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Carbon fiber bundle–Au–Hg dual-electrode detection for capillary electrophoresis

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Abstract

The assembly of a dual-electrode amperometric detection system for capillary electrophoresis is developed. The dual-electrode detector consists of different kinds of electrode material (carbon fiber and Au–Hg). The dual-electrode is placed on the outlet end of the separation capillary. Cysteine, glutathione, ascorbic acid and uric acid can be detected simultaneously and selectively at the two electrodes of the dual electrode, respectively. The capillary electrophoresis–dual-electrode detection system has be used to determine these compounds in human blood samples.
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Keywords: Electrochemical detection; Electrodes; Detection, electrophoresis; Instrumentation; Ascorbic acid; Uric acid; Cysteine; Glutathione

1. Introduction

Capillary electrophoresis (CE) has become a useful and powerful separation technique and many applications are currently in use [1–3]. CE has many inherent features of its operation suitable for analysis of biological substances such as extremely small sample size, high separation speed and efficiency and biocompatible environments. Electrochemical detection (ED) has been shown to be one of the most sensitive detection techniques available for use in CE [4]. The recent advances in CE with electrochemical detection have made operations easier and more effective. Different kinds of electrode such as carbon fiber electrodes, metal electrodes (Pt, Au, Au–Hg, Ni and Cu) and chemically modified electrodes have been applied to CE–ED [5]. Dual-electrode detection can expand the applicability of CE–ED [6–13]. Among their work, the same material such as Au–Hg [6,8], carbon fiber [7], Pt [9], Au [8,10–12] and carbon film [13] is used for the two electrodes in dual electrodes. The most common configuration of dual-electrode detection used with CE–ED is the series mode, where analytes pass over each electrode sequentially. Using this configuration, the upstream electrode oxidizes or reduces the analyte, and then the downstream electrode electrochemically detects its product [6–9]. Another configuration is the parallel dual-electrode mode. In this mode, compounds eluting from the capillary pass over both electrodes simultaneously. If the two electrodes are set at different potentials, the ratio of current response obtained at each electrode can be used to

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verify peak identity and purity. Usually, this technique is used in HPLC. Carbon fiber dual electrodes [7], gold dual electrodes consisted of a disk electrode and an integrated on-capillary electrode [10], gold microband array electrodes [11], gold [12] and carbon film [13] interdigitated array microelectrodes in the dual mode with the same material used in CE were described, which can enhance the current of electrochemical reversible species by redox cycling.

The electrochemical response varies depended upon the combination of the analytes and electrode materials. For example, the electrochemical response of glutathione (GSH) is sensitive at the mercury electrode, but not at the carbon electrode. Ascorbic acid (AA) and uric acid (UA) can be oxidized at the carbon electrode, but not at the mercury electrode. Therefore, they cannot be detected in one run in CE. In order to determine these compounds simultaneously in CE, the dual electrode constructed with different materials should be used. In this work, we developed the carbon fiber bundle–Au–Hg dual-electrode amperometric detection for CE. In this method, the parallel mode was used to measure GSH, cysteine (Cys), AA and UA simultaneously. They were determined at both electrodes of the dual electrode, respectively. The method can be applied to the determination of Cys, GSH and UA in human blood.

2. Experimental

2.1. Apparatus

The capillary zone electrophoresis (CZE) system used in this work was similar to our previous description [14]. Briefly, a reversible high-voltage power supply (Model 9323-HVPS, Beijing Institute of New Technology, Beijing, China) provided a variable voltage of 0–30 kV across the capillary, with the outlet of the capillary at ground potential. Fused-silica capillaries (25 μm I.D.×375 μm O.D.), from Yongnian Optical Conductive Fiber Plant (Yongnian, China) were cut into a length of 50 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 20 kV. ED at two different constant potentials was performed with an electrochemical analyzer (Model CHI800, CH Instruments, Austin, TX, USA) and a voltammetric analyzer (Model JF-01, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, Jinan, China). The detection cell and detector were housed in a Faraday cage in order to minimize the interference from noise of external sources. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber bundle–Au–Hg dual-disk electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a coiled Pt wire (0.5 mm diameter, 4 cm in length) placed at the bottom of the cell as the auxiliary electrode. The Pt wire also served as the ground for the high potential drop across the capillary. The arrangement of the electrochemical detection cell was illustrated in detail in Ref. [14].

