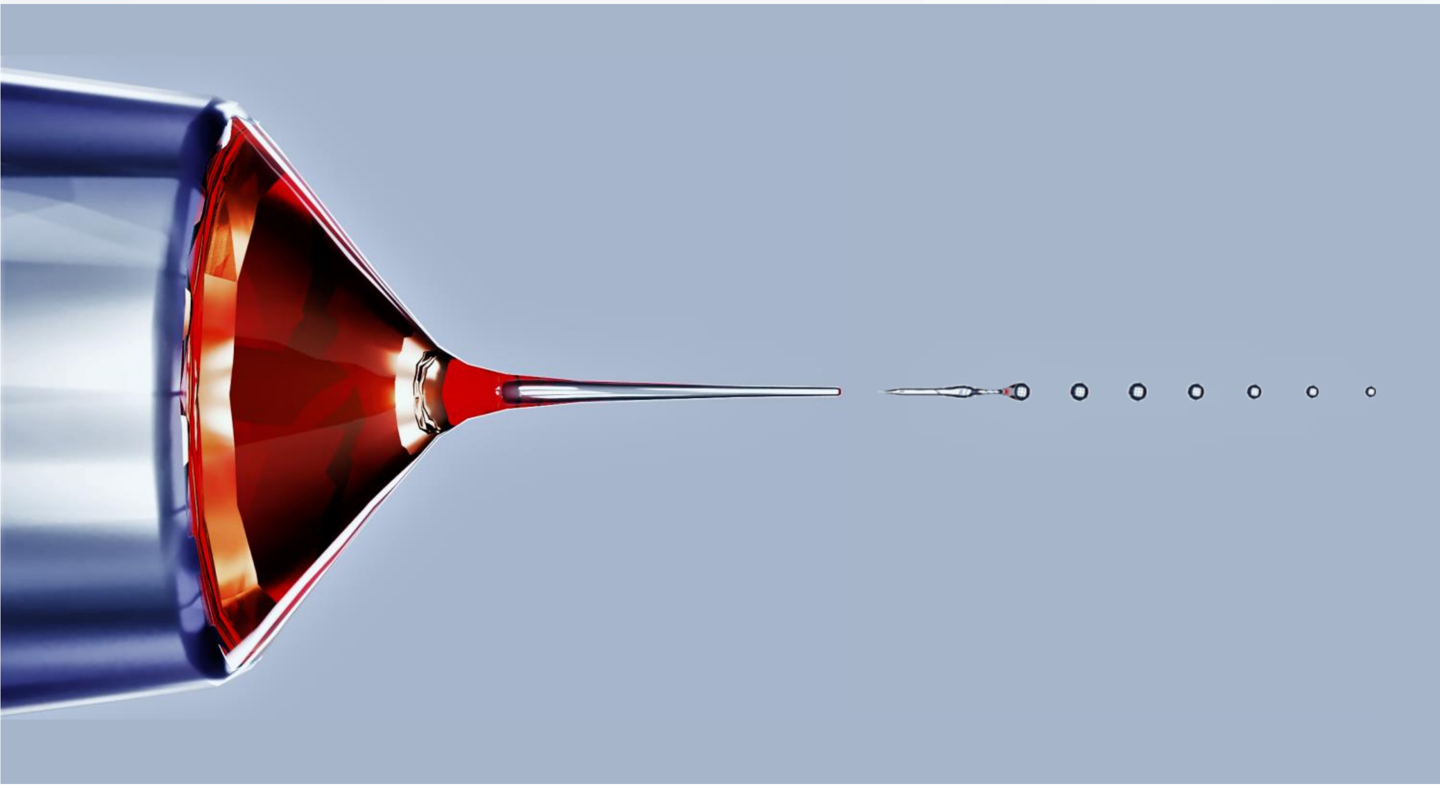
nano-Electrospray in Proteomics, First Principles.

