



Evaluation of a sol–gel derived carbon composite electrode as an amperometric detector for capillary electrophoresis

Xiuhua Sun, Xiurong Yang¹, Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Renmin Street 159, Changchun 130022, Jilin, China

Received 20 September 2002; received in revised form 3 January 2003; accepted 14 January 2003

Abstract

In this paper, we report the construction and application of a sol–gel derived carbon composite electrode (CCE) as an amperometric detector for capillary electrophoresis. The electrochemical properties were characterized and compared with those of conventional carbon fiber and carbon paste electrode (CPE). Experimental results show that peak-to-peak noise of CCE was about 20% of CPE and electrode capacitance was comparatively low. When applied to the detection of dopamine and epinephrine, the optimal detection potential for CCE was 0.1 V lower than CPE under the same separation conditions; CCE with diameter of 75 and 100 μm could achieve a low detection limit of $3 \cdot 10^{-8}$ and $6 \cdot 10^{-8}$ M for the detection of epinephrine, which approaching that of the 33- μm diameter carbon fiber electrode. Also, the linearity for epinephrine at CCE was more than two orders of magnitude, which was slightly wider than that of carbon fiber electrode. Applications to real sample analysis were tested by the determination of betahistine dihydrochloride in tablets and human urine. Using CCE with diameter ≤ 100 μm as an amperometric detector after capillary electrophoresis separation, a low detection limit and a wide linear range combined with excellent reproducibility were obtained. This CCE possesses of many advantages, namely, convenience, ease of fabrication, low cost and high stability.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Electrodes; Carbon composite electrodes; Sol–gel; Electrochemical detection; Detection, electrophoresis; Dopamine; Epinephrine; Betahistine dihydrochloride

1. Introduction

The coupling of capillary electrophoresis (CE) with electrochemical detection offers a highly sensitive and selective tool for the determination of a wide variety of electroactive compounds [1,2]. The successful application of an electrochemical detection system is strongly dependent on the choice of

the working electrode materials, such as carbon [3–5], platinum [6,7], gold [8,9], nickel [10,11], copper [12] and chemically modified electrode [13,14]. However, selection of most electrode materials is still being focused on carbon-based materials owing to their low background, low cost, high stability and resistance to passivation. Carbon-based electrodes have typically served as working electrodes for the detection of oxidizable species in the anodic region. A variety of carbon electrode materials have been developed for CE, with carbon fiber electrode (CFE) and carbon paste electrode (CPE) being most popu-

*Corresponding author. Fax: +86-431-568-9711.

E-mail address: ekwang@ns.ciac.jl.cn (E. Wang).

¹Co-corresponding author.

lar [3–5]. Nevertheless, the application of novel carbon-based electrode materials still remains of interest. Sol–gel carbon composites represent a new class of carbon-based electrode, that are composed of graphite powder homogeneously dispersed in modified silica ceramics [15].

Sol–gel derived carbon composite electrode (CCE) have been reported to possess various desirable properties, e.g. wide operating potential, low and stable background current, which are useful for electrochemical applications [16,17]. Sol–gel carbon materials, thus, appear to be a viable alternative to other carbon-based working electrodes used in liquid chromatography [18]. In comparison, application of CPE in a flowing system is not commonly applied due to the poor baseline produced by the electrode. In this paper, we describe the performance of a wall-jet amperometric detector employing a sol–gel derived carbon composite working electrode for CE system. To our knowledge, a detailed characterization study has never been reported for CCE as an amperometric detector in CE. Though the coupling of CCE with CE has been reported by Tan's group [19,20], a study on the application of such a CCE in a CE system was just confined to the use of a large diameter of several hundred micrometers as a working electrode. Since electrodes with large diameters generated large background noise, which resulted in a poor S/N , it was difficult to achieve a high separation efficiency and low detection limit [21,22]. Moreover, no information was available about the effect of different electrode surface areas on electrochemical properties and analytical performance. To further develop the CCE as an amperometric detector for CE, three sizes of CCE—75, 100 and 250 μm in diameter—were constructed. The electrode capacitance was determined by running a cyclic voltammogram without a redox couple calculated by equation $C = i/VA$, where C represents the observed capacitance ($\mu\text{F}/\text{mm}^2$), i represents the average of the absolute cathode and anode current at certain potential (vs. Ag/AgCl , in A), V represents the scan rate (V/s) and A represents the electrode area (mm^2). Also, peak-to-peak noise together with average current for different electrode materials of CCE and CPE was measured by switching the high voltage on and off. The analytical performance of CCE in terms of the limit of detection, linearity, etc, was evaluated