2.2. Construction of carbon fiber bundle–Au–Hg dual electrode

First, the Au–Hg electrode was constructed using a 100-μm diameter, ca. 3-cm long gold wire. The one end of the gold wire and a 375-μm diameter, ca. 5-cm long copper lead were bound together with a fine copper wire. Epoxy resin was then applied to the junction of the gold wire and the copper lead in order to isolate and protect the electrical junction (Fig. 1A). The gold wire was washed with acetone and water. After drying, the gold wire was coated epoxy resin and blown with hot wind immediately. The surplus epoxy resin can be blown out and the film of epoxy resin was formed on the gold surface. After drying, this process was repeated again. The thickness of the film was about 4 μm. The gold electrode was stored for use. Then about 50 carbon fibers soaking up acetone with 6 μm diameter were carefully inserted into a fused-silica capillary (ca. 250 μm I.D.×375 μm O.D., 0.6 cm long) (Fig. 1B). The gold electrode was inserted into the fused-silica capillary with carbon fibers (Fig. 1C). The gold wire and the carbon fibers were glued to the fused-silica capillary using a low viscosity ethyl α-cyanoacrylate adhesive. Next, the fused-silica capillary with the gold wire and the carbon fibers was inserted into a

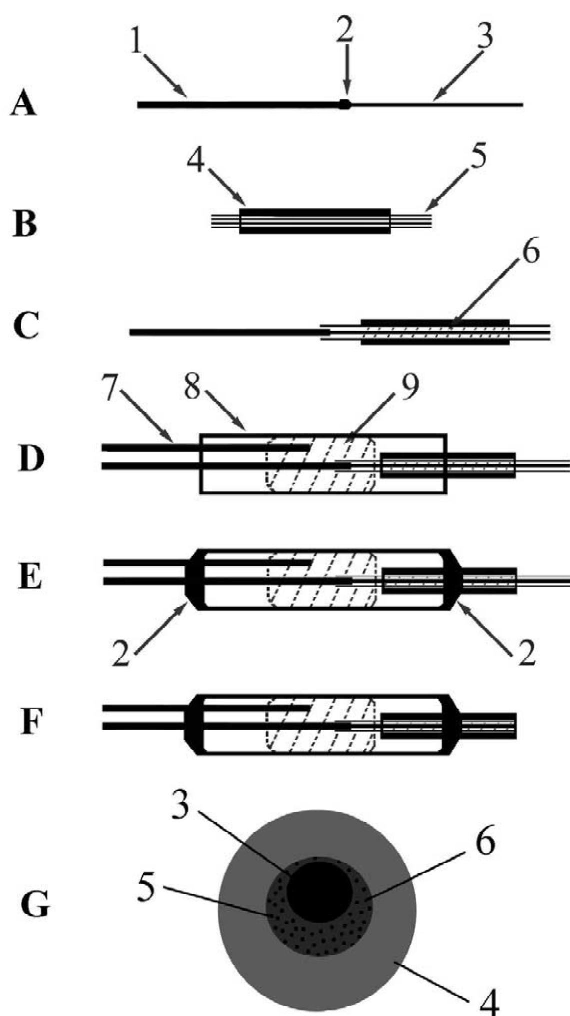

Fig. 1. Manufacturing process of the carbon fiber bundle–Au–Hg dual electrode. (1) Copper lead; (2) epoxy resin; (3) Au–Hg; (4) fused-silica capillary; (5) carbon fiber; (6) ethyl α-cyanoacrylate adhesive; (7) copper lead; (8) glass capillary; (9) mercury.

