# Graphite-Coated Nanoelectrospray Emitter for Mass Spectrometry

2 AUTHORS, INCLUDING:

Troy D Wood

University at Buffalo, The State University of …

**90** PUBLICATIONS **2,101** CITATIONS

# Technical Notes

# Graphite-Coated Nanoelectrospray Emitter for Mass Spectrometry

**Douglas R. Smith and Troy D. Wood***

*Department of Chemistry, Natural Sciences Complex, University at Buffalo, The State University of New York, Buffalo, New York 14260-3000*

**A new, more rapid method for coating nanoelectrospray emitters with graphite is to use a vacuum deposition chamber and a graphite carbon electrode. This method allows for mass production of nanoelectrospray emitters in a short period of time. The emitters are laser-pulled borosilicate glass micropipets and have tapers of around 4 $\mu$m i.d. The conductive coating applied to the emitter is only 20−30 nm thick, allowing for optical transparency with the borosilicate emitters. The conductive coating is stable for a number of hours at the high voltages used for nanoelectrospray ionization and is durable in both positive and negative ion modes—even during electrical discharge. This stability will make it possible to couple these emitters with online separations such as capillary liquid chromatography or capillary electrophoresis.**

As nanoelectrospray ionization continues to grow and gain acceptance as a valuable research tool within the mass spectrometry field, there has been a push to produce nanoelectrospray emitters that are inexpensive and have long lifetimes. "Nanoelectrospray" was first developed by Mann and Wilm in 1994.[1,2] Their technique used a pulled-glass substrate as the electrospray ionization emitter. Nanoelectrospray is a static technique and relies on capillary action induced by the applied electric field to draw the solution to the emitter tip so that it can be electrosprayed;[3] therefore, no forced flow (from a syringe pump or LC pump) is needed, and flow rates are generally in the tens of nanoliters per minute. The droplets produced have 100−1000 times less volume than those produced with conventional electrospray, and desolvation does not require the use of a nebulizing gas to aid the drying of the droplets. These advantages lead to increased sensitivity and decreased limits of detection, making nanoelectrospray the technique of choice when sample volumes and analyte concentrations are limited.

Initial nanoelectrospray emitters used pulled-glass substrates with a metal, typically gold, as the applied conductive medium.[1,2,4]

However, these emitters suffered from the susceptibility of the conductive metal to be ablated from the glass surface through a coronal discharge.[3] Much work has been done to stabilize and protect these metal coatings in an effort to increase the emitter lifetime.[5−8] However, these techniques greatly add to the production time and cost of the emitter. Another approach has been to use polymer-based systems, either a conductive polymer[9−12] or a nonconductive polymer doped with conductive material.[13,14] These emitters have shown better resilience to electrical discharge and the longer lifetimes needed for the coupling of online separation techniques. These systems are generally cheaper than their metal counterparts, and some require less handling during manufacture. Another approach has been to insert a metal wire into the untapered end of the emitter to make electrical contact with the solution.[15−18] This eliminates the need for an external coating. This technique can become labor-intensive, and it might not be suitable for online separations such as capillary LC. It has also been shown that the metal wire used as an electrode can undergo electrolysis and produce additional species whose ions further complicate the observed mass spectrum.[16] A third alternative has been to use carbon or graphite as the conductive medium. Many different types of carbon have been used, such as colloidal graphite,[19]

* To whom correspondence should be addressed. Phone: 716-645-6800. Fax: 716-645-6963. E-mail: twood@acsu.buffalo.edu.

(1) Wilm, M. S.; Mann, M. *Int. J. Mass Spectrom. Ion Processes* **1994,** *136,* 167−180.

(2) Wilm, M.; Mann, M. *Anal. Chem.* **1996,** *68,* 1−8.

(3) Wood, T. D.; Moy, M. A.; Dolan, A. R.; Bigwarfe, P. M., Jr.; White, T. P.; Smith, D. R.; Higbee, D. J. *Appl. Spectrosc. Rev.* **2003,** *38,* 187−244.

(4) Valaskovic, G. A.; Kelleher, N. L.; Little, D. P.; Aaserud, D. J.; McLafferty, F. W. *Anal. Chem.* **1995,** *67,* 3802−3805.

(5) Kriger, M. S.; Cook, K. D.; Ramsey, R. S. *Anal. Chem.* **1995,** *67,* 385−389.