A didactic introduction to the mechanisms of ionization, spray formation, and stability.*



This technical note summarizes the principles of nano-electrospray. The first draft put together the mechanisms that make nano-electrospray ionization so efficient and so delicate. It was for our own internal use. We used it to define design criteria to create the best possible nano-electrospray emitters.

We decided to make a technical note to help you get a better understanding of nano-electrospray, the issues that you may find when producing stable sprays and, and to show how the Sharp Singularity^{TM} emitters improve performance and robustness.

*For simplicity, this document does not include references, but literature on electrospray mechanics is vast. If you have a more specific question, please do not hesitate to contact us and we will be happy to assist you.

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The Sharp Singularity TM

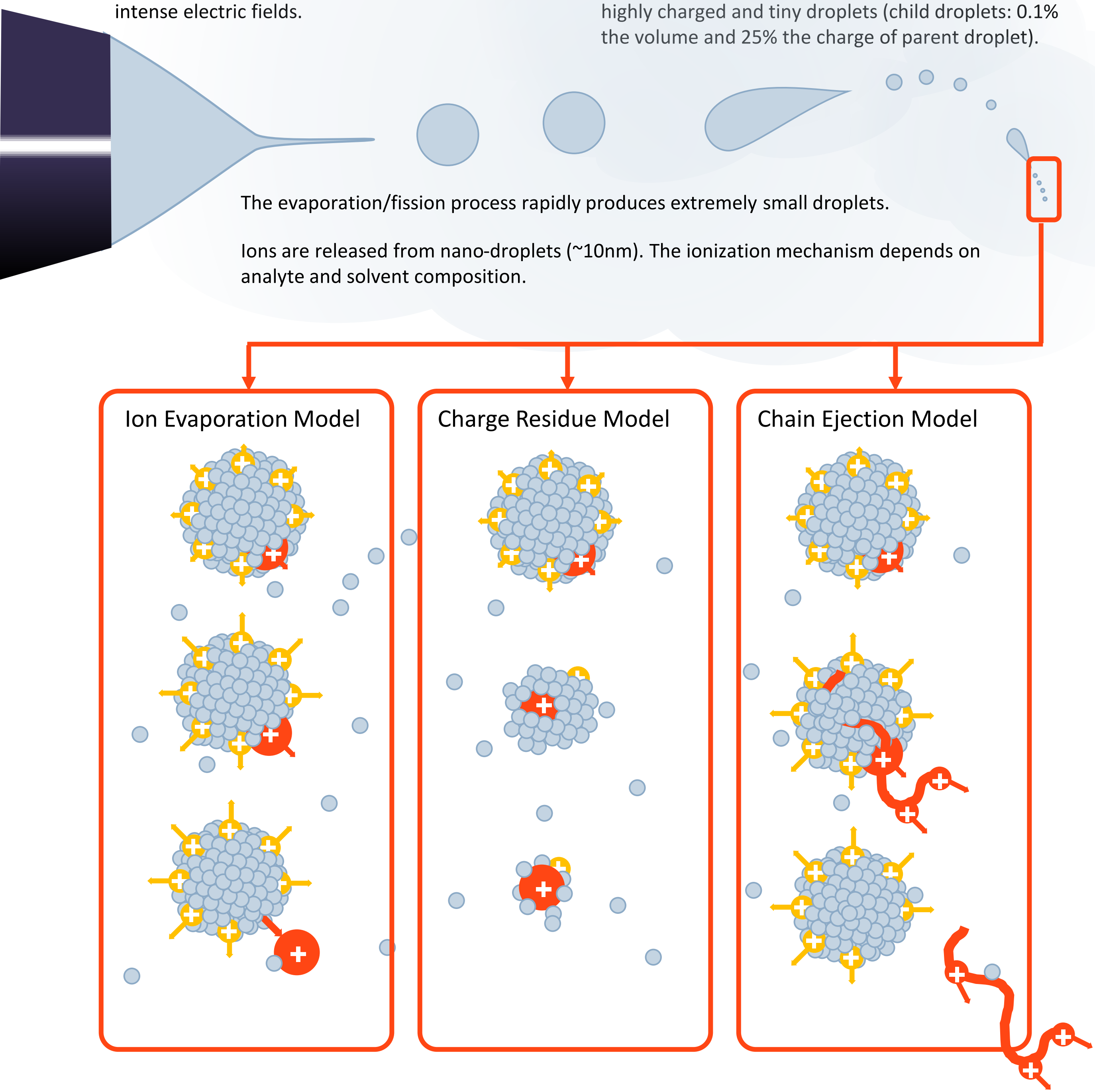
nanoESI, **First Principles**

How it works:

Meniscus: Electrostatic stress equals surface tension. This results in a singularity at the tip, producing extremely intense electric fields.

Jet: resolves the meniscus singularity and breaks up into a stream of droplets.

Droplets: 1st gen droplets are very small, but not enough to shed ions. As solvent evaporates, the droplet charge is confined into a shrinking volume until surface tension can no longer hold the electrostatic stress and the droplet explodes. Coulombic fission events produce highly charged and tiny droplets (child droplets: 0.1% the volume and 25% the charge of parent droplet).



The evaporation/fission process rapidly produces extremely small droplets.

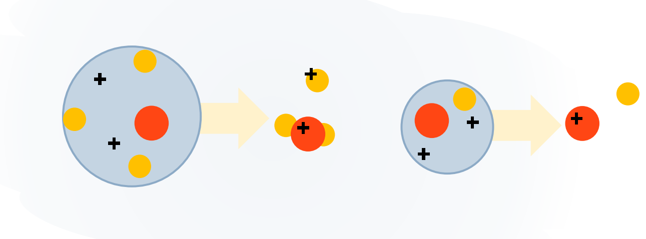