by separation and detection of a test mixture of dopamine (DA) and epinephrine (EP). To investigate the applicability to real sample analysis, betahistine dihydrochloride (BH), a commonly used anti-histamine drug, was determined in human urine and tablets. The analytical results show that a wide linear range and high sensitivity was obtained; no matrix components made an obvious effects on the detection for CCE as well as CFE. In conclusion, as for end-column amperometric detection mode based on wall-jet configuration using 25 μm I.D. capillary, CCE with a diameter of $\leq 100 \mu\text{m}$ exhibited high sensitivity, wider linearity and excellent reproducibility.

2. Materials and methods

2.1. Apparatus

Electrophoresis was driven by a +30 kV high-voltage power supply (9323-HVPS, Beijing, China). Amperometric detection was performed on a CHI 832 electrochemical analyzer (CH Instruments, USA) connected with a detection cell based on a wall-jet configuration. To minimize the interference of external electric noise, the electrochemical cell was housed in a laboratory-made Faraday cage, and the outlet of the capillary was always maintained grounded through a stainless steel tube. The reference electrode was a Ag/AgCl wire, and the auxiliary electrode was a Pt wire. Experiments were carried out using a fused-silica capillary of 25 μm I.D. \times 360 μm O.D. (Hebei, China), cut to 32 cm in length. New capillaries were rinsed with 0.1 M NaOH overnight.

2.2. Reagents and solutions

Methyltrimethoxysilane (MTMOS) and graphite powder (1–2 μm diameter) were obtained from Aldrich. Carbon fiber electrode was constructed using PAN-based (polyacrylonitrile) carbon fiber (33 μm diameter) (Avco Speciality Products, Lowell, MA, USA). L-Epinephrine and L-dopamine were

from Fluka. Betahistine dihydrochloride was purchased from Sigma (St. Louis, MO, USA). Tablets of betahistine dihydrochloride were purchased from a local drug store. Human urine was obtained from a healthy volunteer. All the reagents were used as received without further purification. Stock solutions of 100 mM Na₂HPO₄/NaH₂PO₄ solution were used as buffer. All water used was doubly distilled and all solutions were filtered through 0.22- μ m membranes before injection into the capillary.

2.3. Electrode preparation

CCE was made as described previously [20]. Sol-gel solution was prepared by mixing 70 μ l of MTMOS, 2 μ l of 2 M HCl and 60 μ l of double-distilled water. The above mixture was sonicated for 2 min to ensure uniform mixing. Subsequently, 0.1 g of graphite powder was mixed with the above sol-gel solution to form a homogeneous carbon-sol-gel paste. A section of 75 μ m I.D. \times 360 μ m O.D. fused-silica capillary was used to construct the CCE. Electrical contact was established via a Cu wire, which was threaded into one end of the capillary and fixed by epoxy with the other end of capillary hollow. After curing overnight, the hollow end of capillary with a length of 5 mm was polished on the abrasive paper until its tip was flat. Then the capillary was sonicated and dried. Finally, a portion of the paste was packed into the capillary. The packed electrode was allowed to gel and dry for 3 days in ambient conditions and polished on 2000-grade abrasive paper with 0.05 μ m α -Al₂O₃ before use.

The construction of CPE was similar to that of CCE. It can be basically described as follows: 0.7 g graphite powders were mixed with 0.3 g paraffin oil and filled into a 75- μ m I.D. fused-silica capillary. Following the procedure described above, CCE and CPE with 75, 100 and 250 μ m diameter were made. Carbon fiber electrode was prepared following the procedures described previously [23]. Briefly, a 33- μ m diameter carbon fiber which was sealed in a 75- μ m I.D. capillary connected to a copper wire through carbon powder in a glass tube. Before use, electrode was polished on 2000-grade abrasive paper.