(6) Nilsson, S.; Markides, K. E. *Rapid Commun. Mass Spectrom.* **2000,** *14,* 6−11.

(7) Valaskovic, G. A.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1996,** *7,* 1270−1272.

(8) Barnidge, D. R.; Nilsson, S.; Markides, K. E. *Anal. Chem.* **1999,** *71,* 4115−4118.

(9) Bigwarfe, P. M., Jr; White, T. P.; Wood, T. D. *Rapid Commun. Mass Spectrom.* **2002,** *16,* 2266−2272.

(10) Maziarz, E. P., III; Lorenz, S. A.; White, T. P.; Wood, T. D. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 659−663.

(11) White, T. P.; Wood, T. D. *Am. Biotechnol. Lab.* **2002,** *20,* 16, 18.

(12) White, T. P.; Wood, T. D. *Anal. Chem.* **2003,** *75,* 3660−3665.

(13) Nilsson, S.; Wetterhall, M.; Bergquist, J.; Nyholm, L.; Markides, K. E. *Rapid Commun. Mass Spectrom.* **2001,** *15,* 1997−2000.

(14) Wetterhall, M.; Nilsson, S.; Markides, K. E.; Bergquist, J. *Anal. Chem.* **2002,** *74,* 239−245.

(15) Fong, K. W. Y.; Chan, T.-W. D. *J. Am. Soc. Mass Spectrom.* **1999,** *10,* 72−75.

(16) Van Berkel, G. J.; Asano, K. G.; Schnier, P. D. *J. Am. Soc. Mass Spectrom.* **2001,** *12,* 853−862.

(17) Cao, P.; Moini, M. *J. Am. Soc. Mass Spectrom.* **1997,** *8,* 561−564.

(18) Kelleher, N. L.; Senko, M. W.; Siegel, M. M.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1997,** *8,* 380−383.

carbon particles glued to glass,[13,14] and even a soft pencil.[20,21] However, to date, there has been no mention of evaporated graphite coatings prepared using a vacuum deposition chamber. Early carbon emitters have shown the feasibility of using carbon as a conductive medium, but their manufacture suffers from many of the same drawbacks as other coating techniques. This paper describes a procedure for the mass production of borosilicate emitters using an evaporated graphite coating technique.

Evaporated graphite offers many advantages over other forms of emitter coatings. The most apparent is the ease of coating. Using a graphite rod electrode and introducing an arc, graphite is evaporated onto the surface of the glass. Such simplicity leads to a reduced probability of fracturing the fine tapers of the emitters through operator handling. This is particularly true when one considers the procedure developed by Chang and co-workers with a graphite pencil.[20,21] The pencil might work on thick-walled silica, but any pressure on a pulled taper would cause the emitter to fracture and become unusable. A third advantage is the possibility of mass production. Many emitters can be placed within the vacuum chamber and, provided they are not touching one another, can be coated simultaneously. To date, over 30 emitters have been coated at once, taking about 20 min for the entire procedure. This equates to one emitter taking only 40 s to complete. However, in principle, more than 30 emitters can be coated simultaneously. This yields a tremendous advantage over the "dipping" techniques[12,19] in which the emitters are dipped into a suspension of graphite or polyaniline. This dipping approach lengthens fabrication time to a few minutes per emitter.

To show the usefulness of the evaporated-graphite-coated emitters, various nanoelectrospray experiments were conducted. A series of experiments was performed in both positive and negative ion modes. The first set of experiments determined the functionality of the emitters in each ionization mode. Once functionality had been determined, experiments were performed to show long-term durability over a period of hours. Finally, the mechanical stability of the evaporated coating was tested through an electrical discharge event.

## EXPERIMENTAL SECTION

**Materials and Reagents.** Borosilicate glass (10 cm long, 1.2 mm o.d., 0.9 mm i.d.) was purchased from Sutter Instrument Co. (Novato, CA) and used without further modification. Cytochrome $c$ and gastrin fragment were purchased from Sigma (St. Louis, MO). HPLC-grade methanol was obtained from Aldrich (Milwaukee, WI) and used as purchased. Aqueous samples were prepared using doubly distilled deionized water. Graphite rods ($1/8$ in.) were from Ted Pella, Inc. (Redding, CA) and were sharpened to 1-mm points prior to evaporative coating using an electric pencil sharpener.

