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Short Communication

Capillary electrophoresis with amperometric detection using a porous cellulose acetate joint

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ABSTRACT

A system for coupling amperometric detection with capillary electrophoresis (CE) is described. The interface is formed by fracturing the capillary followed by covering the fracture with a thin layer of cellulose acetate (CA) film. The porous CA joint enables off-column amperometric detection to be performed without adverse effects from the high electrophoretic voltage. The primary advantage of this CA joint is its simple construction. The performance of the CE–amperometric detection system was evaluated with hydroquinone. A detection limit of $8 \cdot 10^{-8}$ *M* or 0.17 fmol with a theoretical plate number exceeding 90 000 was obtained. Application of the system to the separation and detection of aminophenols, catecholamines and catechol was also demonstrated.

INTRODUCTION

In the past few years, capillary electrophoresis (CE) has been shown to be a fast, powerful and efficient analytical separation technique [1]. One of the major areas of research is the development of sensitive detection systems. Owing to the small capillary dimensions encountered and the minuscule sample zone generated in CE, on-column detection methods, such as UV absorption and fluorescence detection, are the most commonly used methods. Recent advances in CE have allowed the use of other non-optical detection techniques, including electrochemistry. CE with electrochemical detection has recently been reviewed by Curry *et al.* [2] and Yik and Li [3].

Among the various electrochemical detection schemes employed in CE, amperometric detection with a microelectrode is currently one of the most sensitive detection modes. However, amperometric detection is not as easily applied to CE as the UV or fluorescence methods. This is because the electric current produced in the separation capillary upon application of a high voltage (10–30 kV) can be several orders of magnitude greater than the electrochemical currents measured at the detector. Therefore, if the detector is not isolated from the high electric field, small fluctuations in the electrophoretic current can create very high noise levels. Several methods have been developed to circumvent this problem, which include the use of a porous glass tube [4], a porous graphite tube [5], or a Nafion

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joint [6] to couple two pieces of capillary column. The joint allows electrical connections to be made to the capillary so that the first segment of the capillary may be used for electrophoretic separation while the second segment may be used for off-column amperometric detection.

We have recently reported an alternative method for constructing a porous joint for CE [7]. The joint is formed by fracturing the capillary followed by covering the fracture with a thin layer of cellulose acetate (CA) film. Owing to its size-exclusion properties, the CA film creates a diffusion barrier which allows only small buffer ions to pass through it. Larger analyte molecules are excluded from permeating through the pores. This conductive joint has been shown to possess the advantages of high performance, long durability, inexpensiveness and easy construction. In this paper, the applicability of the porous CA joint to the amperometric detection in CE is demonstrated.

EXPERIMENTAL

Electrophoresis apparatus

A high-voltage power supply (0–40 kV, Glassman, Whitehouse Station, NJ, USA; Model PS/EH40R02.5) was used to provide the separation voltage. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D., 360 μm O.D. and 50 cm length were used in this study.

Construction of the CA joint was similar to that described previously [7], except that the joint assembly was horizontally mounted in a plastic buffer reservoir. Briefly, a short section (*ca.* 3 mm) of polyimide coating was burned off 2.5 cm from the end of a capillary. The exposed section was cleaned with methanol and then glued to a 5 cm × 2 cm microscope slide. Using a glass-fibre cleaver, a small scratch was made on top of the uncoated silica. The capillary was then pushed up gently from below, directly under the scratch, until a fracture was produced. A small drop (*ca.* 3 μl) of 12% (w/v) CA solution (in acetone) was carefully dripped onto the fracture. Under a gentle stream of air, a thin film of CA was uniformly coated over the fracture region. The film thickness was *ca.* 80 μm, as estimated under a microscope. Subsequently, the fracture assembly was horizontally glued into a 10-ml plastic buffer reservoir, leaving 2 cm of detection capillary sticking out of the vial. A schematic diagram of the CA joint and the detector assembly is shown in Fig. 1.

Amperometric detection

The electrochemical detector was similar to that described by Knecht *et al.* [8]. Amperometric detection was performed in a two-electrode mode. A 9-μm-diameter carbon fibre (SIGRI, Meitingen, Germany) was used as the working electrode and a silver/silver chloride electrode was used as the reference electrode. The whole detector assembly was enclosed in a grounded Faraday cage to reduce noise.

