

A Conductive Polymeric Material Used for Nanospray Needle and Low-Flow Sheathless Electrospray Ionization Applications

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A conductive polypropylene/graphite mixture is used for the production of polymeric nanospray needle emitters and as a coating on fused-silica capillaries that are used for sheathless electrospray ionization (ESI). The described production of these polymeric nanospray needle emitters and sheathless ESI contacts is exceptionally easy and at a very low cost. The described polymeric nanospray emitters have shown excellent features regarding their chemical inertness and spray performance. The long-term stability of the nanospray needles exceeds 24 h of continuous use. Furthermore, the resistance to electrical discharges, which is one of the factors that often limits the lifetime of metal coated tips, has proven to be outstanding. A voltage of up to 5 kV could be applied without loss of spray performance. The use of polypropylene emitters offers a number of desirable features, as compared to silica based emitters. Among these features are mechanical flexibility and simplified regeneration of the nanospray needle. Continuous nanospray of peptides and proteins in conjunction with orthogonal time-of-flight mass spectrometry are shown with signal relative standard deviations of 5%. In addition, the polypropylene/graphite mixture has also been applied as the conductive contact for sheathless ESI in fast capillary electrophoresis separations.

Electrospray ionization (ESI) is one of the most used methods for on-line coupling of liquid-based separation techniques, such as capillary electrophoresis (CE), capillary electrochromatography (CEC), and liquid chromatography (LC), to mass spectrometry (MS). The combination ESI-MS has had a vast impact on the analysis of large biomolecules and has now become one of the leading techniques in proteomics. Recent research in ESI-MS has focused on the reduction of sample consumption, increased sensitivity, and resolution in the analysis. These requirements have led to low-flow rate approaches, such as nanospray¹ needles, for which the flow rate is approximately 20–40 nL/min and low-flow sheathless ESI having flow rates <300 nL/min. This can be compared to conventional ESI having flow rates in the microliter-per-minute range. The reduction of the flow rate yields smaller

droplets.² Small droplets have a high surface-to-volume ratio, which in turn implies a number of desirable features.³ Among these features are increased sensitivity and a higher tolerance to salts in buffers and samples. As a consequence, a lot of emphasis has been put on the production of high-performing, physically and chemically stable nanospray needles and low-flow ESI emitters.^{4–8} The limiting factor for the stability of these emitters is the robustness and lifetime of the conductive material needed for the electrical contact to the sprayed solvent. Gold is most commonly used as the conductive material, and it is often sputtered on the ESI tip using additional adhesive or protecting materials in order to enhance the stability.^{6,8,9} These emitters are, however, susceptible to decomposition as a result of electrical discharges and electrochemical stress, which in turn decreases the lifetime. Furthermore, the production of these emitters is often tedious, time-consuming, and expensive. Our group has reported an alternative method, the so-called “fairy dust” technique,¹⁰ by which the lifetime of the emitters has been significantly increased, and the production of the emitter is considerably simplified.

Despite the fact that gold-coated emitters might give high performance and good stability, the production will never become inexpensive. There is a need for a low-cost material and production method, especially for disposable nanospray needle tips and future microfabricated disposable formats. The ultimate demands on the emitters are, thus, high performance, robustness, and low cost. We recently reported a development of the fairy dust technique for low-flow ESI in which graphite is used as the conductive material.¹¹ These emitters, called the “black dust” emitters, have shown the properties required for future demands. Alternative materials, such as polyaniline¹² and carbon coatings,¹³ have also