**Production of Emitters.** Fabrication of the nanoelectrospray emitters used a protocol previously reported by this laboratory.[12] To summarize, a Sutter Instruments P-2000 $CO_2$ laser-based micropipet puller was used to pull borosilicate nanoelectrospray emitters with open emitter ends with an orifice i.d. of 4 $\mu$m. A 10-cm length of micropipet tubing was placed within the horizontal
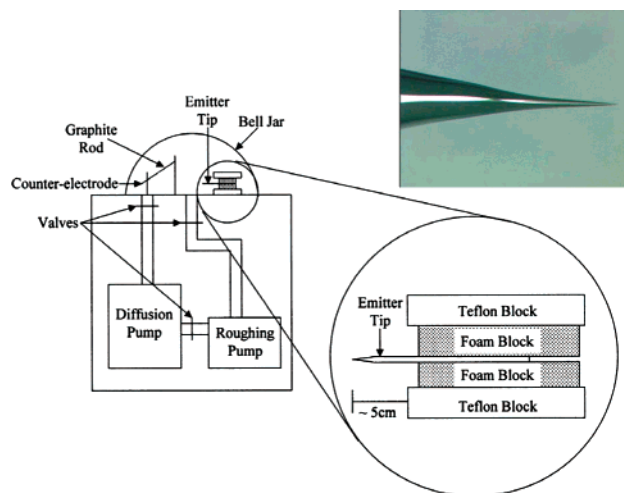


**Figure 1.** Schematic of the evaporated graphite coating apparatus with an expanded view of the emitter mount. A typical evaporated-graphite-coated nanoelectrospray emitter is depicted in the micrograph in the top right.

pulling arms of the laser puller. Heat was supplied by the laser until the glass reached a predetermined softness. At that point, the pulling arms exerted a force that pulled the heated segment apart, yielding two tapered emitters. Parameters were optimized to produce emitters with short tapers, relatively thick-walled orifices, and open emitter ends with orifices of 4 $\mu$m and a total emitter length of 5.5 cm. Each emitter takes approximately 40 s to fabricate, starting from loading of the glass into the laser puller to removing of the pulled emitters from the evaporation chamber.

**Coating of Emitters.** A general schematic of the coating apparatus is shown in Figure 1. Each batch of emitters (ranging from 20 to 30 emitters each) was secured by double-sided tape to a Teflon plate (8 cm $\times$ 20 cm) onto which a layer of foam had been mounted. A second Teflon plate with foam was placed on top of the emitters to form a sandwich. The two plates were secured to one another using transparent tape. Each emitter protruded approximately 5 cm beyond the Teflon, yielding a graphite coating of about 5 cm. The Teflon plates aided in preventing accidental breakage of the tips and eased the coating procedure, while the foam prevented crushing of the glass during handling. The evaporative coating was generated using a Denton Vacuum (Moorestown, NJ) model DV502 graphite evaporation chamber. A 4-mm length of sharpened graphite was used for evaporation. The chamber reached a vacuum of below 100 mTorr before current was supplied to the graphite rod. A 10-V, 50-A current was passed through the graphite for approximately 5 s until the sharpened length was completely evaporated. This evaporative process creates a graphite coating layer calculated to be 20−30 nm in thickness (as determined by the applied voltage, current, and length of time of evaporation). The chamber was then returned to atmospheric pressure. After one side of the emitter had been coated, the plates could simply be flipped over to allow for quick and easy coating of the other side, helping to ensure total coverage of the emitter tip. After each side had been coated, the emitters were removed and stored until used. However, no curing time is needed, as is required with polymeric coatings, and the tips can be used immediately.

**Mass Spectrometry.** Mass spectrometry experiments were performed using a commercially available PE Sciex API-3000 triple-