Ions are released from nano-droplets (~10nm). The ionization mechanism depends on analyte and solvent composition.

Reasons to aim for small droplets:

The sequence of evaporative droplet shrinkage and coulombic fissions leads to nano-droplets capable of shedding ions even if the spray produces large droplets.

Considering that nano-electrospray comes with some added difficulty, why bother to produce nano-electrospray tiny droplets? Here are some reasons:

- Improve **ionization efficiency**: As solvent evaporates, contaminants including salts accumulate in the resulting residue. The number of contaminants accompanying the molecule of interest is greatly reduced by starting with a smaller droplet,



- Improve **ion suppression susceptibility:** The coulombic fissions transfers hydrophobic substances to the next generation more favorably because they stay in the surface of the droplet, which is more available during the fission event. This can lead to ion suppression of the more hydrophilic molecules. The number of fission events required to reach the ion shedding regime is reduced by starting with smaller droplets.
- Improve **sample utilization**. Coulombic fission events leave low-charge droplets as a byproduct. These droplets do not produce ions and waste a lot of sample. They repel the ions of interests, saturate the ion optics and contaminate the ion path. This ‘zombie’ load is reduced by starting with smaller droplets.

nano-ESI dynamics and droplet size:

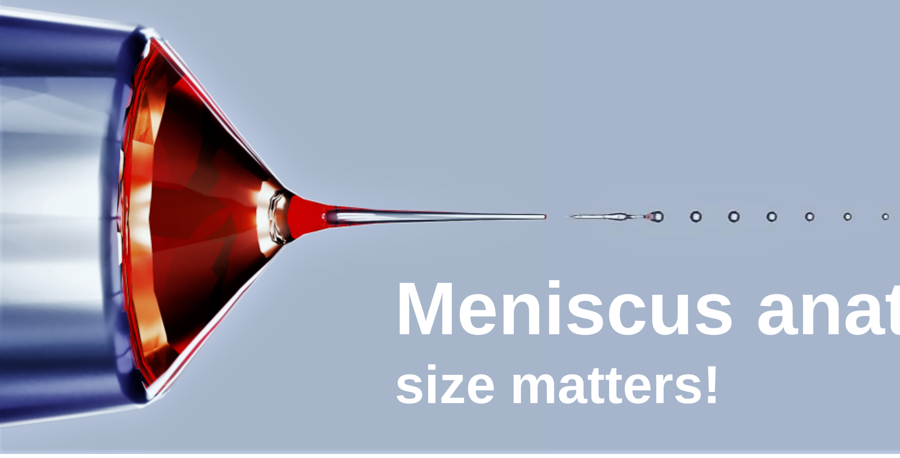
In normal nLC-MS proteomics workflows, the droplet size is about 200-500 nm *. This is well below the ID of the emitter, and it only requires about one fission step to reach the ion shedding scale (~10nm).