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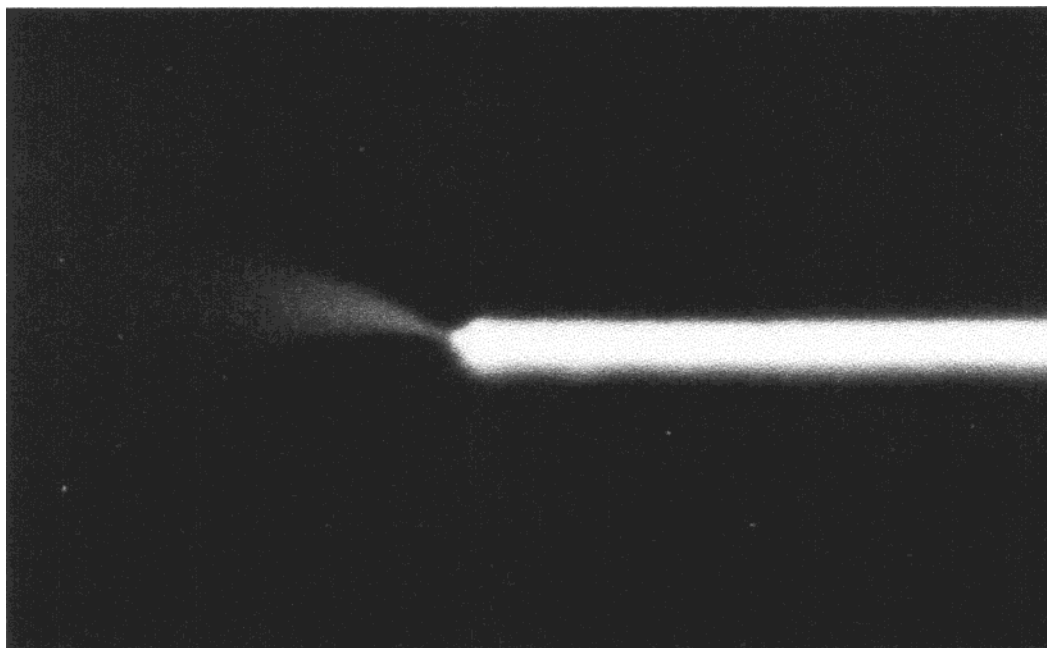


Figure 1. Close-up of the polymeric nanospray needle emitter. The emitter consists of an electrically conductive mixture of polypropylene/graphite. The flow rate was ~ 45 nL/min at a voltage of 1.8 kV.

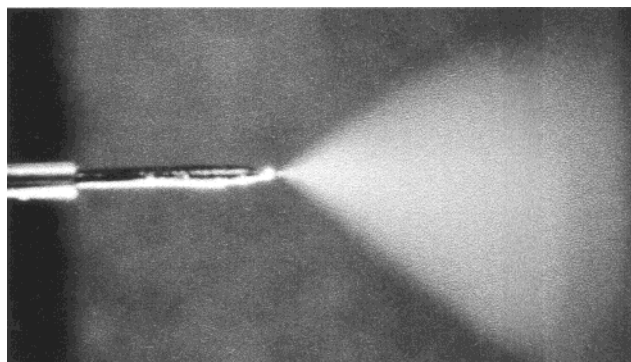


Figure 2. Close-up of the black jack sheathless ESI emitter. The electrically conductive polypropylene/graphite mixture was applied on the tapered emitter end of a $25\text{-}\mu\text{m}$ -i.d. $360\text{-}\mu\text{m}$ -o.d. fused-silica capillary. The flow rate was 500 nL/min, and the voltage was 3 kV.

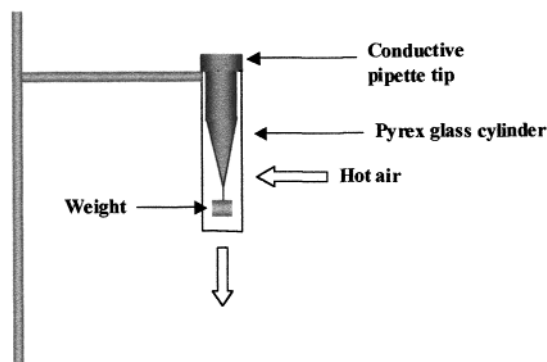


Figure 3. Schematic figure for the production of the polymeric nanospray needle emitter. A conductive pipet tip is inserted into a Pyrex glass cylinder, which is heated with air. A small weight pulls the pipet into a long and narrow hollow fiber.

been reported for the production of nanospray needles and low-flow ESI emitters, although these emitters have shown limitations regarding the lifetime.

In this study, we present the design and fabrication of a new polymeric nanospray needle emitter (Figure 1) consisting of a conductive mixture of polypropylene and graphite. Furthermore, this polypropylene/graphite mixture has been applied as a coating on fused-silica capillaries to produce low-flow ESI in fast CE separations (Figure 2). This sheathless ESI interface has been named the "black jack" emitter.