3. Results and discussion

3.1. Characterization of electrochemical behavior

3.1.1. Characterization of electrode capacitance

Electrode capacitance is a crucial factor for electrode characterization in electrochemical experiment related to the capacitive current. The rough electrode capacitance was calculated by equation $C = i/VA$, C represents the observed capacitance (μ F/mm²), i represents the average of the absolute cathode and anode current at 300 mV (vs. Ag/AgCl, in A), V represents the scan rate (V/s) and A represents the electrode area (mm²). By measuring the charge current at a given scan rate, the capacitance of the electrode was determined. The CCE, CFE and CPE made according to the procedures described above were cycled in 1 M KCl solution with a scan rate of 70 mV/s. The calculated capacitance of CCE shown in Table 1 is higher than that of CFE and CPE due to the porous structure of the composite sol-gel carbon electrode. Because the surface area of CCE was much larger than the inner surface of the capillary, the actual capacitance of CCE should be much smaller than the calculated value. It is noted that the excellent stability was demonstrated from the current response in cyclic voltammograms at varied time. However, for CPE, high capacitance current and irreversible electrochemical behavior were exhibited from the cyclic voltammograms after 24 h immersion in solution compared to the freshly prepared electrode, since the paste usually leached from the capillary.

3.1.2. Effect of electrode area on peak-to-peak noise and average current

Peak-to-peak noise was usually as a parameter to investigate the effect of separation voltage on de-

Table 1
Capacitance of three types of electrodes^a

Electrode material (diameter/ μ m)	Electrode capacitance (μ F/mm ²)
CCE (75 μ m)	4.83
CPE (75 μ m)	0.52
CFE (33 μ m)	1.61

^a Electrode was cycled in 1 M KCl solution from 0.35 to 0 V with a scan rate of 70 mV/s.

tection. Since the separation field can change the interfacial potential at the working electrode and, consequently, alter the background current observed [21]. In our work, a 15 kV high voltage was exerted on the separation capillary with the cathode end grounded by a stainless steel tube, while keeping a distance of 40 μm from the capillary end to the electrode surface throughout the experiment. The effect of the electrode surface area on peak-to-peak noise and average current was investigated by switching the high voltage on and off. The experimental results are listed in Table 2 show that there existed a general increase of peak-to-peak noise and average current with the increase of electrode area for CCE whether the high voltage was on or off. However, a comparison of the value for the grounded system with that obtained when no separation voltage was applied indicated that the separation voltage was not completely isolated from the detection zone since there was a significant increase of the peak-to-peak noise when the separation voltage was on. As for the same size electrode, it should be noted, however, that a much larger background noise was generated at the CPE compared to the same size CCE due to the instability of CPE in a flowing system. Nevertheless, the noise level of the background current for CCE was not as small as the literature values [24]. This may be due to the fact that the actual electrode area was larger than the inner diameter of the capillary because of the porous structure of CCE.

3.2. Analytical performance of CCE

3.2.1. Hydrodynamic voltammograms

Because of the carbon composite character of these sol–gel derived electrodes, their performance is compared with that of common carbon paste-based detectors. DA and EP were commonly used in CE coupled with electrochemical detection. The dependence of the anodic current response on the applied potential was assessed by means of hydrodynamic voltammograms (HDVs). Fig. 1 compared the peak current of the two neurotransmitters under varying detection potential. The HDVs curves obtained under CE conditions show that sol–gel carbon composite detector offers a substantial decrease in the potential of the voltammetric curve for the two compounds compared with CPE. The HDVs of EP show that the anodic current rises rapidly from 0.5 V and a limiting current plateau is reached at potentials 0.85 V for CCE, while limiting current of CPE is at a potential of 0.95 V. The HDVs of DA exhibited a similar trend. In summary, the detection potential corresponding to the limiting current for CCE detector was lower than that of CPE, which could be explained as the catalytic action of the silicon oxide component in the sol–gel materials.

3.2.2. Dependence of the electrode area on the separation and detection

The results obtained for the test mixture of DA and EP with three sizes of CCE are illustrated by the

Table 2
Effect of electrode surface area on peak-to-peak noise^a and average current^b

Electrode diameter (μm)	High voltage (15 kV)	Peak-to-peak noise (pA, 4 s)	Average current (nA, 4 s)
75 (CPE)	On	70.5	2.6
	Off	52.8	2.9
75 (CCE)	On	18.6	2.3
	Off	11.9	2.8
100 (CCE)	On	23.6	6.8
	Off	15.1	7.8
250 (CCE)	On	53.2	11.7
	Off	31.9	16.6

The separation voltage was 15 kV, and the detection potential was 1.0 V.