When the meniscus forms a stable jet, the droplet size depends on the liquid properties and the flow rate:
- High conductivity, low electrical permittivity and low surface tension lead to smaller droplets.
- Smaller flow rates also reduce the droplet size, but there is a minimum flow rate that can sustain a stable electrospray.

The key is at the Meniscus!

The quality of the signals depends on the size of the 1st generation nano-electrospray droplets, which is defined by the nano jet, which emerges from the electrospray meniscus, whose shape is determined by the emitter. The first step in this chain of cause and effect is at the electrospray meniscus. The key for a stable high performing nano-electrospray is a stable Taylor cone.
In short, to produce a stable signal, you need to produce a stable meniscus. Our goal is to facilitate the task with better emitters, but let’s see more about the meniscus in the next section.

*Scaling laws predict the size based on liquid properties and spray flow rate (for more details, check some of the literature on 1st generation electrospray droplet)



Meniscus anatomy,

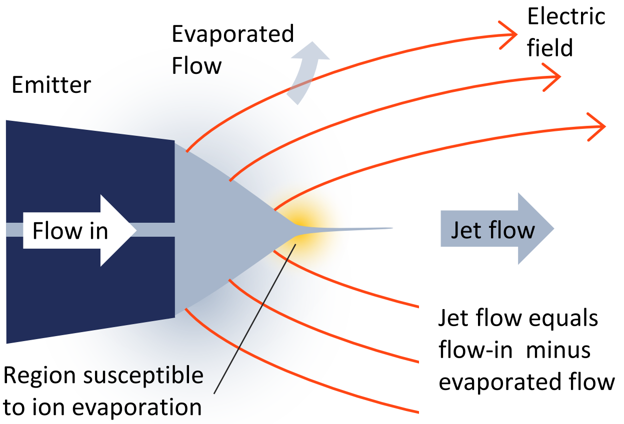
size matters!

Reasons to reduce the meniscus size:

- **Solvent evaporation** is desirable at the droplets but too much evaporation at the meniscus increases the concentration of contaminants reaching the jet. This enhances ion suppression effects and changes the properties of the liquid, affecting the droplet size, and the optimum the flow and voltage conditions. Smaller meniscus means less solvent evaporation.

- **Ion evaporation** is desirable at the droplets but, at the vicinity of the nano-jet, this further ionize the gas because of high energy collisions induced by the strong electric fields. These gas ions reduce the droplets charge and hence their ionization efficiency. Ion evaporation is enhanced by solvent evaporation. Smaller meniscus means less ion evaporation.

- **Corona discharges** form in the gas surrounding the meniscus when the voltage is too high. Ions formed at the discharge are attracted to the droplets and reduce their net charge. The voltage required to form an electrospray depends on the meniscus size. Small meniscus means low voltages an no corona discharges



Ion transport: Smaller meniscus produce weaker electric fields pushing the ions forward. For this reason, nano-electrospray must be located very close to the MS inlet. The smaller the meniscus, the smaller the emitter to MS inlet distance.