EXPERIMENTAL SECTION

Chemicals and Materials. Deionized water was obtained from a Milli-Q⁺ system (Millipore Corp., Marlborough, MA). All organic solvents, acids, and bases used were from Merck (Darmstadt, Germany). Peptide standards were obtained from Sigma

Chemical Co. (St. Louis, MO) and were used without further purification. The CE column-coating reagent, 3-aminopropyltrimethoxysilane, was obtained from Aldrich (Milwaukee, WI). Fused-silica capillaries, $25\text{ }\mu\text{m}$ i.d. \times $360\text{ }\mu\text{m}$ o.d., were purchased from Polymicro Technologies (Tucson, AZ). Eppendorf (Hamburg, Germany) kindly provided conductive pipet tips ($300\text{ }\mu\text{L}$) consisting of a mixture of polypropylene/graphite.

Production of the Polymeric Nanospray Needle Emitters.

A conductive pipet tip was inserted into a Pyrex glass cylinder. A small weight (400 mg) was put in the elution end of the pipet tip. The lower part of the glass cylinder was heated with hot air ($400\text{ }^{\circ}\text{C}$) using an air gun (Bosch, Gerlingen, Germany), thus ensuring nearly equal cross-sectional heating of the elution part of the pipet tip. As the heated tip started to soften, the weight pulled it into a very long and narrow tip. After cooling to ambient temperature, the emitter end was cut with a razor blade and was ready to use. The emitter end can be cut at various lengths, which results in a choice regarding the inner diameter, and hence, the ability to vary the flow rate is achieved. Further investigations regarding the

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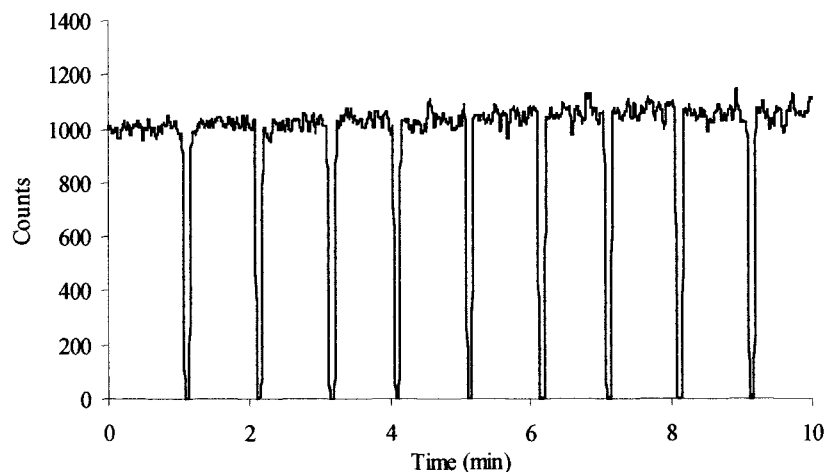


Figure 4. Selected ion profile (SIP) of methionine-enkephalin (574–575 m/z) showing the possibility of turning the spray off and on without compromising the spray performance.