Potential control between the working and reference electrodes was accomplished using a polarographic analyser (EG & G Princeton Applied Research, Princeton, NJ, USA; Model 264A). A picoammeter (Keithley Instruments, Cleveland, OH, USA; Model 485) was connected as a preamplifier at the working electrode input. Both the reference and auxiliary leads were connected to the silver/silver chloride electrode. The voltage output from the polarographic analyser was passed through a 1-s *RC* low-pass

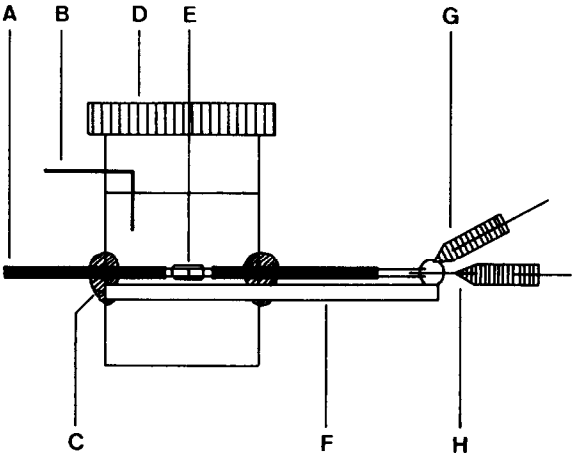

Fig. 1. Schematic diagram of CA joint and amperometric detector assembly. (A) Fused-silica capillary; (B) platinum-wire electrode; (C) epoxy glue; (D) buffer reservoir; (E) CA joint; (F) microscope slide; (G) Ag/AgCl reference electrode; (H) carbon fibre electrode.

filter and recorded on a strip-chart recorder (Pantos, Tokyo, Japan; Model U-228).

Prior to each analysis, the carbon fibre electrode was electrochemically pretreated by repetitive scanning the potential from 0.0 to +1.2 V *versus* silver/silver chloride reference at 0.1 V/s for 2 min while simultaneously flowing operating buffer past the detector. This pretreatment ensured constant electrode sensitivity.

Reagents

2-Morpholinoethane sulphonic acid (MES) buffer, hydroquinone, 2-, 3- and 4-aminophenol were obtained from Merck (Darmstadt, Germany). Dopamine, epinephrine and catechol were purchased from Sigma (St. Louis, MO, USA). All solutions were prepared in distilled, deionized water, and were filtered (0.2 μm) before use.

RESULTS AND DISCUSSION

The performance of the CE–amperometric detection system with a porous CA joint was tested with hydroquinone. Fig. 2 shows the electropherogram of $5 \cdot 10^{-7}$ *M* hydroquinone. The high efficiency achievable with this system is apparent, with the number of theoretical plates calculated from the peak half-width for hydroquinone exceeding 90 000. Linear regression analysis for concentrations ranging from $5 \cdot 10^{-7}$ *M* to $2 \cdot 10^{-4}$ *M* provided a calibration graph with a correlation coefficient of 0.997 ($n = 8$). From the electropherogram shown in Fig. 2, the detection limit ($S/N = 3$) for hydroquinone was determined to be $8 \cdot 10^{-8}$ *M*. With an injection volume of 2.1 nl (based on electroosmotic flow), the detection limit corresponds to 0.17 fmol. Relative standard deviations for the reproducibility of the migration time and the peak current for hydroquinone were 1.1% and 1.8%, respectively ($n = 6$).

Fig. 3 shows the separation and detection of three aminophenol isomers, *viz.* 4-, 3- and 2-aminophenol and hydroquinone. This was obtained in a 20 m*M* MES buffer with a pH of 5.7. At this pH, hydroquinone behaves as a neutral

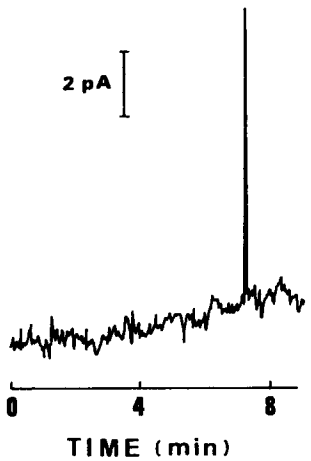

Fig. 2. Electropherogram of $5 \cdot 10^{-7}$ *M* hydroquinone. Column, 50 cm total length × 50 μm I.D. × 360 μm O.D.; 20 m*M* MES buffer (pH 4.0); separation voltage, 200 V/cm (4.0 μA); sample injection, 1 s at 10 kV; detection potential, 0.9 V *vs.* Ag/AgCl reference.

