

Fundamental Exploration of Electrospray Ionization Methods

Introduction

Electrospray ionization (ESI) is an ambient ionization technique widely used for the analysis of polar molecules from aqueous solutions [1]. In the pharmaceutical industry, LC-ESI-MS is used extensively in pharmacokinetic studies for drugs in preclinical stages. Several different mechanisms have been proposed for ESI, but the mechanism is still highly debated [1]. Such fundamental studies have resulted in the emergence of several ESI variant techniques, such as nanoelectrospray ionization (nESI) for improving ionization efficiency to achieve low limits of detection, and paper-spray ionization (PS-MS) for analyzing biological samples [2,3]. Both spray-based techniques are performed in air and require only minimal sample preparation, characteristics which have facilitated their use as highly robust and rapid analysis methods.

Traditional ESI is performed by applying a high DC voltage to a metal electrode which is in contact with an analyte-containing solution. The high voltage causes charges to form, separate, and repel each other in solution. Charge repulsion results in the formation of a Taylor cone at the sharp end of an emitter. Charged droplets are emitted from the Taylor cone and undergo rapid desolvation due to the addition of heat and a nebulizing gas. During desolvation, the charged droplets undergo many successive fission events, resulting in the generation of smaller and smaller highly charged droplets until only free, gaseous analyte ions remain. Many parameters, such as solvent composition, greatly affect how ions are generated. Spray solvents are normally composed of both aqueous and polar-organic solvents, but nonpolar organic solvents like chloroform are used depending on the need. Water alone is not often used as a spray solvent because of its high surface tension, but it can be used if there is a sufficient concentration of electrolytes. Other parameters which affect ion generation include the voltage polarity used, the location of the applied voltage, the type of emitter used, and the shape of the emitter, features which also can drastically affect the type of data acquired during an experiment.

Electrospray ionization suffers from known disadvantages. One example in particular is that low volumes of solution, such as the cytoplasm contained in a single cell, are difficult to electrospray because of the requirement to make contact between a low volume of solution and an electrode. To overcome this problem, a new technique called relay electrospray ionization (rESI) was developed by the Cooks research group [4]. In rESI, charge is deposited onto an emitter by a discharge from an APCI needle, charged droplets from a nanospray emitter, or charged particles from a piezoelectric gun (Figure 1b) [4]. This technique avoids direct solution contact with an electrode, allowing low volumes of solution contained in an emitter to be effectively electrosprayed. Applying a voltage to the outside of an emitter also eliminates any possible contamination resulting from contacting a solution with an electrode, lending to the possibility of quantitative measurements.

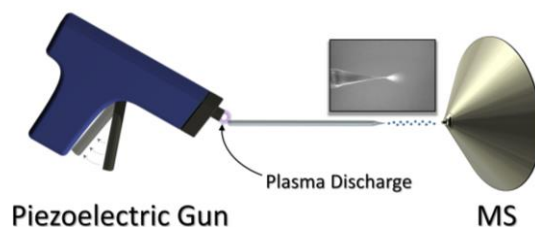


Figure 1. Relay electrospray ionization occurs by depositing charged ions onto the outside of an emitter.

Paper spray is another variant of electrospray which allows for the direct analysis of complex samples like blood or urine. Analysis is performed by depositing blood or another biofluid on paper or some other porous substrate. A small volume of solvent is applied to the paper, which is cut to a sharp point. The solvent wicks through the substrate and sample by capillary action. The tip of the paper is positioned a few millimeters away from the atmospheric pressure inlet of a mass spectrometer and a high voltage (3-5 kV) is then applied to the moist paper, which induces an electrospray from the sharp tip of the paper. The entire analysis requires less than one minute and requires only the paper substrate on which the blood is already stored, a small amount of solvent, and an electrical connection to a low-current high voltage power supply. Paper spray analysis can be performed manually by cutting paper into a triangle using scissors and applying solvent with a pipette. A small automated attachment that fits onto the mass spectrometer in place of the commercial ESI/APCI source as well as single-use cartridges with pre-cut paper are now available commercially (Prosolia Inc., Indianapolis, IN).

