Mass Spectrometry

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On-Demand Ambient Ionization of Picoliter Samples Using Charge Pulses**

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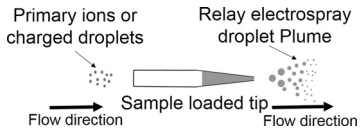
Abstract: *Relay electrospray ionization (rESI) from a capillary containing a sample solution (or from an array of such capillaries) is triggered by charge deposition onto the capillary. Suitable sources of primary ions, besides electrosprays, are plasma ion and piezoelectric discharge plasma sources. With no requirement for physical contact, high-throughput sample screening is enabled by rapidly addressing individual secondary (sample) capillaries. Sub-pL sample volumes can be loaded and sprayed. Polar analytes, including neurotransmitters, phosphopeptides, oligonucleotides, illicit drugs, and pharmaceutical compounds are successfully ionized by rESI with concentration sensitivities (0.1 ppb for acetylcholine) which are similar to nanoESI but absolute sensitivities are orders of magnitude better. Nonpolar analytes (steroids, alkynes) are ionized by rESI using an open-tube secondary capillary and injecting electrolytically generated metal cations from the primary electrospray.*

Electrospray is an electrohydrodynamic phenomenon discovered in the twentieth century.[1] Today it has important applications in mass spectrometry,[2] propulsion,[3] and materials fabrication.[4] In ESI, electrical contact with a voltage supply is necessary to generate a continuous spray of charged droplets from a solution. The electrical contact adds dead volume and adsorption surfaces. It also complicates the apparatus configuration, especially for arrays of ESI emitters. Desorption electrospray ionization,[5] extractive electrospray,[6] acoustic wave nebulization,[7] and laser ablation electrospray[8] avoid electrode contact with samples but require a sheath gas, laser, or acoustic wave to break up the sample solutions. Dielectric induction is an alternative way to avoid solution contact.[9]

In this work, we present a relay electrospray ionization (rESI) technique, performed by depositing charge (ions) onto or into an ESI emitter loaded with sample solution. The impinging ions, generated by a primary electrospray or plasma ionization source, pass charge to the electrically floated sample loading capillary, causing an immediate electrospray to occur from the tip of the secondary capillary (Scheme 1).



Scheme 1. Relay electrospray ionization: charge is supplied into (open configuration) or onto the outside (closed configuration) of the sample capillary as ions or charged droplets from a primary source (needle discharge plasma, piezoelectric discharge plasma, or electrospray ion source). The relay generates ions from the analyte solution for mass spectrometric analysis.

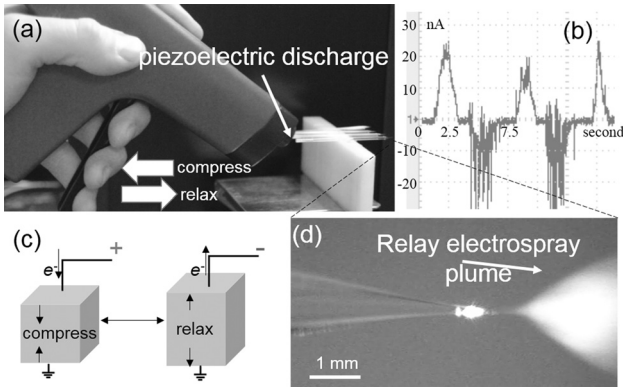


Figure 1. Hand-held piezoelectric direct discharge plasma generator as primary ion source in relay ESI. a) Photograph of set-up; b) rESI current through three positive and two negative cycles; c) electrical operating schematic and d) photograph of relay spray plume.

Using a hand-held piezoelectric direct discharge plasma generator as a primary ion source, rESI ion signal was generated (Figure 1). In one triggering–releasing cycle, the piezoelectric direct discharge plasma generates cations and anions/electrons consecutively (Figure S1). The triggered relay ESI has corresponding positive and negative ion currents of 10–20 nA in typical experiments. Various analytes of interest, including acetylcholine, cholesterol, phosphopeptides, and DNA oligomers were successfully ionized in both the open and closed configurations (Figure 2). Even in the open mode, the impinging plasma ions do not degrade the analytes in most samples tested, except for phosphopeptides for which a small amount of dephosphorylation was observed

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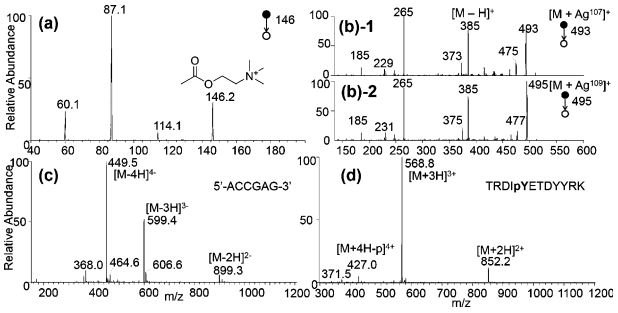

Figure 2. Relay electrospray MS analysis of a) ca. 1 pL of 0.5 ppb acetylcholine, MS/MS of *m/z* 146. This single scan data is taken using a 50 ms injection time window. The whole experiment (dip and spray) takes less than 30 s on a total sample size of 2000 molecules, b) 100 ppb cholesterol, MS/MS of isotopic $[M+Ag]^+$ ions, c) 1 μM DNA oligomer in negative ion mode, and d) 1 μM phosphopeptide. In (b), silver ions from a primary electrolytic ionization source[17] were used to cationize the analyte.