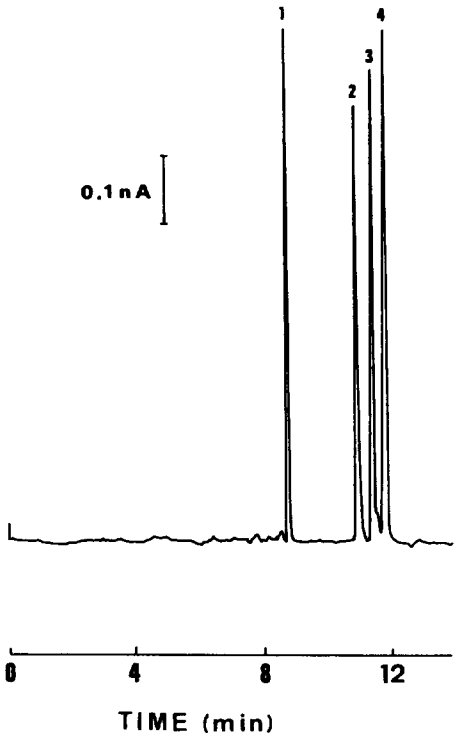

Fig. 3. Electropherogram of (1) 4-aminophenol (10 μM), (2) 3-aminophenol (12 μM), (3) 2-aminophenol (12 μM) and (4) hydroquinone (15 μM) with amperometric detection. Column, 60 cm total length; 20 m*M* MES buffer (pH 5.7); separation voltage, 250 V/cm (2.3 μA); other conditions as in Fig. 2.

marker and all three aminophenols are cations. The high efficiency obtained with this system is evident from the average number of theoretical plates, which exceeds 100 000 based on the half-width. Detection limits for the three isomers were all in the lower fmol range.

Application of this system to the separation and detection of catecholamines and catechol is shown in Fig. 4. The sample mixture contained dopamine (85 μM), epinephrine (82 μM) and catechol (27 μM). Injected amounts ranged between 170 fmol for dopamine and 54 fmol for catechol, in an injection volume of 2.0 nl. Owing to their structural proximity and similar net charge at the buffer pH (6.0), complete separation of dopamine and epinephrine was not achieved. It has been shown that a high concentration (*e.g.* 0.1 M) of borate is crucial in the CE separation of some biologically active molecules differing only by a single hydroxyl group with no change in net charge, including several catecholamines [9]. However, borate was found not to be an ideal electrolyte for amperometric detection in CE because it generated higher electrophoretic currents, which increase the detector noise. This has also been reported by Wallingford and Ewing [4] and O'Shea *et al.* [6]. In the present work, MES buffer, which has a higher resistance, was used. The high efficiency obtained with this system is evident in the catechol peak, which exhibits approximately 47 000 theoretical plates based on the half-width. The rather pronounced peak tailing found in Fig. 4 is thought to be due to electrostatic interactions between analyte and capillary wall [10]. The detection limits for dopamine, epinephrine and catechol were determined to be 12, 14 and 6 fmol, respectively.

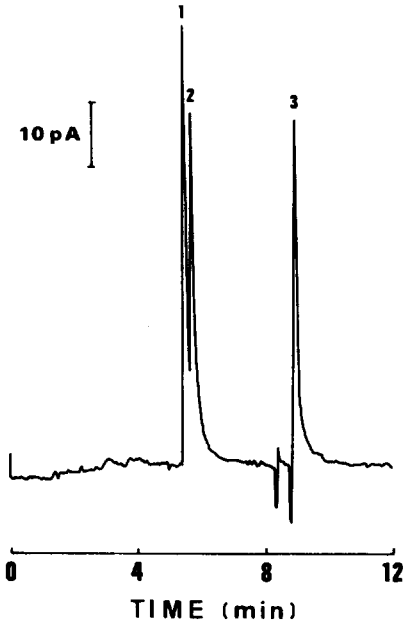

Fig. 4. Electropherogram of catecholamines and catechol with amperometric detection. 20 mM MES buffer (pH 6.0); detection potential, 0.75 V *vs.* Ag/AgCl; other conditions as in Fig. 2. Peak identities: 1 = dopamine (85 μM); 2 = epinephrine (82 μM); 3 = catechol (27 μM).

CONCLUSIONS

From the above studies we have demonstrated the feasibility of using the porous CA joint for amperometric detection in CE. In comparison with other conductive joints (*e.g.* porous glass tube, porous graphite tube or Nafion joint), the primary advantage of the CA joint is its simple design and construction. In addition, the CA joint is expected to have minimal band-broadening effect. According to other workers [4–6], the conductive joints were always constructed by threading a small section of porous glass, graphite or Nafion tube over the capillary fracture, followed by sealing both ends of the tube with epoxy glue. The inner diameter of the porous tube must be slightly larger than the outer diameter of the fused-silica capillary; therefore, a small dead volume will form around the fracture region. On the other hand, the CA membrane is tightly adhesive to the fracture region in a porous CA joint, leaving no stagnant zone formed between capillary surface and membrane. This "zero dead volume" nature partially contributes to its high performance. Since the main obstacle to the wide use of amperometric detection for CE lies in the difficulties of preparing a reliable conductive joint, we hope that the CA joint can provide an easy way for the researchers who are interested in CE–amperometric detection.

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