glass capillary (ca. 2 mm I.D.×3 mm O.D., 3 cm long), into which a small mount of mercury had been drawn, and protruded ca. 2 cm from the glass capillary. The other end of carbon fibers was connected to a copper lead via the mercury junction by pushing the copper wire down (Fig. 1D). Then, epoxy resin was applied to the two ends of the glass capillary to seal the fused-silica capillary and the two copper leads to it (Fig. 1E). Finally, the gold wire and the carbon fibers protruded from the fused-silica capillary were cut (Fig. 1F). Before use, the surface of the gold wire and the carbon fibers were ground with emery paper. Then it was cleaned in ethanol and water for 2 min, respectively, by a supersonic wave cleaner. After drying with filter paper, the electrode was dipped into pure mercury for approximately 2 min. Thus, the carbon fiber bundle–Au–Hg dual electrode was constructed. The cross-sectional view of the dual-electrode is shown in Fig. 1G.

2.3. Reagents and solutions

AA (analytical grade) from Xian Chemical Reagents Factory (Xian, China). A 1.00×10^{-3} mol/l stock solution of AA was prepared weekly by dissolving appropriate amount of AA in 0.1 mol/l $HClO_4$ deaerated and was stored at 4 °C. UA (content 99%) was from Aldrich (St. Louis, MO, USA). A 1.00×10^{-3} mol/l stock solution of UA was prepared by dissolving appropriate amount of ascorbic acid in 0.1 mol/l $HClO_4$. Cys (content>98.5%) was obtained from Shanghai Kangda Amino Acids (Shanghai, China). GSH (content>98%) was obtained from Acros Organic (NJ, USA). A 1.00×10^{-2} mol/l stock solution of Cys or 1.00×10^{-2} mol/l stock solution of GSH was prepared by dissolving appropriate amount of Cys or GSH in 0.02 mol/l Na_2H_2EDTA and was stored at 4 °C. Sulpho-5-salicylic acid (SSA) was obtained from Shanghai First Reagents Factory (Shanghai, China). Unless stated otherwise, all other reagents were of analytical grade and purchased from standard reagent suppliers. Dilute solutions were obtained by a serial dilution of the stock solution with the running buffer. The running buffer was 3.7×10^{-2} mol/l NaH_2PO_4–3.2×10^{-3} mol/l Na_2HPO_4 of pH 5.8. All solutions were prepared with double-distilled water.

2.4. Procedure

The carbon fiber bundle–Au–Hg dual electrode was cemented onto a microscope slide, which was placed over a laboratory-made *xyz* micro-manipulator and glued in place. The position of the carbon fiber bundle–Au–Hg dual-electrode was adjusted (under a microscope) against the end of the capillary with a distance of 10 μm. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the

capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double-distilled water, 0.1 mol/l NaOH, double-distilled water and the corresponding separation electrolyte, respectively, by means of a syringe. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electro-osmotic flow reached a constant value, the electro-migration injection was carried out and the electropherogram was recorded. All potentials were measured versus SCE.

In the end-column amperometric detection with the dual-electrode, the current detected is critically dependent on the proper alignment of the dual-electrode center with the bore of the capillary. The alignment procedure is similar to that described in our previous work [15]. In order to accomplish this, the working electrode center was aligned to the capillary center in horizontal direction with a distance of 10 μm between the dual-electrode and outlet of the capillary by using the *xyz* micro-manipulator. With the aid of a mirror, then, the vertical alignment was carried out using the *xyz* micro-manipulator. In order to guarantee the reproducibility, the alignment must be checked before each experiment by paying attention to the following steps: the elution curves of 5.00×10^{-5} mol/l cysteine at the dual-electrode were recorded. The place of the dual-electrode was adjusted using the *xyz* micro-manipulator again. The manipulation was repeated until the ratio of the two peak currents at the Au–Hg electrode and the carbon fiber bundle electrode of the dual electrode approached $2.5\pm10\%$.

2.5. Preparation of human blood samples

Six hundred μl fresh blood with anticoagulant, 150 μl 0.02 mol/l Na_2H_2EDTA and 250 μl SSA were combined in a 5-ml centrifuge tube, causing the erythrocytes to lyse and protein to precipitate. The tube was vortexed briefly. After standing for 5 min in the dark to precipitate proteins, the mixture sample was centrifuged for 10 min at 4000 rpm, 3061 *g*. The supernatant liquid was transferred to a 1.5-ml centrifuge tube and stored at 4 °C.