^a Average deviation of baseline current in 4 s.

^b Average of the base line current in 4 s.

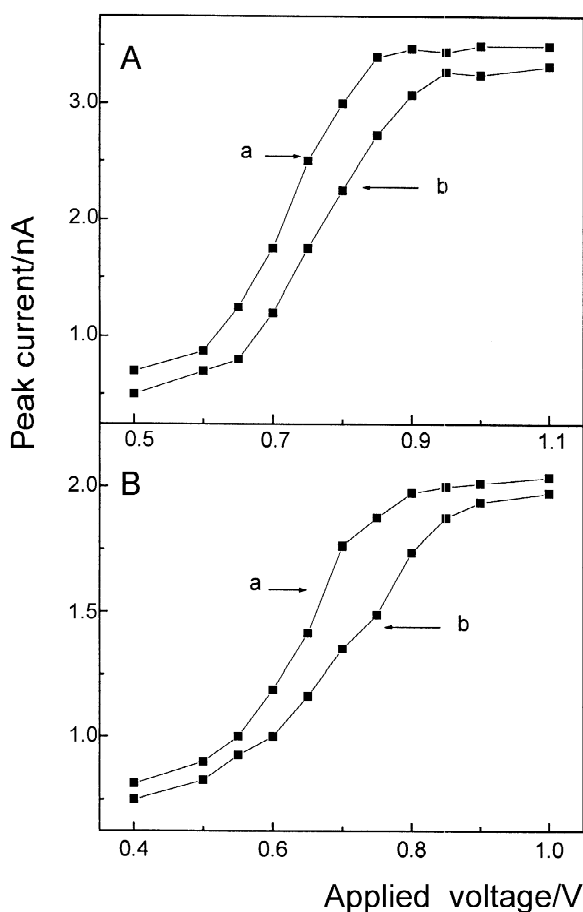


Fig. 1. Hydrodynamic voltammograms of $5 \cdot 10^{-5}$ M DA and EP. Characteristics: 25 mM phosphate buffer, pH 6.5; separation voltage: 15 kV. (A) $5 \cdot 10^{-5}$ M EP; (B) $5 \cdot 10^{-5}$ M DA: a = 75 μ m CCE; b = 75 μ m CPE.

electropherograms in Fig. 2 and summarized quantitatively in Table 3. For CCE, limit of detection at 10^{-8} level and N at 10^4 level approaching that of CFE were acceptable; wide linear range more than two orders of magnitude and excellent reproducibility were much better than CFE. Electropherograms in Fig. 2 show that DA and EP were all separated and well-detected at three sizes CCE. More importantly, the two peaks appear quite sharp and well-shaped, and the efficiency as indicated by a theoretical plate number N estimated to be at least $6.0 \cdot 10^4$ for the 75- μ m diameter CCE was respect-

able. Even for the larger 250- μ m CCE, a perfectly acceptable electropherogram was still observed, with a modest decrease in N because of slightly increased band-broadening due to the larger electrode surface area. The larger electrode was able to electrolyze more of the sample and therefore gave larger currents for the same concentrations. Nevertheless, no improvement in signal-to-noise ratio or detection limit occurred as a result. Because with the increase of electrode diameter, the background noises tended to increase more rapidly than the peak current, hence decreased detection sensitivity was observed. Though the size of the CCE seemed to be larger than the commonly used ultramicroelectrode with diameters of several micrometres [25], the alignment of capillary with this larger diameter CCE can be judged by naked eye, while the entire operation typically required a few minutes to carry out, however, alignment of the 10- μ m diameter was much time consuming, requiring the trial-and-error adjustment of the electrode position. CCE with diameter ≤ 100 μ m was favorable as an amperometric detector as a compromise between background noises and peak current of analyte. In addition to its ability to maintain critical performance characteristics such as detection limit, linear range, and separation efficiency at high levels, the normal size CCE compared with the ultramicroelectrode appeared to offer some significant practical benefits which include the following: easier alignment of electrode and capillary, larger detection current, greater roughness, and, hence, increased reproducibility from one electrode to another and from one CE run to another.