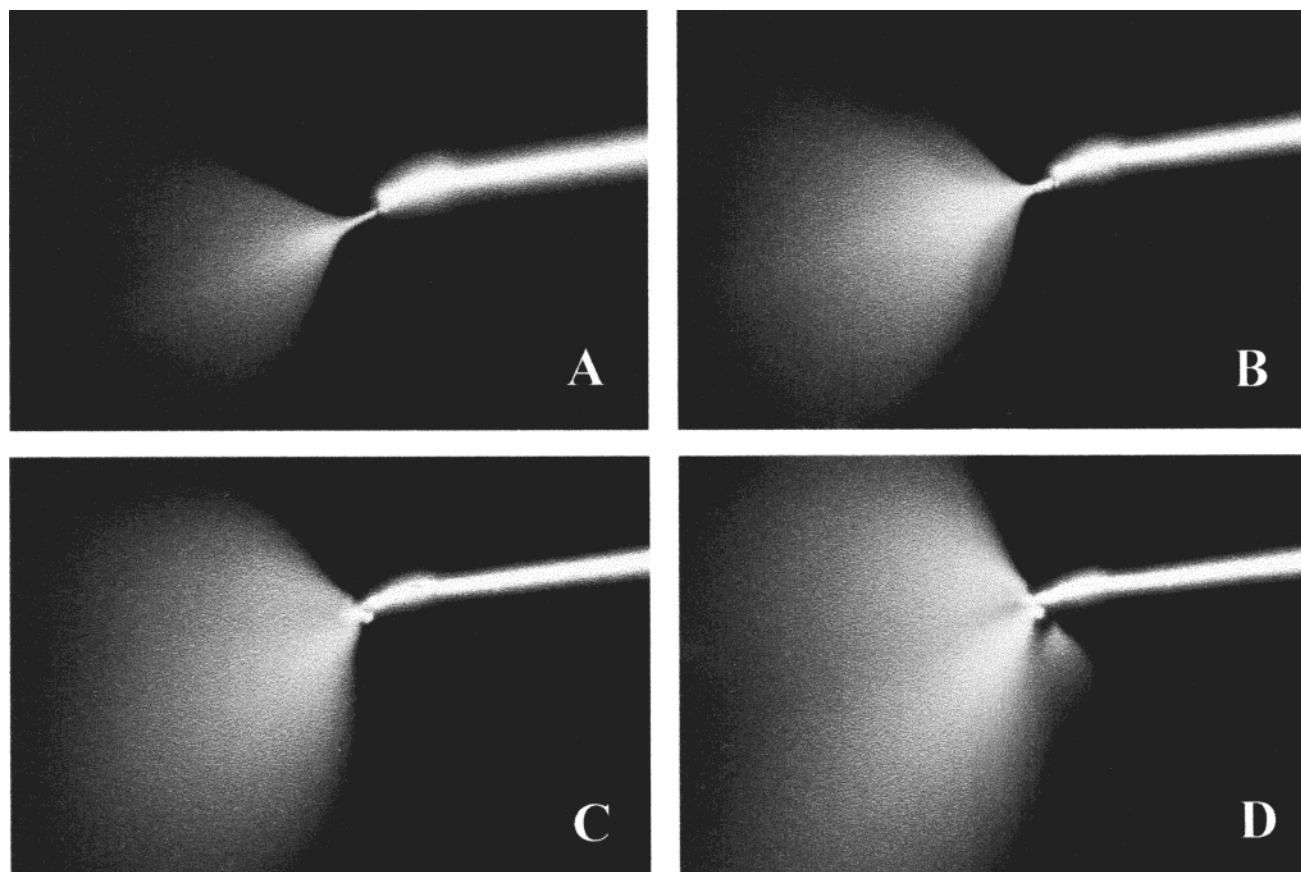


Figure 5. Close-up of the polymeric nanospray needle emitter where (A) 2, (B) 3, (C) 3.5, or (D) 5 kV has been applied on the emitter. The flow rate was slightly higher (~ 100 nL/min) than in Figure 1 as a result of the higher field.

inner diameter of the emitter will be conducted using a scanning electron microscope (SEM). The inner diameter of the emitter has been visually estimated to be <20 μm . Figure 3 shows a schematic figure of the production of the polymeric nanospray needle emitters. The time required to produce one emitter was less than 1 min. The production of the polymeric nanospray needle emitter requires some practice, because it is conducted manually.

Off-Line Evaluation of the Polymeric Nanospray Needle Emitter Performance. The evaluation of long-term stability, flow rate, and resistance to electrical discharges of the emitters was conducted off-line using a benchtop ESI source. In these experi-

ments, the ESI voltage was applied directly to the back end of the pulled conductive polymeric nanospray needle emitter. A flat (4 cm high and 6 cm wide) piece of grounded stainless steel was placed as a counter electrode at a distance of approximately 5 mm from the emitter. For positive spray, a potential of 1.8–2.0 kV was applied to the emitter by a Spellman SL10 high voltage supply (Hauppauge, NY). The buffer solution that was used consisted of MQ water:acetonitrile:glacial acetic acid (49.5:49.5:1, v:v:v) and the flow rate was dictated only by the electrospray process itself; no additional pumps or siphon forces were applied. Visual inspections of the nanospray needle performance were