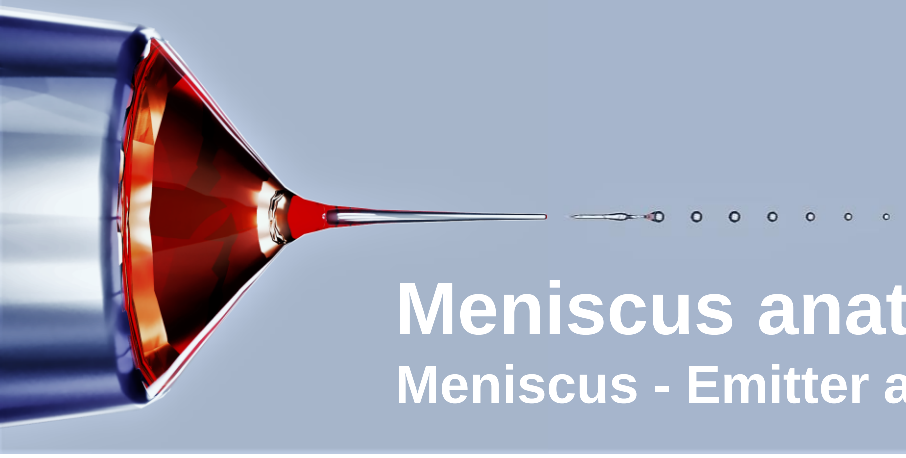
Stability:

Provided that the meniscus size is well defined and sufficiently small, the stability of the spray is mainly affected by:

- **The spray voltage:** There is a range of voltages that produce stable sprays, which depends on the meniscus size. In nano-ESI proteomics workflows, this is typically 2.5 to 3.5 KV.
- **Flow rate.** The theoretical minimum jet flow for proteomics workflows is below 1nL/min, but the evaporated flow is 10-20 nL/min for online 10-20µm ID emitters. In practice, the flow rate is limited by solvent evaporation.

Other common sources of instability include:

- **Lateral wetting instabilities:** The meniscus points sideways.
- **Longitudinal wetting instabilities:** unstable meniscus size changes with moving anchorage line.
- **Clogging:** residues accumulate at the tip.
- **Bubbles:** as the liquid flows through the column and the emitter, dissolved gases tend to precipitate. As bubbles expand, they distort the flow rate reaching the meniscus, its geometry, and break the electric conductivity.



Meniscus anatomy,

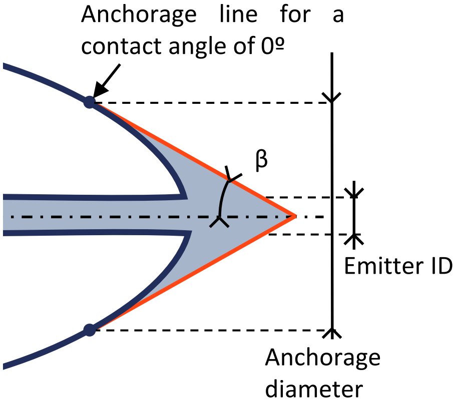
Meniscus - Emitter anchorage

How the base of the meniscus is formed:

The meniscus is anchored at the point where the meniscus cone meets the emitter at the contact angle.

In practice, the contact angle is very low because the emitter surface rapidly becomes wettable due to the harsh environment. This means that the anchorage line forms at the point where the meniscus is tangent to the emitter.

The meniscus cone angle (β) varies with the spray voltage, flow rate, and the liquid properties. The meniscus size is defined by its angle and the emitter outer geometry, not the emitter Inner Diameter (ID)!

