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A Colloidal Graphite-Coated Emitter for Sheathless Capillary Electrophoresis/Nanoelectrospray Ionization Mass Spectrometry

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A colloidal graphite-coated emitter is introduced for sheathless capillary electrophoresis/nanoelectrospray ionization time-of-flight mass spectrometry (CE/ESI-TOFMS). The conductive coating can be produced by brushing the capillary tip to construct a fine layer of 2-propanol-based colloidal graphite. The fabrication involves a single step and requires less than 2 min. Full cure properties develop in ∼2 h at room temperature and then the tip is ready for use. The coated capillary tip is applied as a sheathless electrospray emitter. The emitter has proven to bear stable electrospray and excellent performance for 50 μm i.d. × 360 μm o.d. and 20 μm i.d. × 360 μm o.d. capillaries within the flow rate of 80−500 nL/min; continuous electrospray can last for over 200 h in positive mode. Baseline separation and structure elucidation of two clinically interesting basic drugs, risperidone and 9-hydroxyrisperidone, are achieved by coupling pressure-assisted CE to ESI-TOFMS using the described sheathless electrospray emitter with a bare fused-silica capillary at pH 6.7. It is found that the signal intensity of *m*/*z* in sheathless CE/ESI-TOFMS at pH 6.7 is ∼50 times higher than that at pH 9.0 for the two analytes, although the electroosmotic flow (EOF) at pH 9.0 provides sufficient flow rate (∼150 nL/min) to maintain electrospray.

Over the past decade, due to its high separation efficiency, short analysis time, and small size sample consumption, capillary electrophoresis (CE) has played an important role in the field of separations. However, there is a lack of qualitative information when traditional UV detection is employed in CE. Interfacing CE to a mass spectrometer (MS) can offer the attractive advantage of mass identification of unknown analytes. The benefit of combining CE with MS detection is that it provides a second dimension to the separation by combining mass-to-charge ratio information with charge-to-size information. MS detection can also help to improve the general sensitivity of CE analysis.

A variety of coupling methods have been attempted since Olivares et al. first reported the interface of CE and electrospray ionization (ESI) MS.[1] Sheath flow and sheathless interfaces are most commonly used in CE/ESI-MS. For sheath flow interfaces, a coaxial sheath flow that serves as the source of electrical contact to the tip of the capillary outlet has been introduced.[2] In the presence of sheath flow, the electrospray depends less on electroosmotic flow (EOF). Thus, this leads to a broader range of possible CE separation conditions. Although a sheath flow interface has several advantages including reliability and ease of use, it suffers from unavoidable dilution of the sample by sheath flow, leading to a decrease in sensitivity. In addition, the use of a sheath liquid other than the separation buffer can cause variations in migration time of analytes[3] and unwanted reactions at the capillary tip.[4] Sheathless interfaces have drawn much attention recently, because unlike a sheath flow interface, there are no sample dilution effects and there is an increase in sensitivity.

The electrical contact for both CE separation and electrospray can be created by coating conductive material on the capillary tip. Gold is an ideal material that is most often sputtered on the capillary tip for sheathless CE/ESI-MS;[5−9] it can also be electroplated.[10,11] However, the coating layer is susceptible to degradation and is physically unstable on the surface of fused-silica capillary.[12] Therefore, the coated tips have a short lifetime in general. In addition, the fabrication of the coating is time-consuming and requires special instrumentation. To improve the resistance of the gold coating on the capillary tip, Barnidge et al. first applied a thin polymeric film on the tip before adhering 2-μm-diameter gold particles on the surface.[13] The main limitations of this technique are that gold is expensive and it requires curing at a high temperature to form a stable coating. As an alternative to gold

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particles, Nilsson et al.[14] and Wetterhall et al.[15] used graphite as a coating material. However, the polymer's high viscosity made coating difficult, and high temperatures were still required for curing. Interestingly, Chang et al. used a soft pencil for the quick preparation of the carbon-coated capillary tip.[16,17] Unfortunately, the thin and uneven coating could not guarantee a stable electrospray for more than 8 h. In addition, use of organic solvents may damage the supporting layer made by a marker pen underneath the carbon coating.