(19) Zhu, X.; Thiam, S.; Valle, B. C.; Warner, I. M. *Anal. Chem.* **2002**, *74*, 5405−5409.
(20) Chang, Y. Z.; Her, G. R. *Anal. Chem.* **2000**, *72*, 626−630.
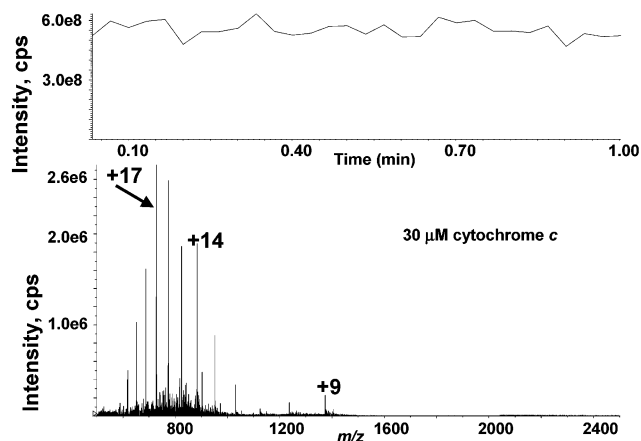(21) Chang, Y. Z.; Chen, Y. R.; Her, G. R. *Anal. Chem.* **2001**, *73*, 5083−5087.

**Figure 2.** TIC (top) and nanoelectrospray mass spectrum (bottom) for 30 $\mu$M cytochrome $c$ in positive ion mode using a vacuum-deposited-graphite-coated nanoelectrospray emitter. A charge state distribution of +9 ($m/z$ 1374) to +20 ($m/z$ 619) is observed.

quadrupole mass spectrometer. A home-built nanoelectrospray source was designed and manufactured specifically for use with nanoelectrospray on the API-3000 instrument and has been described elsewhere.[22] A standard solution of 30 $\mu$M cytochrome $c$ dissolved in 50/50 MeOH/H$_2$O was used for all positive-ion-mode experiments, and 30 $\mu$M gastrin fragment dissolved in 50/50 MeOH/H$_2$O was used in the negative ion mode. Individual emitters were placed 1−2 mm from the inlet orifice to the mass spectrometer, a voltage of 4.5 kV was supplied to the emitter tip to conduct nanoelectrospray (except where noted), and the endplate counter-electrode potential was 10 V. Borosilicate emitters used nanoelectrospray with a flow rate determined to be about 0.25 $\mu$L/h. Mass spectra were acquired using a Q1 scan from $m/z$ 500 to 2500 for cytochrome $c$ and from $m/z$ 300 to 1500 for gastrin fragment with a step size of 0.1 $m/z$ at 2 s/scan.

## RESULTS AND DISCUSSION

To test for emitter usability, 5 $\mu$L of a standard cytochrome $c$ solution was loaded into an emitter using a 10-$\mu$L Hamilton syringe. No arcing was evident, and mass spectra were recorded. Figure 2 shows a representative spectrum and total ion chromatogram (TIC) acquired using an evaporated-graphite-coated nanoelectrospray emitter for cytochrome $c$. As can be seen in the TIC, the nanoelectrospray generated is relatively stable over 1 min, yielding a spectrum with a signal-to-noise ratio of 11:1 for the 728.5 (+17) peak. The mass spectrum generates a charge state distribution from +9 ($m/z$ 1374) to +21 ($m/z$ 589), with a heme peak at $m/z$ 616.5.

To show long-term usability, another emitter was loaded with 1 $\mu$L of cytochrome $c$ solution and run continuously for 7 h. Figure 3 shows the TICs and mass spectra recorded every 2 h over the course of the experiment. Each spectrum is a 1-min sum of 30 scans taken at 2, 4, and 6 h. As can be seen, the TIC is somewhat variable, but it can be argued that, over 7 h, the TIC is relatively stable (total ion intensity $\pm$20% average over 15-min intervals). Each of the mass spectra shows the same charge state distributions of +9 ($m/z$ 1374) to +19 ($m/z$ 652). However, there is a
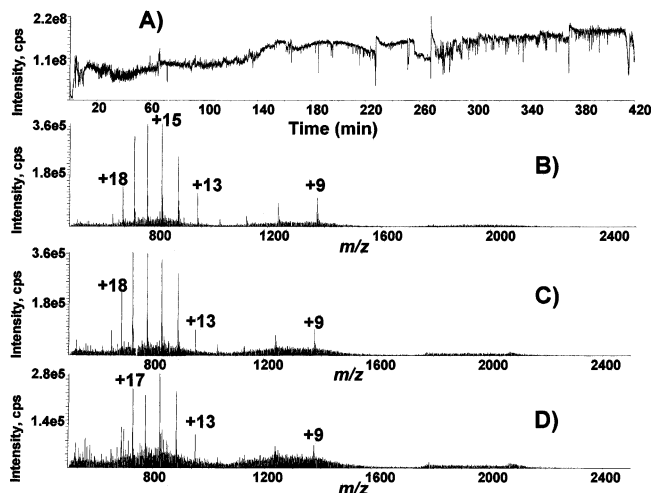
(22) Smith, D. R.; Sagerman, G.; Wood, T. D. *Rev. Sci. Instrum.* **2003**, *74*, 4474−4477.