A more recently developed technique in the Cooks research group demonstrates that an electrospray plume can be generated directly from a Whatman filtration membrane. This technique is another example of how electrospray ionization can be used to simplify analytical methodologies. In this technique, solvent is added to a filtration membrane which has been placed below a sharp needle or wire (Figure 2a-b). The solvent then passes through the membrane and pools on the underside of the membrane (Figure 2c). A high voltage applied to the needle or wire, creating a high electric field, causes an electrospray plume to form from this pool of solvent. The entire setup is then positioned above a mass spectrometer inlet capillary with a 90° bend so that the opening of the capillary is directly below the electrospray plume (Figure 2d). The utility of this technique is that the filtration membrane can be used, for example, to isolate certain components from whole blood. These components can then be analyzed by mass spectrometry directly from the filtration membrane. There is no need for further sample processing. This saves time, allows for automation, and reduces the risk of sample processing errors.

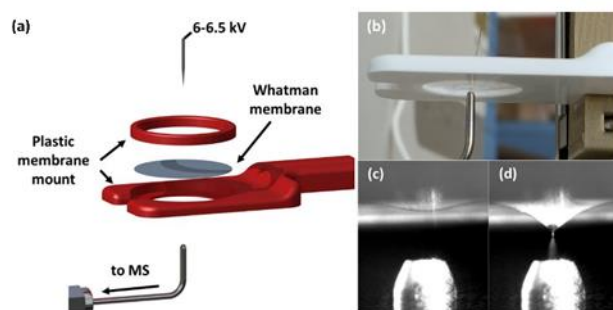


Figure 2. (a) Illustration of membrane holder. (b) Picture of ESI plume generated from a polycarbonate membrane. ESI plume (c) before and (d) after voltage is turned on.

Aims

Electrospray plumes are difficult to see with the naked eye. However, spray plumes can be readily observed under illumination from a strong light source, such as a light microscope or a laser pointer and camera combination. Using such equipment, participants will observe the Taylor cone and electrospray solvent plume formed during paper spray ionization in real time. Various parameters such as the substrate properties and shape, solvent, and applied voltage will be varied and their effect on the experiment will be observed. A relay electrospray ionization experiment will also be performed so that participants can visualize the electrospray burst generated. Following relay electrospray, a membrane spray experiment will be performed so that participants can observe how an electrospray plume can be generated from a filter membrane.

Experimental procedure

1. Introduction to paper spray.
 - Use a digital microscope to observe Taylor cone formation during paper spray MS analysis.
 - Observe other ways that charge is carried during this process (e.g. breakdown and corona discharge). Discuss why these (normally unwanted) phenomena occur.
 - Change substrate porosity, polarity, etc. and observe the effect on spray formation.
 - Change solvent properties (such as the organic to aqueous ratio) and observe the effect on spray formation.
2. Introduction to relay electrospray ionization.
 - Basic description of the piezoelectric effects involved in generating ions.
 - Describe the setup required to observe spray plumes.
3. Use the piezoelectric gun to demonstrate spray generation from different emitters, including a nanoelectrospray emitter, paper used for PS-MS, a sharpened wooden tip, and a theta tip.
4. Demonstrate low-volume sampling methodology with relay electrospray.
 - Dip the tip of a nanospray emitter into a solution and aspirate <100 nL.
 - Observe the aspirated volume under camera.
 - Spray solution using a piezoelectric gun.
5. Introduction to generating an electrospray directly from a Whatman membrane.
 - Describe the setup required to generate the electrospray plume.
 - Brief description of the electrical effects involved in generating a spray from a Whatman membrane.
 - Deposit solvent on top of the membrane.
 - Apply high voltage to the APCI needle and observe how the solvent transverses the membrane and generates a large electrospray plume below the membrane.
 - Observe the electrospray plume with the naked eye.

References

1. Konermann L, Ahadi E, Rodriguez AD, Vahidi S. Unraveling the Mechanism of Electrospray Ionization. *Analytical Chemistry* **85**, 2-9 (2013).
2. Espy RD, Muliadi AR, Ouyang Z, Cooks RG. Spray mechanism in paper spray ionization. *International Journal of Mass Spectrometry* **325** 167-171 (2012).

3. Wilm M, Mann M. Analytical Properties of the Nanoelectrospray Ion Source. *Analytical Chemistry* **68**, 1-8 (1996).
4. Li A, Hollerbach A, Luo Q, Cooks RG. On-Demand Ambient Ionization of Picoliter Samples Using Charge Pulses. *Angew. Chem. Int. Ed.* **54**, 6893–6895 (2015).