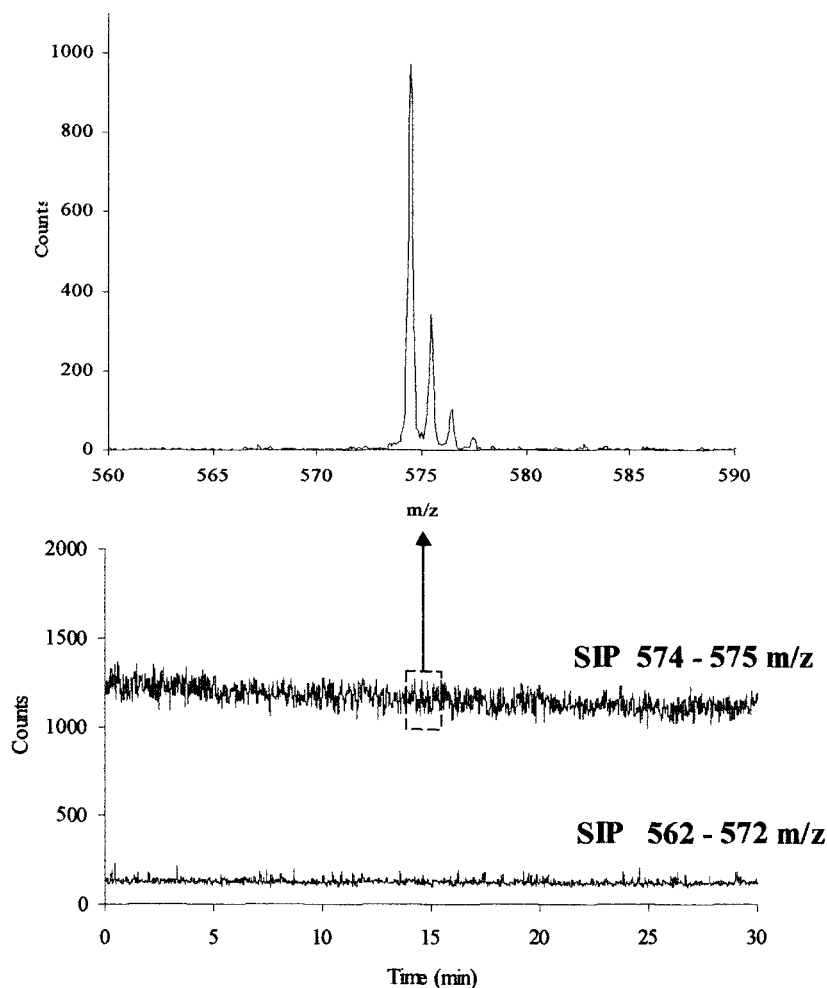


Figure 6. Selected ion profile of a continuous nanospray infusion of 1.7 μ M methionine–enkephalin (SIP, 574–575 m/z) and chemical background (SIP, 562–572 m/z). The enlarged mass spectrum shows the peak for approximately 1.3 fmol of methionine–enkephalin.

conducted using a binocular microscope, and photographs were taken by a Canon EOS 500 camera through the microscope.

The flow rate was estimated by filling the polymeric nanospray needle emitter with a known and density-calibrated volume of buffer. Continuous nanospray was run, and the remaining volume in the emitter was weighed every 30 min. The correction for solvent evaporation was performed using the same emitter. It was filled with an equal volume of calibrated buffer, and the remaining volume in the emitter was weighed every 30 min. The calculated flow rate was corrected for volume losses due to solvent evaporation.

The spray performance was studied at increased ESI voltages. An ESI voltage up to 5 kV was applied on the polymeric nanospray needle emitter.

Production of CE Capillary ESI Tips. Fused-silica capillaries of 35-cm lengths (25 μ m i.d. \times 360 μ m o.d.) were mechanically tapered⁸ and APS-coated.¹⁴ The emitter end of the APS-coated capillaries was covered with the polypropylene/graphite mixture. The coating was performed by melting (\sim 160 $^{\circ}$ C) the mixture and pulling the emitter end through the melt, thus providing a smooth conductive polypropylene/graphite layer that supplied the electrical contact for electrospray. A flow of nitrogen was purged

through the capillaries while applying the conductive layer. It is worth noting that the ESI conductive coating was applied after the APS coating. This demonstrates the possibility of applying the ESI conductive coating on capillaries that already have a heat-sensitive derivatized inner surface or that consist of a heat-sensitive bulk material.