**Figure 3.** (A) TIC and (B−D) nanoelectrospray mass spectra for a long-term experiment using 30 $\mu$M cytochrome $c$ in positive ion mode with a vacuum-deposited-graphite-coated nanoelectrospray emitter. Mass spectra are 1-min sums taken (B) 2, (C) 4, and (D) 6 h after initiation of nanoelectrospray.
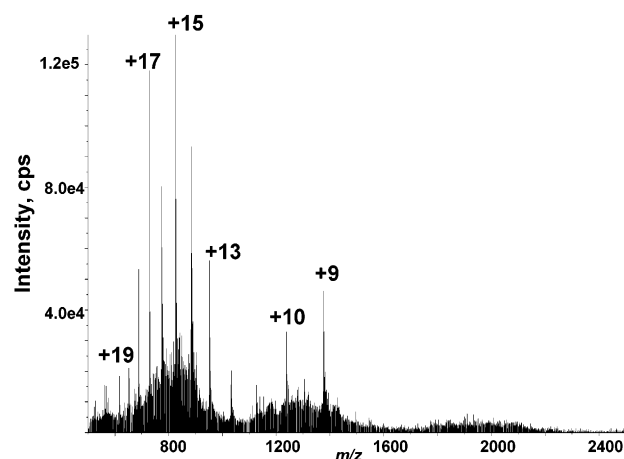


**Figure 4.** One-minute sum of scans taken after a 7-h run from the TIC shown in Figure 3. The applied voltage was raised from 4.5 to 6 kV.

slight decrease in signal-to-noise ratio at the 6-h mark. By 7 h, the signal-to-noise ratio approaches 3:1 for the most intense peak, although the TIC continues to gradually increase. However, after 7 h, increasing the spray voltage from 4.5 to 6 kV causes the spectrum generated to have roughly the same profile as the 6-h spectrum (see Figure 4). It is not entirely clear why the signal level is restored upon increasing the voltage. One might speculate that redox products (e.g., formation of H$^+$ from oxidation of water) accumulate over time. However, there is no evidence from the mass spectra that pH (i.e., the H$^+$ concentration) has changed during the course of the experiment, as the charge state distributions are little changed over this period of time. It should be noted that, even after 7 h, the 1 $\mu$L of loaded sample has not been completely consumed. An appreciable amount of sample remains in the taper of the tip. A possible explanation is that, early in the experiment (before 6 h), a large enough amount of solution is in the nanoelectrospray tip to provide enough pressure to induce capillary action at the lower voltage (4.5 kV). As the sample is consumed (the volume in the emitter decreases), there is no longer sufficient pressure to push the solution through the tip.
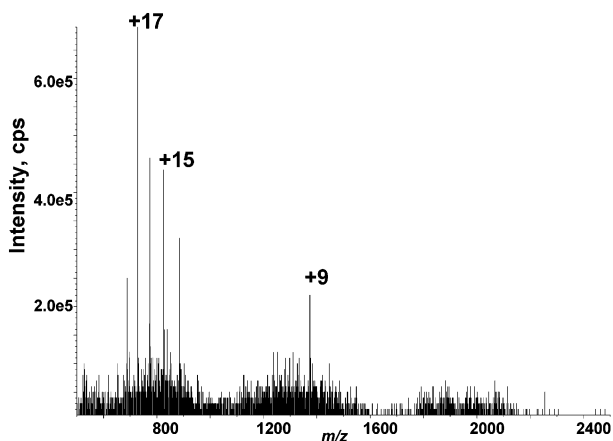
**Figure 5.** Single scan from Figure 3. Assuming a flow rate of 3 nL/min, only 3 fmol of sample was consumed to generate the spectrum. A signal-to-noise ratio of 8:1 is seen for $m/z$ 728 (+17).
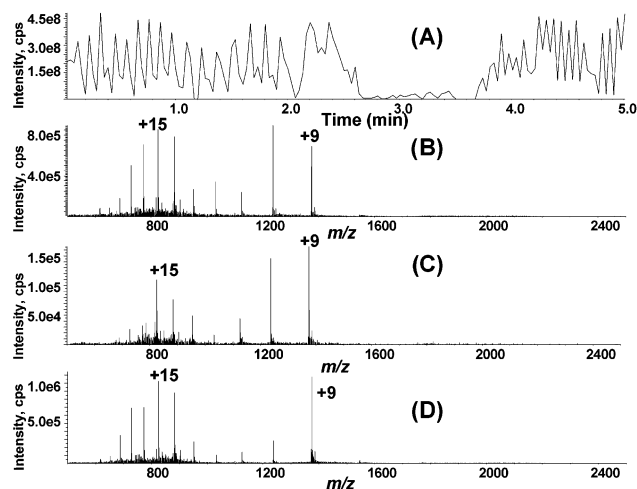