As an alternative to the above approaches, a nanospray capillary tip was connected to a CE column by low-dead-volume tee[18] or polysulfone microdialysis tubing for sheathless interface.[19] Because of the dead volume where the two capillaries were attached, peak broadening occurred. Another method for sheathless interface was to insert an electrode into the CE column through a small aperture near the terminus[20] or through the column outlet[21,22] to obtain electrospray. Unfortunately, the redox reaction of water on the electrode may cause bubble formation, thereby interrupting the electrospray. Moini et al. investigated the use of a buffer additive for bubble suppression.[23] Moini also investigated a split-flow technique for sheathless CE/ESI-MS.[24] However, it was mechanically difficult.

Overall, an improved process for production of a coated emitter for sheathless CE/ESI-MS is still desirable in terms of low cost, ease of fabrication, and long-lasting stable electrospray. In this paper, we introduce a colloidal graphite-coated emitter for sheathless CE/ESI-MS for the first time. The simple production of the stable coating at room temperature involving a single step makes this method promising. The performance of electrospray and application of this sheathless CE/ESI-MS for analysis of two basic drugs risperidone and 9-hydroxyrisperidone are discussed.

EXPERIMENTAL SECTION

Chemicals and Materials. Glacial acetic acid, methanol, and acetone of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ) and used without further purification. Ammonium hydroxide was purchased from Mallinckrodt Baker (Paris, KY). Ultrapure grade ammonium acetate was purchased from Amresco Inc. (Solon, OH). Risperidone and 9-hydroxyrisperidone were kindly provided as a gift by Janssen Pharmaceutica (Beerse, Belgium). The 2-propanol-based colloidal graphite, consisting of approximately 5% proprietary thickener, 5% propylene glycol methyl ether, 5% *n*-butyl alcohol, 5% hexylene glycol, 50% 2-propanol, and 20% graphite, was obtained from Energy Beam Sciences (Agawam, MA). Water deionized by a USFilter system (Lowell, MA) was used for preparation of all solutions. All other chemicals were analytical grade. Two types of fused-silica capillary, 50 μm i.d. × 360 μm o.d. and 20 μm i.d. × 360 μm o.d., were purchased from Polymicro Technologies (Phoenix, AZ).

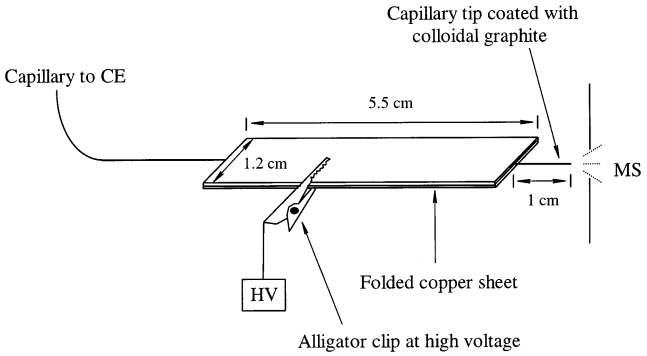

Figure 1. Schematic representation of the sheathless interface for CE/ESI-TOFMS.

Preparation of the Colloidal Graphite Coating on the Capillary Tip. The procedure for making the colloidal graphite coating on the capillary tip is rather simple. The tip, ~4 cm in length from the capillary terminus, was cleaned by use of acetone to remove dust. The colloidal graphite was then brushed gently on the tip to form a thin coating. To prevent clogging during the coating procedure, a low flow of nitrogen was applied through the capillary. It is worth mentioning that it takes less than 2 min to complete this coating. Finally, it took ~2 h to develop the cure properties of the coating at room temperature before the tip was ready for use. The tip was employed as a sheathless ESI emitter.