Mass Spectrometry. All nanospray needle and CE experiments were conducted using a Jaguar O-TOFMS instrument (Leco, St. Joseph, MI). The instrument has a nonreflectron flight tube with quadratic field profile, giving a resolution over 2000 for reserpine (609 m/z) and a mass range of 0–6000 m/z . The pulsing frequency was set to 5 kHz, and the potential on the interface plate was set at 600 V. The flow of nitrogen curtain gas for the source region was set to 600 mL/min at a temperature of 80 $^{\circ}$ C for nanospray and 100 $^{\circ}$ C for CE.

Nanospray MS. Samples of methionine–enkephalin (573.2 Da) with the concentration of 1.7 μ M and cytochrome *c* (horse heart, 12384 Da) having a concentration of 1.0 μ M were prepared in a buffer consisting of MQ water:acetonitrile:glacial acetic acid (49.5:49.5:1, v:v:v). These samples were used for continuous nanospray with the polymeric nanospray needle emitters. The prepared emitter was filled with sample and mounted in an *x*, *y*, and *z* micromanipulator. The emitter end was positioned 5 mm in front of the orifice. High voltage for spraying was provided by

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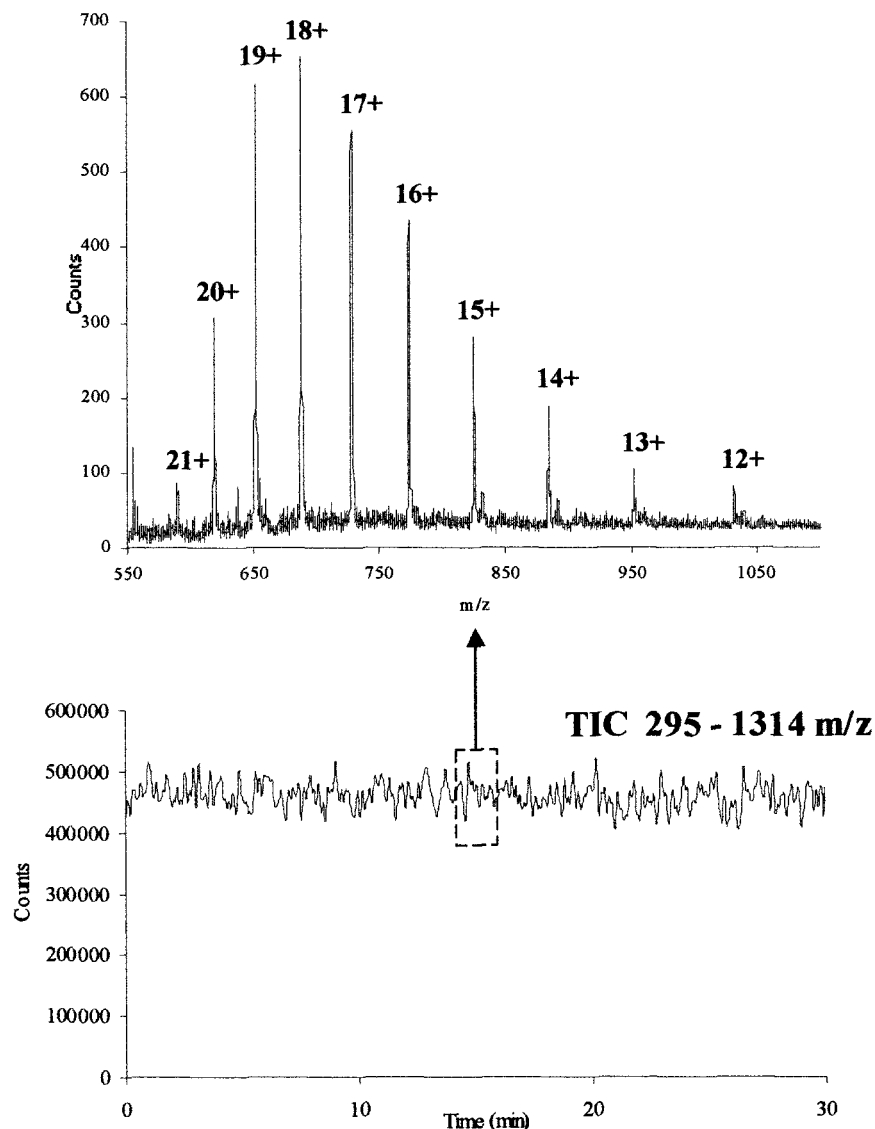


Figure 7. Total ion chromatogram (TIC, 295–1314 m/z) of continuous nanospray infusion of 1.0 μM cytochrome *c*. The enlarged mass spectrum shows the charge distribution of ~ 750 amol of the protein.

the MS instrument, and a potential of 2.3–2.4 kV was applied to the back of the emitter. Only the electrospray process itself without additional pumps or siphon forces produced the flow. Continuous infusion experiments were run for several hours, and mass spectra with various spectral sums were collected.