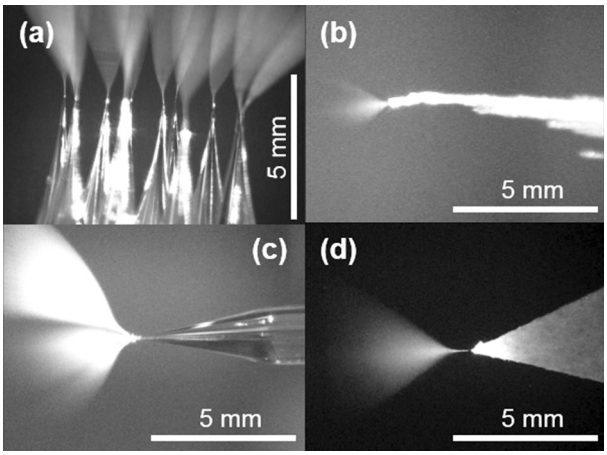

Figure 3. Relay spray from several different emitters: a) bundle array of 11 nanoESI emitters; b) sharp end of a wooden pick; c) pulled theta-shaped tip, and d) filter paper triangle.

(Figures 2c and S2). Solutions of acetylcholine (0.1 ppb) analyzed by rESI using multiple reaction monitoring (MRM) gave signals ten times that of the blank solution. Although a needle discharge plasma source produces constant rESI currents over a longer time, for most rESI analyses, the 2–5 s piezoelectric discharge triggering is adequate, portable, and free of safety concerns.

When the proximal end of the secondary emitter is sealed either with epoxy or by melting the glass, rESI emission is still generated when triggered by a primary ion source (Figure S3). MRM analysis revealed a 40% decrease in signal intensity when compared with the results from an open emitter (Figure S4). However, rESI can only be avoided altogether when the entire secondary capillary is grounded (e.g. by sputter-coating a layer of Pd/Au; Figure S3). This suggests that charge accumulation in the sample solution or on the outside of the glass capillary will lead to relay electrospray, corresponding to the inside contact[10] and outside contact (through a coated gold layer)[11] configurations of conventional ESI. The rESI phenomenon occurs with all the types of emitters tested, including capillaries of borosilicate, quartz, or fused silica, steel needles,[12] theta-shaped capillaries,[13] and those made of porous materials like wooden tips[14] and paper.[15] The on-demand spray from a paper substrate represents an alternative way of performing paper spray ionization. Bundled (close-packed) emitter arrays also demonstrate rESI, allowing for high-throughput screening and scaled-up ion soft landing[16] experiments in the future (Figure 3).

The rESI emitters can be used as micro aspiration tools. Due to the requirement of electrical contact, conventional ESI emitters are usually loaded using LC pumps[18] or centrifugation[19] from their wider end. When this end is sealed, thermal expansion of air can be used to aspirate solution volumes in the range of pL to μL by capillary action. Ultralow sample volumes (from 50 fL up to several μL) were achieved (Figure S5). Zero dead volume rESI is demonstrated by electrospraying all of the solution from a capillary. Volumes of about 1 pL (0.5 ppb, ≈0.5 attogram, ca. 2400 molecules) of acetylcholine produced reliable ion signals observable using a linear ion trap mass spectrometer (Figure S6, Videos 1 and 2). Finally, the application of a flame to the sharp tip can seal the emitter, transforming the emitter into a micro vessel for sample storage.

In a typical relay experiment stable ion currents of 10 nA were generated by the relay (secondary) tip when using a 12 nA primary ESI emitter current to generate primary charged droplets (Figure S7). This corresponds to ca. 80% current transmission efficiency (Figure S8). Because it is isolated from the sample solutions, the primary electrospray ionization source also provides opportunities for versatile chemistries.[20] As one example, noble metal ions from electrolytic spray ionization of silver[21] and gold[16b,17] in the primary ion source were used as cationization reagents[22] for the soft ionization of olefins and alkynes in steroids, vitamin D3, and 3-octyne (Figures 2c, S9 and S10).

Selective and sequential activation of elements in arrays of emitters, as well as multiple stage serial relay electrosprays, has also been demonstrated (Figures S11 and S12). All these capabilities associated with rESI bring opportunities to develop portable, high-throughput biochemical analysis systems[23] and to perform small-volume reactions[24] and reaction intermediate studies.[25] Recent interest in ultra-microscale chemical reactions,[24b] including experiments in which arrays of drug candidates are tested against biological substrates and the products analyzed in high-throughput fashion could also be impacted. The ability to measure mass spectra from samples consisting of several thousand molecules will advance these objectives and other low-level measurements including single-cell mass spectrometry.[26]

Experimental Section

Primary electrospray voltage was 1.0–3.5 kV; needle discharge plasma was 3–4.5 kV; piezoelectric discharge gun (Zerostat) was triggered by hand. The spray plumes were illuminated by a 405 nm laser and recorded using a Watec camera (WAT-704R). For ultralow volume sampling, an optical microscope (Olympus BX-51) was used

to monitor the spray ejection of the sample solution. Mass spectra were recorded using a linear ion trap mass spectrometer LTQ (Thermo Scientific) using conditions given in the Supporting Information.

Keywords: electrohydrodynamics · electrospray ionization · mass spectrometry · microaspiration · piezoelectric

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