CE/ESI-TOFMS Instrumentation. A Hewlett-Packard[3D] CE instrument (Palo Alto, CA) and an electrospray ionization time-of-flight mass spectrometer (ESI-TOFMS) Mariner Biospectrometry Workstation from Applied Biosystems (Framingham, MA) were employed. A solution containing 1% acetic acid in 50:50 (v/v) water/methanol was supplied to the ESI emitter in direct infusion using a Harvard Apparatus syringe pump (Holliston, MA). The ESI voltage range was from 3 to 5 kV in positive mode. Data acquisition was performed in the range of m/z 50−1000 with a scan rate of 3 s/spectrum. The flow rate of curtain gas was optimized at 0.6 L/min, and no nebulization gas was applied to support the electrospray. Figure 1 shows the configuration of the sheathless interface for CE/ESI-TOFMS. The coated capillary tip was placed in a folded copper metal sheet that was 5.5 × 1.2 cm. About 1 cm of the tip extended from the metal sheet, and the end of the tip was 1.0−1.5 cm away from the nozzle of the mass spectrometer. The folded copper sheet was clamped with an alligator clip, which is connected to the high-voltage source of the ESI-MS. The 75-cm-long capillary for CE/ESI-TOFMS was rinsed with 1 M sodium hydroxide for 15 min, followed by a 30-min rinse with deionized water before coating the tip. A 10 mM ammonium acetate solution was prepared in 20% methanol/water (v/v) and adjusted to pH 6.7 and 9.0 with 1 M acetic acid and 1 M ammonium hydroxide, respectively. These solutions were used as the running buffer for the CE/ESI-TOFMS separation. Between runs, the capillary was rinsed with the running buffer for 2 min. Each analyte was first dissolved in methanol at a concentration of 1 mg/mL and then diluted further with the running buffer. Pressure was applied at 50 mbar for 5 s for sample injection.

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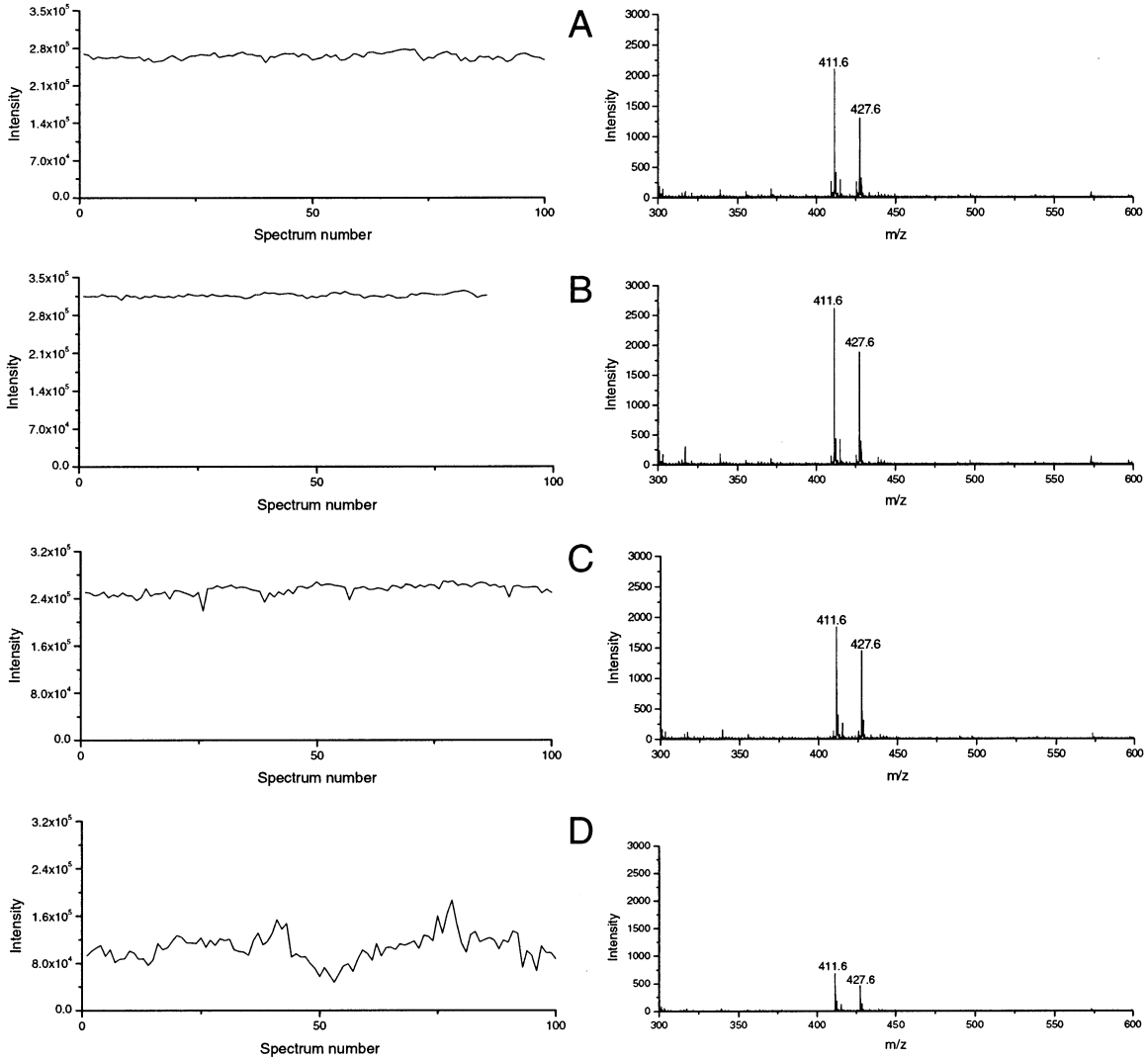