3.2.3. Application to real sample analysis

The CCE was evaluated by the determination of BH in tablets and human urine, which is a drug resembling naturally occurring histamine in some of its effects and is used as the treatment for Muniere's disease. BH tablets were crushed into fine particles; accurately weighed 8 mg of powder were dissolved in 50 ml water, and the solution was filtered and diluted to 200 ml. Diluted human urine (urine–water, 1:4, v/v) was spiked with $5 \cdot 10^{-5}$ M BH. We used CCE as the working electrode to investigate the optimal separation and detection condition and quantitatively determine the content of BH in tablets. In comparison, a parallel experiment utilizing CFE was

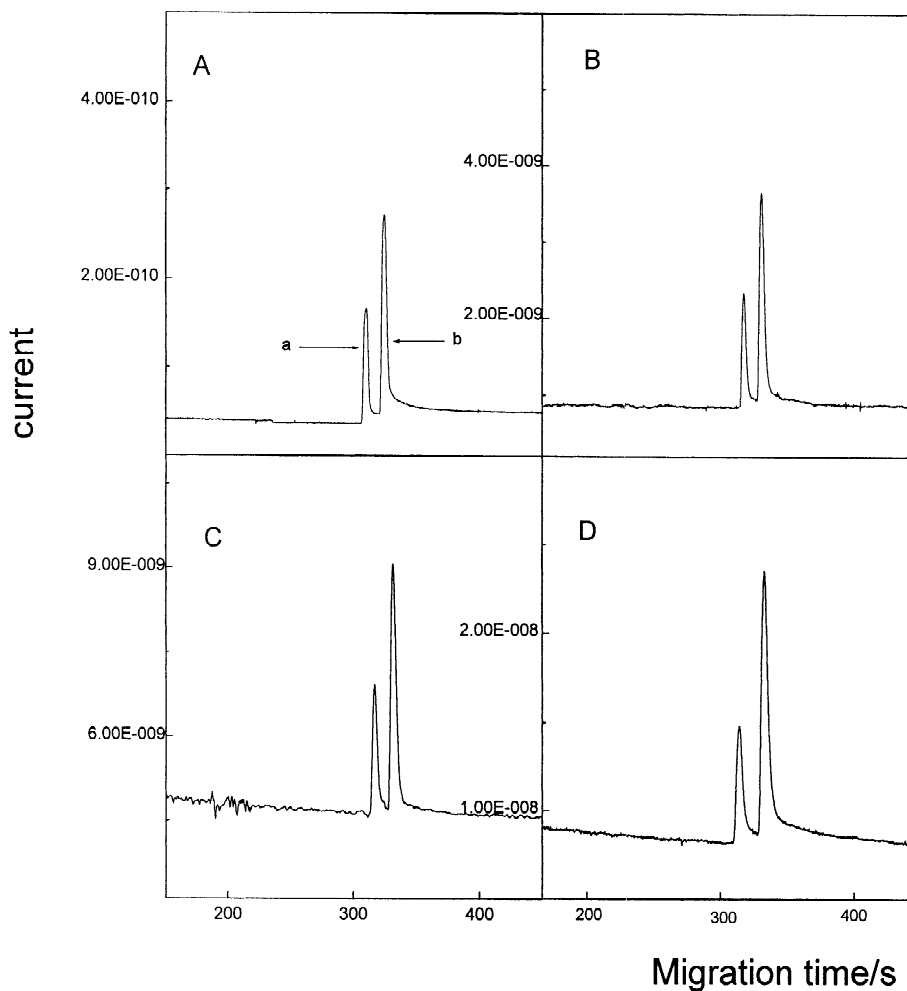


Fig. 2. Effect of the electrode surface area on separation and detection. (A) 33 μm CFE; (B) 75 μm CCE; (C) 100 μm CCE; (D) 250 μm CCE; separation voltage: 15 kV; 25 mM phosphate buffer, pH 6.5; detection potential: 0.85 V; a = $5 \cdot 10^{-5}$ M DA; b = $5 \cdot 10^{-5}$ M EP.