3. Results and discussion

3.1. Electrophoresis of Cys, GSH, UA and AA

In previous work [16,17], It was found that Cys and GSH can be oxidized at the Au–Hg electrode at pH 2.4–8.0. The electrochemical characteristics have been used for their determination. When a constant detection potential of more positive than 0 V versus SCE is applied to the Au–Hg electrode, deoxygenation is not necessary. However, the electrochemical response of GSH is not sensitive at the carbon fiber electrode; this makes the procedure much simpler in CE. It is noted that UA and AA can be oxidized at the carbon fiber electrode in weak acid and neutral solutions, but not at the Au–Hg electrode. In order to measure them simultaneously in the same solution, a weak acid running buffer consisted of 3.7×10^{-2} mol/l NaH_2PO_4–3.2×10^{-3} mol/l Na_2HPO_4, pH 5.8, was selected. In the electrophoretic experiments, a separation voltage of 20 kV, and 5 kV injection voltage and 10 s injection time were used. A detection potential of 0.1 V was applied to the Au–Hg electrode and a detection potential of 0.8 V was applied to the carbon fiber bundle electrode of the dual electrode. Fig. 2 shows the electropherograms of the standard solution containing 5.00×10^{-5} mol/l Cys, GSH, UA and AA at the dual electrode. Three electrophoretic peaks with the migration times of 285, 470 and 550 s appear in the electropherogram detected by the carbon fiber bundle electrode (curve 1), which correspond to Cys, UA and AA, respectively. Two electrophoretic peaks with the migration times of 285 and 453 s appear in the electropherogram detected by the Au–Hg electrode (curve 2), which correspond to Cys and GSH, respectively. It is noted that the peak current of Cys detected by the carbon fiber bundle electrode is lower than that detected by the Au–Hg electrode. If the concentration of GSH is increased 10 times, a small peak of 0.86 pA, eluting at 453 s, is detected at the carbon fiber bundle electrode. These results indicate that the detection sensitivity of Cys and GSH is very different at the both electrodes. However, UA and AA cannot be detected at the Au–Hg electrode. This means that the dual electrode benefits simultaneous determination of the four substances

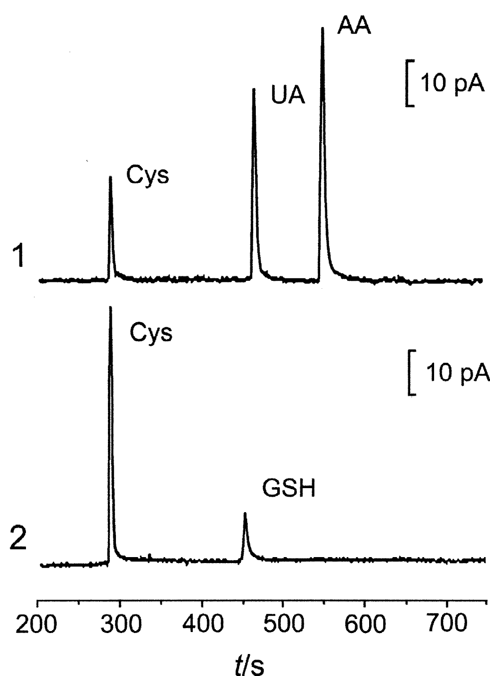

Fig. 2. Electropherograms of the standard solution containing 5.00×10^{-5} mol/l Cys, GSH, UA and AA at the dual electrode. (1) Using a carbon fiber bundle electrode; (2) using an Au–Hg electrode; 3.7×10^{-2} mol/l NaH_2PO_4–3.2×10^{-3} mol/l Na_2HPO_4 (pH 5.8); capillary, 50 cm×25 μm I.D.×375 μm O.D.; injection, 5.0 kV for 10 s; separation voltage, 20 kV; detection potential, 0.80 V for the carbon fiber bundle electrode, 0.10 V for the Au–Hg electrode.

that cannot be detected simultaneously at a single electrode in one run.