Figure 2. TIC and mass spectra at different electrospray flow rates using 50:50 (v/v) methanol/water with 1% acetic acid, a bare fused-silica capillary 75 cm long, 50 μm i.d., 360 μm o.d.; an ESI voltage of 3800 V; and an analyte concentration of 2 μg/mL: (A) 500, (B) 300, (C) 200, and (D) 80 nL/min.

RESULTS AND DISCUSSION

An ideal electrospray emitter for sheathless CE/ESI-MS should be stable and rugged. Recent studies in the literature show that even if the capillary tip is shaped mechanically, there is not a big difference in terms of electrospray performance in comparison with an unmodified capillary tip.[13] As a capillary tip's outlet diameter decreases, the electric field at the tip increases. Enhancement of the electric field at the capillary tip can also occur by increasing the applied potential or decreasing the distance between the MS nozzle and the end of the capillary tip. We did not modify the capillary tip mechanically before coating in our experiments.

To evaluate the possible risk of contamination or degradation byproducts from the material used to coat the capillary tip, we compared the mass spectrum of a blank solution of 50:50 methanol/water (v/v) with 1% acetic acid when using the colloidal graphite-coated emitter with those obtained when using a stainless steel electrospray needle (data not shown). There is no significant difference in spectra between the two tips.

The total ion chromatograms (TIC) and mass spectra obtained from direct infusion at different flow rates of risperidone and 9-hydroxyrisperidone in 50:50 water/methanol with 1% acetic acid using a 50 μm i.d. $\times$ 360 μm o.d. capillary are shown in Figure 2. When the flow rate was decreased from 500 to 80 nL/min, the TIC baseline became noisy, and an apparent decrease of mass signal was found. The total ion counts at a flow rate of 500 nL/min are a little lower than that at 300 nL/min. This may result from a shift toward larger diameters in the droplet size distribution, thus leading to lower ionization efficiency. Similar results were obtained for a 20-μm-i.d. capillary.

Figure 3 shows the influence of applied voltage at the capillary tip on the MS signal. During electrospray, there is a loss of the mass signals of the two analytes at a voltage of 5 kV, possibly because of the corona discharge. The discharge gives rise to instability of the static Taylor cone (depicted as a triangle exiting the tip of the sprayer) and degrades the electrospray performance. The spectrum represents the analyte ion molecule reactions. At the same time, many ions present in solution can no longer be observed. The mass signals produce a noisy TIC baseline when the voltage is down to 4.5 kV. The optimum voltage is observed at 4.0 kV where high mass signal intensity and a stable baseline