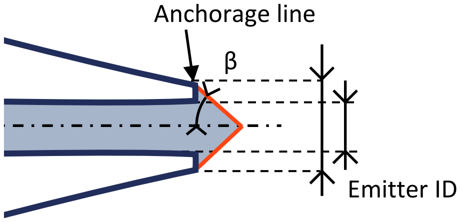


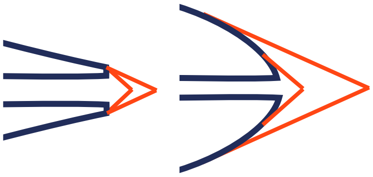
What is the optimum emitter geometry:

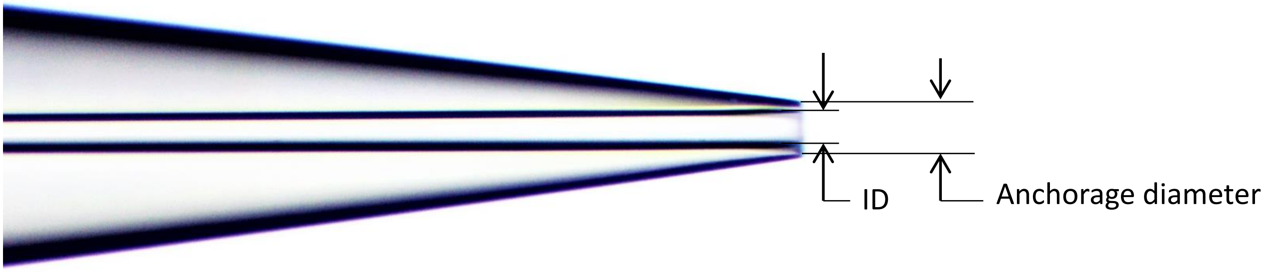
Very acute angle: stable meniscus anchorage requires the emitter cone (α) to be smaller than the meniscus cone angle (β). Sharper emitters can accommodate a wider range cone angles.

Well-defined edge: with this abrupt geometry, the meniscus sits at the same anchorage diameter regardless of the angle (β). In contrast, the meniscus size varies with β in rounded tips. This edge eliminates a source of variability, thus improving the consistency of the results.

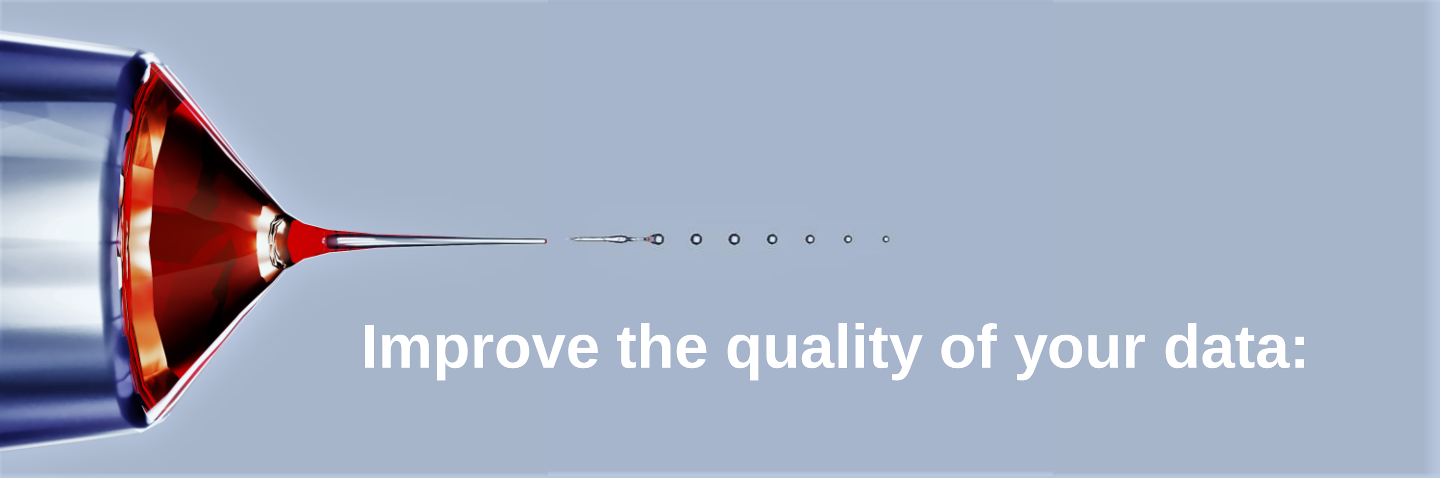
Emitter Inner Diameter: the emitter ID capillary is a just tiny pipe that feeds the meniscus as its content gets sprayed. For robustness, the ID should be as large as possible to prevent clogging.