**Figure 6.** (A) TIC and (B−D) nanoelectrospray mass spectra for an electrical discharge experiment in positive ion mode using a vacuum-deposited-graphite-coated nanoelectrospray emitter. The mass spectra in (B) and (D) are at optimized conditions, with the mass spectrum in (D) being summed after an emitter was placed in the inlet orifice for 1.5 min. The mass spectrum in (C) was summed while the emitter was within the inlet orifice. Electrical discharge was not visually apparent, even when the emitter was within the inlet orifice.

To reestablish signal, it is necessary to increase the applied voltage to exert a greater external force on the sample solution. Taking this into consideration, an upper limit to the flow rate is 3 nL/min. However, the actual flow rate must be somewhat lower, as an appreciable volume remains in the emitter tip even after 7 h. Figure 5 shows a single 2-s scan. Using a 30 $\mu$M sample, this correlates to only 3 fmol of sample consumed with a signal-to-noise ratio of 8:1 for $m/z$ 728 (+9).

To date, emitter stability has been a major issue, particularly the hardiness of the applied conductive coating. To determine the robustness of the evaporated graphite coating, experiments were conducted in which electrical discharge was intentionally induced and maintained while signal was being acquired. Figure 6 shows a representative result of these experiments. Each spectrum is a 30-s sum of 15 scans taken during the 1.5−2.0-, 3.0−3.5-, and 4.5−5.0-min time periods as described below. Signal response was optimized via lateral emitter position to maximize ion current, and mass spectra were acquired for 2.5 min. The emitter was then
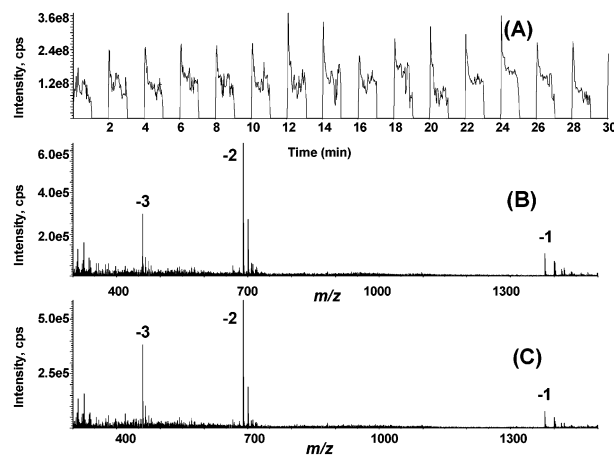
**Figure 7.** (A) TIC and (B−D) nanoelectrospray mass spectra for a pulsed-voltage experiment using a vacuum-deposited-graphite-coated nanoelectrospray emitter. Voltage was switched between −5 kV and 0 V every minute for 30 min. Mass spectra are from the 2−3- and 28−29-min time periods.

directed into the inlet orifice of the mass spectrometer. Upon visual inspection, there did not appear to be any arcing between the emitter and the endplate electrode. The emitter was left within the inlet orifice for 1.5 min when it was then returned to its optimal position, and signal was acquired for an additional 1.5 min. As can be seen, signal was maintained, and a usable mass spectrum was acquired even when the emitter was within the endplate orifice. The overall intensity was lower while the emitter was in the endplate, but the signal returned to its previous level once the emitter had been returned to its optimized position. The apparent lack of arcing might be due to the decreased applied potential compared to that encountered in other types of nano-electrospray emitters, the electrochemical stability of the graphite, or even the relatively thin layer compared to most metallized and polymer-coated conductive films on nanoelectrospray emitters.