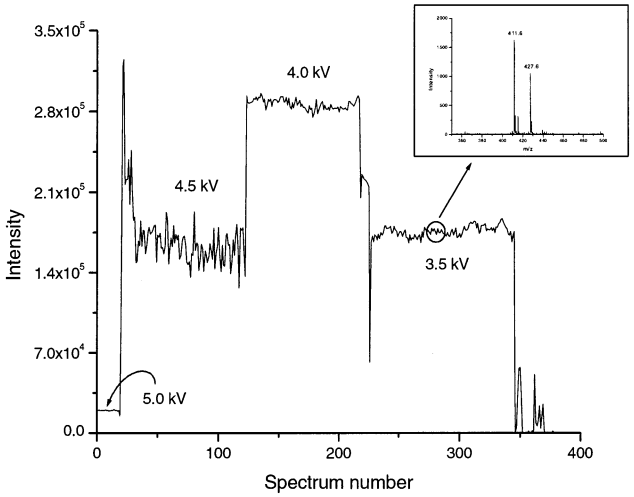

Figure 3. Effect of ESI voltage on spray performance, using 50:50 (v/v) methanol/water with 1% acetic acid; a bare fused-silica capillary 75 cm long, 50 μm i.d., 360 μm o.d., a flow rate at 200 nL/min and an analyte concentration of 2 μg/mL.

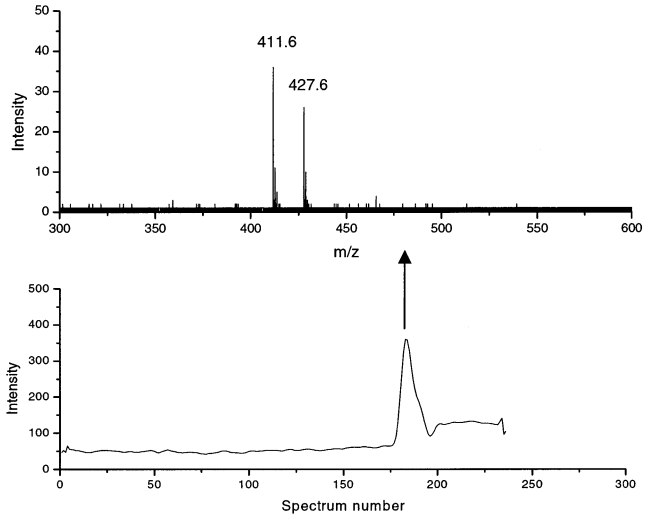

Figure 4. SIC and mass spectrum of CE/ESI-TOFMS separation of risperidone ($[M + H]^+ = m/z$ 411.6) and 9-OH risperidone ($[M + H]^+ = m/z$ 427.6) using the 75-cm-long bare fused-silica capillary with 50 μm i.d., 360 μm o.d. 10 mM ammonium acetate in 20% methanol/water solution as running buffer, pH 9.0; the concentration of each analyte is 10 μg/mL; CE separation voltage 25 kV, current 5.5 $\pm$ 0.1 μA; ESI voltage 3250 V. About 120−130 fmol of each analyte is injected.

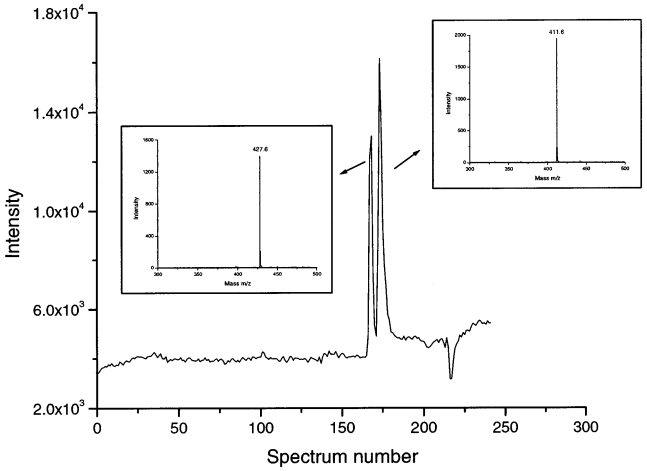

Figure 5. SIC and mass spectrum of CE/ESI-TOFMS separation of risperidone ($[M + H]^+ = m/z$ 411.6) and 9-OH risperidone ($[M + H]^+ = m/z$ 427.6) using the 75-cm-long bare fused-silica capillary with 50 μm i.d., 360 μm o.d. 10 mM ammonium acetate in 20% methanol/water solution as running buffer, pH 6.7; the concentration of each analyte is 10 μg/mL; CE separation voltage 25 kV, current 5.2 $\pm$ 0.2 μA; ESI voltage 3250 V. About 120−130 fmol of each analyte is injected.