CE/MS. A sample consisting of the five neuropeptides, methionine–enkephalin (573.2 Da), leucine–enkephalin (555.3 Da), angiotensin II (1046.2 Da), neurotensin (1672.9 Da), and luteinizing hormone releasing hormone (LHRH, 1181.6 Da), was prepared at a concentration of 1 $\mu\text{g}/\text{mL}$ of each peptide in MQ water:acetonitrile (20:80, v:v). A home-built CE instrument with pressurized injections was used in combination with a Bertan ARB-30 high voltage supply (Bertan, Hicksville, NY). An x, y, z micromanipulator (Protana, Odense, Denmark) in which a stainless steel tube, fitting 360- μm -o.d fused-silica capillaries, provided the ESI contact. The electrospray high voltage supply was connected to the conductive coating through the stainless steel tube to which the conductive coating made physical contact. Running buffer was 5 mM acetic acid with 50% acetonitrile. A sample volume of 3.5 nL (3.5 pg of each peptide) was injected (2

psi for 10 s), and the CE separation was performed using -30 kV applied on the injection end and $+3$ kV (ESI voltage) on the emitter end, resulting in a total electric field of -0.94 kV/cm.

Safety Considerations. All acids and bases should be handled with great care. The CE column-coating reagent 3-aminopropyl-trimethoxysilane is highly reactive. Acetonitrile is combustible, volatile, and slightly toxic if exposed to lungs or skin. To avoid electrical chock, the high-voltage power supplies should be handled with extreme care. The peptides used are biologically active and should be handled using protective gloves.

RESULTS AND DISCUSSION

Off-Line Evaluation of the Nanospray Needle Emitter Performance. The polymeric nanospray needle emitters have shown excellent long-term stability. Continuous nanospray, using the same emitter, has been performed for more than 24 h. The flow rate for three emitters was estimated at 19, 47, and 70 nL/min. The variation in flow rate is due to a difference in inner diameter, due to the manual production. In Figure 4, another useful feature is demonstrated. The ESI voltage can be turned on

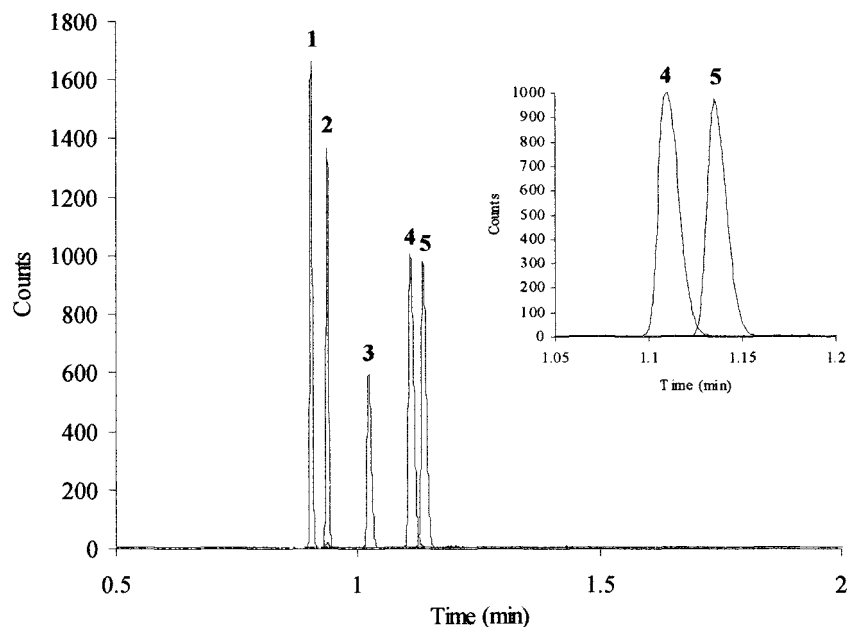


Figure 8. Selected ion profiles (SIP) for a five-peptide mixture separated by capillary electrophoresis. The elution order was 1, methionine–enkephalin; 2, leucine–enkephalin; 3, angiotensin II; 4, neurotensin; and 5, LHRH. The enlarged SIP shows the peaks of neurotensin and LHRH.