The Sharp Singularity^{TM} Emitters provide a well-defined anchorage diameter and a robust ID



Improve the quality of your data:

- As a skilled user, you need to interpret spectra and adjust your equipment to produce high-quality data. For this you need a reliable system.
- The Sharp Singularity^{TM} emitters provide performing and predictable ionization so that you can optimize your methods.

Causes of instability and strategies implemented:

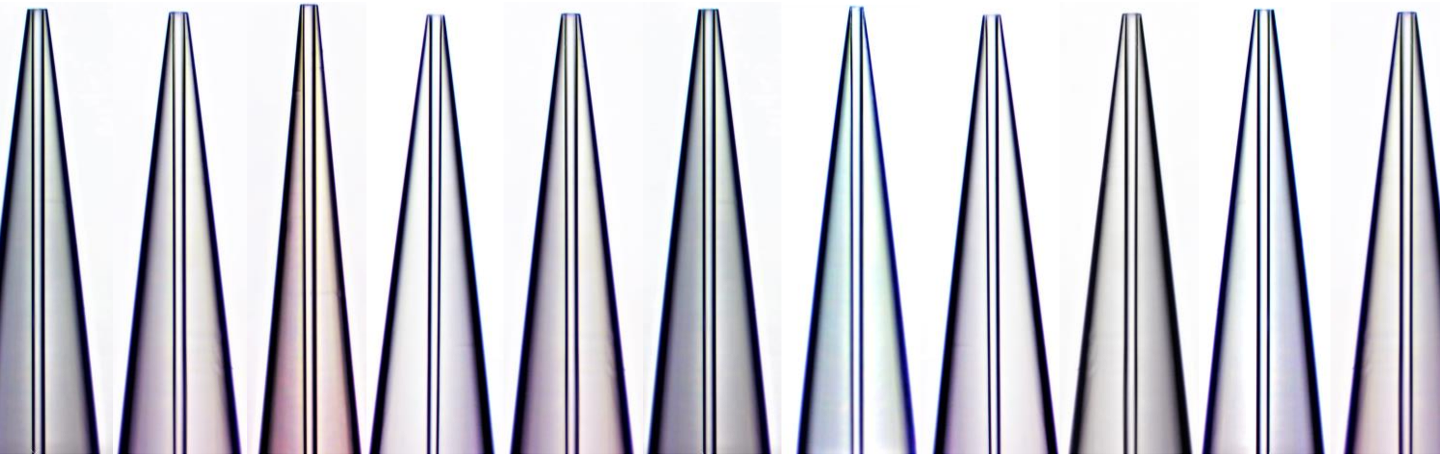
Wetting	The position and the size of the meniscus are fixed by the acute angle and the well-defined edge of the emitter.
Voltage & flow rate	The onset voltage and other transition voltages depend on the meniscus size. The meniscus is very stable because its size is extremely small and stable. This means that the right voltage will change little during runs. This also applies to the flow rate.
Clogging	This is minimized by choosing the largest possible ID for a given anchorage diameter. Different diameters are available so that you can choose the best combination of robustness and ionization efficiency.
Bubbles	Gas is normally dissolved in the liquid, either because it isn't properly degassed, or because it forms when voltage is applied to the liquid. Bubbles form when the gas nucleates at tiny imperfections. The back of the emitters is straight cut and polished at the specified length to ensure a smooth flow-path that prevents bubble nucleation.

nanoESI **Emitters,** the Sharp Singularity™

(Technical note)

Robust and repeatable nano-electrospray ionization for high quality data:

- very acute angle,
- well-defined edges,
- constant ID,
- geometric reproducibility
- full traceability and quality control



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