Table 3
Performance characteristics of CCE

	CFE (33 μm)	CCE (75 μm)	CCE (100 μm)	CCE (250 μm)
Detection limit (10^{-8} M)	1.0	3.0	6.0	70
Linear range (M)	$1.2 \cdot 10^{-6} - 4.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-6} - 8.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-6} - 1.0 \cdot 10^{-3}$	$7 \times 10^{-5} - 2.0 \cdot 10^{-3}$
N^a	$1.2 \cdot 10^5$	$6.0 \cdot 10^4$	$5.1 \cdot 10^4$	$4.0 \cdot 10^4$
% RSD ^b	8.5	3.2	2.2	2.0

Sample: EP; separation voltage: 15 kV; 25 mM phosphate buffer, pH 6.5; detection potential: 0.85 V.

^a The number of theoretical plates was calculated by the formula $N = 5.54(t_m/w_{1/2})^2$.

^b Calculated for six replicate injections without any flushing process.

also performed. It was proved that the CCE exhibited a similar trend in response to the changing buffer concentration, pH, etc. An optimized condition of 25 mM phosphate buffer with pH of 8.0, detection potential at 1.20 V was finally used. Electropherograms are shown in Fig. 3, the peak was well-shaped and no interference was observed. The content of BH in tablets was calculated to be 0.25 mg/mg.

3.2.4. Reproducibility

A reactivation process was crucial for obtaining good reproducibility of peak current for CCE as well as for CFE. After each injection, double-distilled water, 0.1 M NaOH, double-distilled water and buffer solution were sequentially injected for 60, 60,

60 and 120 s, respectively, while running cyclic voltammograms from -0.2 to $+1.3$ V, then maintaining the detection potential for 2 min.

Reproducibility in preparation of the electrodes was determined by measuring the current response for a $5 \cdot 10^{-5}$ M solution of BH at 10 individually constructed electrodes, the relative standard deviation of 2.7% was obtained. This reproducibility is better than that CFE with RSD of 4.5% with a parallel experiments. We attribute this RSD to the larger cross-sectional area of the disk-shaped working electrode.

The precision and long-term stability of the CCE was investigated. RSD for the current response of six consecutive injection of $5 \cdot 10^{-5}$ M BH was calculated to be 2.5%. The long-term stability was measured over a consecutive 7-day period and no deterioration was apparent. The CPE exhibited a decrease in sensitivity over the first 2 h of operation. As far as CE with electrochemical detection was concerned, the same CCE was used without any further pretreatment over a day. After a day's operation, the CCE can easily be activated by the cyclic voltammetry from -0.5 to $+1.5$ V to achieve the sensitivity. Also, this electrode surface could easily be renewed by a simple polishing step and could be used for at least 1 month.

In our work for constructing the CCE, we have attempted to pack the carbon ceramic paste into a 50- μ m inner diameter capillary according to the mixing ratio described above, but the paste was a little dry and difficult to pack. Only 30% of electrodes were successfully made. When we change the mixing ratio by reducing the carbon powder content, though the electrode can be made, but the conductivity was unsatisfactory. Hence, it is desirable to construct all kinds of shapes and sizes to meet the practical demands.

4. Conclusion

The results clearly demonstrated that a sol-gel derived carbon composite electrode was a convenient and reliable amperometric detector for capillary electrophoresis. Sol-gel derived carbon electrode displayed favorable electron-transfer kinetics and a wide potential window. It is a physically rigid,