and off numerous times without compromising the stability of the spray. Figure 5 shows the influence of increased ESI voltage on spray performance. The applied voltage was raised from 2 kV to 5 kV and then lowered back to the starting potential. It can be seen that multiple jets are formed with increased potential, yet the emitter is still spraying, despite the excessive applied voltage. The spray was restabilized when returning to the starting potential. This demonstrates that an excessive voltage does not significantly disrupt the performance of the spray. Furthermore, it was possible by cutting a small part of the emitter end to regenerate a good spray with minimal loss of sample after the nanospray tip had experienced discharges at 7 kV.

Nanospray MS. The ability of the polymeric nanospray needle emitter to provide a stable nanospray is demonstrated in Figures 6 and 7. A selected ion profile (SIP) of a 30-min continuous infusion MS run (acquisition speed, 3.1 spectra/s; flow rate, ~ 45 nL/min) of $1.7 \mu\text{M}$ methionine–enkephalin is shown in Figure 6. The data were acquired after 1 h and 30 min of continuous spray. The enlarged mass spectrum shows an acquisition of 1 s, thus representing a mass spectrum of 1.3 fmol. It can be clearly seen that the signal is stable during the run (signal relative standard deviation (RSD), 5%) and that a low-femtomole concentration of the peptide is readily detected with a good signal-to-noise ratio (S/N 100). Figure 6 shows a 30-min continuous infusion of $1.0 \mu\text{M}$ cytochrome *c*, with an acquisition rate of 3.1 spectra/s. The total ion chromatogram (TIC) shows a stable spray during the analysis (TIC RSD, 4.5%), and the charge distribution of the protein can readily be seen in the enlarged mass spectrum, representing a total of 750 amol of the protein. No interfering peaks originating from the emitters could be detected in the mass spectra. We have tried to dissolve the polymer in various organic solvents, such as chloroform, hexane, DMSO, and acetonitrile, without observing any impact on the emitter. The specifications¹⁵ from Eppendorf

regarding the chemical stability of polypropylene indicate that the material is compatible with the most commonly used solvents, acids, and bases in mass spectrometry.

CE/MS. The successful use of the polypropylene/graphite as a coating on the tip of a CE capillary for low-flow ESI is demonstrated in Figure 8. A representative electropherogram of the CE/MS separation of a five-peptide standard is shown in the figure, including an enlargement of peaks 4 and 5. The electropherogram shows almost complete baseline separation of the five peaks within 68 s, with peak widths at the base ranging from 0.84 to 1.76 s. The number of theoretical plates for methionine–enkephalin is 1.6×10^5 , with a migration time of 54 s. The CE separation points out that the polypropylene/graphite mixture successfully provides the ESI contact for a stable electrospray for CE/ESI-MS experiments using APS-derivatized capillaries.

CONCLUSIONS

The polypropylene/graphite mixture has been shown to possess a number of desirable features for ESI and nanospray. The polymeric nanospray needle emitters have shown very good long-term stability. The production of these emitters is very easy, fast, and at a low cost. Furthermore, the polymeric nanospray needle emitter has shown a stable spray performance, despite an excessive voltage. The material is mechanically stable and flexible; for example, it does not break as easily as silica-based needle emitters. The tip of the emitter can easily be regenerated if it is plugged or otherwise damaged without losing the sample in the emitter. These emitters can also be packed with various adsorption media, thus facilitating on-line preconcentration and desalting within the emitter itself prior to elution and MS analysis. Initial studies on packed polymeric nanospray needle emitters have been conducted and will soon be presented in a separate paper. The polypropylene/graphite mixture also provides a good ESI contact on fused-silica capillaries, thus providing the sheathless interface

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for CE, CEC, and capillary LC separations. Furthermore, the presented interfaces could be implemented on microfabricated separation chips, thus rendering it a robust and cheap product that truly is disposable.

Future studies regarding the sensitivity, salt tolerance, electrochemical stability, and dynamic range for organic modifiers and pH range will be conducted. A more extensive comparison to commercially available nanospray emitters regarding these parameters, as well as post genome applications, will be performed. A filed patent covers all presented emitter material.

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