are indicated for a 50-μm-i.d. capillary at the flow rate of 200 nL/min. A minimum ESI voltage of 3.0 kV is required in order to maintain the spray at the capillary tip. The electrospray performance is also tested after a 10-h spray by repeatedly switching the ESI voltage on and off. A stable spray remains without any change in signal intensity (data not shown).

In the event of a loss of electric contact and spray performance, the electrospray can be restored by again applying the same coating on the capillary tip. In contrast to methods that cure at high temperature,[13−15] the mild conditions used to cure this graphite-coated emitter will be beneficial to any coating material on the inner wall of the capillary that might spoil in the process of curing at high temperatures. Unlike silver and gold, graphite is not easily oxidized in the atmosphere. These advantages make this method outstanding compared to other techniques for the fabrication of sheathless ESI emitters. Moreover, we have demonstrated that the graphite emitter can maintain a stable electrospray for over 200 h in positive mode.

A bare fused-silica capillary is used for the separation of risperidone and 9-hydroxyrisperidone. Since the pK_a values of the two analytes are ~8.0, the two analytes are slightly charged at pH 9.0. As shown in Figure 4, the mass signals at m/z 411.6 and 427.6 represent the molecular ion $[M + H]^+$ of risperidone and 9-hydroxyrisperidone, respectively. While a buffer with pH 9.0 can generate sufficient EOF (~150 nL/min) for a stable electrospray, it is not suitable for CE separation because the separation depends mainly on EOF at this pH, as shown by the single peak in Figure 4. A lower pH buffer is needed for optimum separation of the two analytes; however, the EOF produced fails to provide a sufficient flow rate for electrospray, even at pH 6.7. A minimum flow rate through the capillary is required in order to maintain a stable electrospray in sheathless CE/ESI-MS, because a low flow rate will result in no spray. To reach a compromise between CE separation and a flow rate for electrospray, pressure-assisted CE is employed. In this case, 50 mbar of pressure, which can enhance the flow rate by ~60 nL/min as calculated with a 75-cm-long, 50-μm-i.d. capillary, is applied to compensate for the low flow rate used during the separation when the pH of the running buffer is 6.7. A satisfactory selected ion chromatogram (SIC) and two mass spectra are shown in Figure 5. Risperidone migrates after 9-hydroxyrisperidone, as judged from the m/z value. The efficiency of electrospray ionization at different pHs in CE/ESI-TOFMS for the two analytes is remarkable (see Figure 4 and Figure 5). The number of the positively charged species of the two analytes at pH 6.7 is much higher than that at pH 9.0, according to the pK_a values of the two analytes. Therefore, the signal intensity of m/z for both analytes at pH 6.7 is much higher (~50 times) than that at pH 9.0.

CONCLUSIONS

Colloidal graphite is introduced as a coating material for electrospray emitters used in sheathless CE/ESI-TOFMS. Fabrication of the emitter is simple, fast, and inexpensive and can be performed at room temperature. In comparison with other curing methods that require high temperatures, these mild conditions will be beneficial to capillary inner wall coatings, which may spoil during high-temperature curing if an internal coating capillary is employed. The emitter has a long lifetime and stable electrospray. Its lifetime may be extended by simply coating the capillary tip once more with colloidal graphite. The advantages of the emitter shown here make it a promising approach for sheathless interface of CE/ESI-MS. It has been proven that the sheathless CE/ESI-TOFMS described in this paper is reliable for analysis. This technique can also be applied to sheathless interface for CEC/MS, chip/MS, and capillary LC/MS. For practical use, 50-μm-i.d. capillaries are suggested, especially for packed CEC/MS.

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