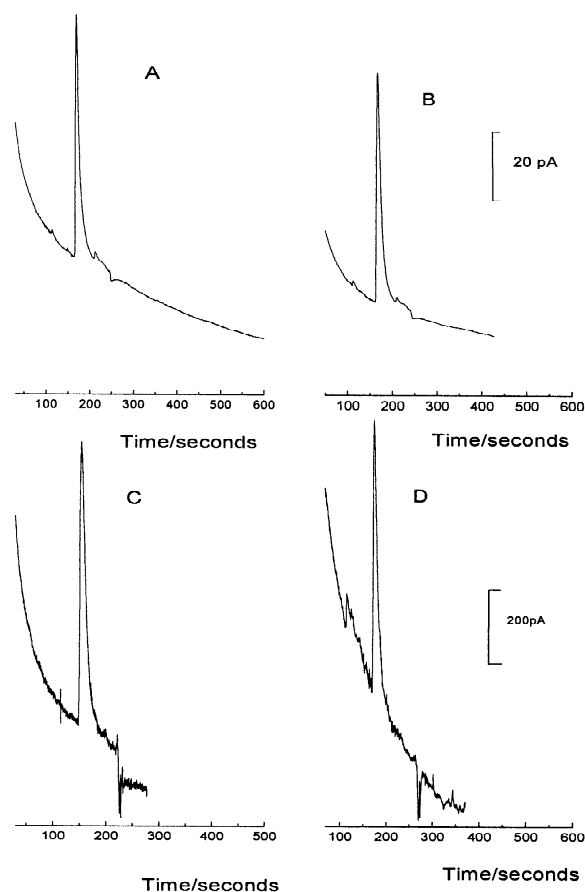


Fig. 3. Determination of BH in tablets (A, C) and spiked human urine (B, D). (A, B) 33 μ m CFE; (C, D) 75 μ m CCE; (B, D) spiked with $5 \cdot 10^{-5}$ M BH; 25 mM phosphate buffer, pH 8.0; separation voltage: 15 kV; detection potential: 1.20 V.

renewable surface, amenable to chemical or biological modification, and stable in various solvents. Such attractive features combined with the versatility of the sol–gel process could offer additional advantages compared with the convention carbon fiber and carbon paste electrode.

Acknowledgements

This work was supported by the National Natural Science Foundation of China with grants Nos. 39990570 and 20299030.

References

- [1] S.C. Beale, *Anal. Chem.* 70 (1998) 279R.
- [2] A.G. Ewing, J.M. Mesaros, P.F. Gavin, *Anal. Chem.* 66 (1994) 527A.
- [3] S. Sloss, A.G. Ewing, *Anal. Chem.* 65 (1993) 577.
- [4] J. Zhou, S.M. Lunte, *Anal. Chem.* 67 (1995) 13.
- [5] X. Huang, W.T. Kok, *J. Chromatogr. A* 716 (1995) 347.
- [6] M.C. Chen, H.J. Huang, *Anal. Chem.* 67 (1995) 4010.
- [7] R.D. Voegel, W. Zhou, R. R Baldwin, *Anal. Chem.* 69 (1997) 951.
- [8] W. Lu, R.M. Cassidy, *Anal. Chem.* 65 (1993) 2878.
- [9] R.L. Weber, S.M. Lunte, *Electrophoresis* 17 (1996) 302.
- [10] A.M. Fermier, M.L. Gostkowski, L.A. Colon, *Anal. Chem.* 68 (1996) 1661.
- [11] M.C. Chen, H.J. Huang, *Anal. Chim. Acta* 341 (1997) 83.
- [12] J. Ye, R.R. Baldwin, *Anal. Chem.* 66 (1994) 2669.
- [13] T.J. O'Shea, S.M. Lunte, *Anal. Chem.* 66 (1994) 307.
- [14] J. Liu, W. Zhou, T. You, F. Li, E. Wang, S. Dong, *Anal. Chem.* 68 (1996) 3350.
- [15] M. Tsionsky, G. Gun, V. Glezer, O. Lev, *Anal. Chem.* 66 (1994) 1747.
- [16] J. Li, L.S. Chia, N.K. Goh, S.N. Tan, *J. Electroanal. Chem.* 460 (1999) 234.
- [17] G. Gun, V. Glezer, O. Lev, *Anal. Chem.* 66 (1994) 1747.
- [18] P.V.A. Pamidi, C. Parrado, S.A. Kane, J. Wang, M.R. Smyth, J. Pingarrón, *Talanta* 44 (1997) 1929.
- [19] L. Hua, S.N. Tan, *Anal. Chim. Acta* 403 (2000) 179.
- [20] L. Hua, S.N. Tan, *Anal. Chem.* 72 (2000) 4821.
- [21] R.A. Wallingford, A.G. Ewing, *Anal. Chem.* 59 (1987) 1762.
- [22] X. Huang, R.N. Zare, *Anal. Chem.* 62 (1990) 443.
- [23] T. You, X. Yang, E. Wang, *Analyst* 123 (1998) 2357.
- [24] W.T. Kok, Y. Sahin, *Anal. Chem.* 65 (1993) 2497.
- [25] W. Lu, R.M. Cassidy, *Anal. Chem.* 65 (1993) 1649.