Finally, emitter performance was investigated using negative ion mode. It has been noted that electrical discharge is more likely to occur when operating in the negative ion mode.[23] To show emitter durability when high voltage is applied, a pulsed-voltage experiment was conducted. This experiment entailed switching the applied voltage from −5 kV to 0 V back to −5 kV every minute over 30 min. As can be seen in Figure 7, the signal intensity dropped to zero as the applied voltage was switched to 0 V. However, the signal response returned once −5 kV was reapplied. The emitter response is nearly identical for each "spike". The spectra are 1-min sums between the 2−3- and 28−29-min time periods. Each spectrum shows the −1 ($m/z$ 1379) to −3 ($m/z$ 459) charge states, along with salt adducts.

Finally, because electrical discharge is a major issue in negative ion mode, intentional discharge was induced to illustrate the robustness of the coating. Figure 8 shows the TIC and mass spectra for a discharge experiment. Each spectrum is a 1-min sum of 30 scans taken during the 1.5−2.5-, 4.5−5.5-, and 7.5−8.5-min time periods as described below. The emitter response was optimized, and mass spectra were acquired for 4 min. The emitter was then moved toward the inlet orifice until arcing was discernible. The emitter was left in the arcing field for 3 min until it was

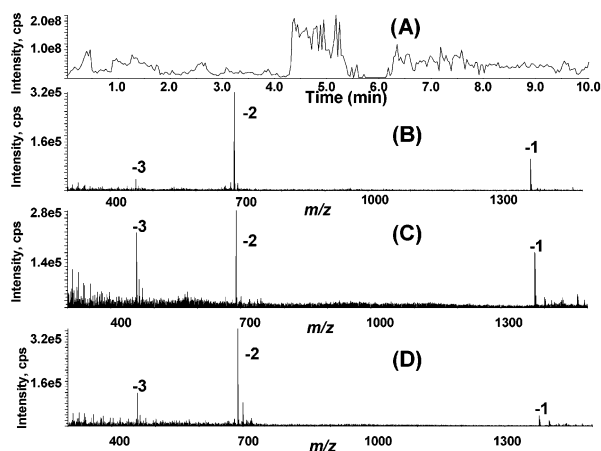(23) Bruins, A. P. *J. Chromatogr. A* **1998**, *794*, 345−357.

**Figure 8.** (A) TIC and (B–D) nanoelectrospray mass spectra for an electrical discharge experiment in negative ion mode using a vacuum-deposited-graphite-coated nanoelectrospray emitter. The mass spectra in (B) and (D) are at optimized conditions, with the mass spectrum in (D) being summed after an emitter was discharged for 3 min. The mass spectrum in (C) was summed while the emitter was discharging. While discharging, the emitter glowed a brilliant blue/violet color.

repositioned at its optimal location for another 3 min. During arcing, visual inspection showed the emitter tip to be a brilliant blue color due to constant electrical discharge. However, as can be seen from the mass spectra, the emitter did not fail, and indeed, the response was nearly identical to that observed at the optimized location. Thus, not only can these emitters survive electrical discharge (even continuously for 3 min), they can produce usable mass spectra while arcing is taking place. Thus far, evaporated graphite films are the only coatings to exhibit such performance during electrical discharge during nanoelectrospray.

## CONCLUSIONS

A new conductive coating for nanoelectrospray emitters has been developed and tested. The evaporated graphite coatings are stable in both negative and positive ionization modes. Moreover, this coating is not susceptible to electrical discharge under normal operating conditions. Even if electrical discharge takes place, the coating is durable and continues to generate useful spectra. The lower applied potential needed by these tips might allow for lower flow rates. This would lead to a great reduction in required sample volume. Finally, the chief advantage of this coating is the speed and ease of the coating procedure. Emitters can be coated in less than 1 min per piece with minimal operator handling. Because this is a vapor deposition technique, there is little chance of clogging the emitter orifice, and more importantly, there is no need for gas pressure while the emitter is being dipped into a solution. These advantages will be important as current work in our laboratory investigates packed-silica capillaries for online capillary LC and CEC/MS. Using this approach, the silica capillary functions directly as the nanoelectrospray emitter, removing the need for a liquid junction or sheath flow maintaining chromatographic integrity.