Optimization of Spray Capillary

Non-electrospray ionization

SEAN CLEARY
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Step 1: Sample Conditions

The first consideration to achieve the desired spray has nothing to do with the capillary itself, but rather the conditions of the sample that you are trying to analyze. An extensive guide on sample preparation is beyond the scope of what is being discussed here, but in general, the following should be considered with sample conditions to achieve optimal spray:

- The most commonly used buffer in native mass spectrometry is 200 mM ammonium acetate with a pH of roughly 7.0. Ammonium acetate is often the salt of choice for native mass spectrometrists due to the volatile nature of the salt, in that it greatly reduces sodium adductions, which can aid in spectral clarity and reducing ion suppression. These buffering conditions are not always required, and the salt concentration, pH, and even type of salt (for example, charge reducing agents) can be adjusted on an experiment-by-experiment basis. However, 200 mM ammonium acetate at pH 7 is generally a good place to start.

  Concentration of the analyte of interest is also important when considering spray optimization. Too low of a concentration can make it difficult to get a strong enough signal to see the analyte, and too high of concentration can induce non-specific oligomerization of the analyte. Optimal concentration is again analyte specific, but a good place to start is typically 1 uM.

Step 2: Ensure the capillary is in proper working order

The second consideration to achieve desired spray is the quality of the capillary itself. Again, an extensive guide on capillary pulling is beyond the scope of this guide on spray optimization, but those interested in more information on this topic are encouraged to see our guide on pulling capillaries:

- Air bubbles and foreign objects (such as small particles of broken glass) are the most common issue that will arise with spray optimization. These issues can dramatically affect both the abundance of signal and the stability of the spray. If a capillary has a spray that is unstable, it is recommended to check under a microscope to see if one of these is causing an issue. While air bubbles can often be removed, foreign objects often cannot, and thus a new capillary may need to be made to achieve desired spray if this is the case. It should be noted that, on occasion, these issues may not actually cause issues to the spray. Furthermore, at a moment's notice, capillaries can cease to function properly for a variety of other reasons. It is often recommended to pull a new capillary if something strange suddenly occurs with the spray and see if this remedies the issue.
- Once pulled, avoid at all costs touching the tip of the capillary to any surface. If this occurs, assume the tip as gone bad and new capillary needs to be pulled. Remember, while it may not be visible to the naked eye, if the tip touches any surface, it is most likely broken and no longer optimized for nESI.

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Step 3: Initial Capillary Position

The next two steps involve how to optimize the capillary spray from the instrument. It should be noted that small adjustments to the procedure described below may need to be made for any given instrument, including the same model by the same manufacture, so consider the following a good place to start rather than an extensive list:

- A platinum wire is generally used to supply charge to the solution during the nESI process, so care is advised to not bend the wire when placing it in the capillary.
- The wire should be placed in capillary until the end of the wire meets the beginning of the taper of the capillary.
- Once the capillary is placed on the wire, the initial position of the tip of the capillary should be moved close to the inlet of the mass spectrometer. This will be moved back as we optimize spray, but this is a good place to start.

Step 4: Optimizing Spray

Now that tip of the capillary is in the correct position, it is now time to try to optimize the spray. Spray is initiated by applying a potential difference between the capillary and the front of the instrument. The charged solution is then pulled from the capillary in the form of tiny charged water droplets which evaporate on the way to the instrument. Optimizing the parameters that control this can lead to more desired results:

- While not a hard fast rule, in general, a good operational range for the potential difference for our UHMR orbitrap is 0.6-1.0 kV. Again, this may be instrument specific, so these values may be different according to your set up.
- A beneficial way to initiate spray is by starting high and then tapering off. So, in the example above, starting at 1.0 kV and then slowly ramping down to 0.6 kV by 0.1 kV can be a good place to start. You can even start higher than the operational range (1.2 kV for example) but be advised that starting too high can induce air bubbles to form in your capillary. While not always the case, a lower capillary voltage is often preferred, as this can reduce salt adductions and improve spectral clarity.
- Once the spray has been initiated, and the voltage reduced to operational range, slowly move the position of the tip backwards. Most likely, you will initially see the amount signal increase as the capillary moves further back. However, as you continue to move backwards, there will come a point where the intensity of the signal starts to decrease again. Right before this point is most likely the optimal position for your capillary.