3.2. Limit of detection, linear range and reproducibility

The linear ranges of Cys and GSH at the Au–Hg electrode, and Cys, UA and AA at carbon fiber bundle electrode are listed in Table 1. Their linear ranges are over two orders of magnitude. Using least-squares treatment of these data, the slopes, y-intercepts and the correlation coefficients yielded are also listed in Table 1. Their limits of detection (LODs) calculated from the electrophoretic peak currents obtained for the concentration at the low end of their linear range are summarized in Table 1, when the signal-to-noise ratio is 3. It can be seen that the LOD of Cys at Au–Hg electrode is lower than that at carbon fiber bundle electrode. The relative standard deviations (RSDs) of the method for determining these compounds are between 2.0 and 3.1% for the migration time and 2.6–5.0% for the peak current, respectively, for a series of six injections of 2.5×10^{-5} mol/l each compound (see Table 2).

3.3. Analysis of human blood sample

After the prepared sample solution of human blood of 150 μl was diluted to 1 ml with the run buffer, it was injected into the separation capillary by 5 kV for 10 s. Fig. 3 shows the electropherograms of human blood sample without and with the standard solution containing Cys, GSH, UA and AA at the dual-electrode. One small peak and one high peak appear on the electropherogram (curve 1) detected by the carbon fiber bundle electrode. On the basis of the migration time, they can be identified to be Cys and UA, respectively. The Au–Hg electrode can detect two electrophoretic peaks (curve 2). By comparison with Fig. 2 (curve 2), the two peaks should be Cys and GSH. In order to affirm the four peaks, the standard solution containing Cys, GSH, UA and AA

Table 1
Analysis data of Cys, GSH, UA and AA

Compound	Linear range (mol/l)	LOD (mol/l)	Slope (pA/μmol/l)	y-intercept (pA)	Correlation coefficient
Cys[a]	5.00×10^{-6}–5.00×10^{-4}	4.50×10^{-6}	0.395	3.14	0.9998
Cys[b]	1.00×10^{-6}–5.00×10^{-4}	8.76×10^{-7}	1.21	−0.351	0.9999
GSH[b]	5.00×10^{-6}–5.00×10^{-4}	5.00×10^{-6}	0.235	0.697	0.9998
UA[a]	5.00×10^{-6}–5.00×10^{-4}	2.35×10^{-6}	0.840	1.77	0.9993
AA[a]	2.50×10^{-6}–5.00×10^{-4}	1.10×10^{-6}	1.10	0.169	0.9997

[a] Using carbon fiber bundle electrode.
[b] Using Au–Hg electrode. Conditions as in Fig. 2.

Table 2
Migration time, t_m, peak current, I_p, and their RSD of 2.50×10^{-5} mol/l Cys, GSH, UA and AA ($n=6$)

Compound	t_m (s)	RSD of t_m (%)	I_p (pA)	RSD of I_p (%)
Cys[a]	274, 276, 279, 284, 288, 291	2.4	6.2, 6.1, 6.2, 5.7, 5.6, 5.6	5.0
Cys[b]	274, 276, 279, 284, 288, 291	2.4	15.1, 14.7, 14.5, 13.6, 13.8, 13.6	4.5
GSH[b]	431, 434, 439, 447, 459, 465	3.1	2.9, 2.7, 2.8, 2.7, 2.6, 2.7	3.8
UA[a]	459, 460, 467, 472, 479, 482	2.0	11.3, 11.1, 10.9, 11.0, 10.7, 10.5	2.6
AA[a]	526, 533, 547, 550, 553, 558	2.3	14.2, 13.8, 13.5, 13.6, 13.2,13.1	3.0

[a] Using carbon fiber bundle electrode.
[b] Using Au–Hg electrode. Conditions as in Fig. 2.

were added to the blood sample. The peak currents of the four peaks detected by the dual electrode increase (curves 3 and 4). This can also prove that they correspond to Cys, GSH and UA, respectively. However, we could not find the electrophoretic peak of AA on the electropherograms of the sample solution, although its peak could be detected in the sample solution with its standard solution (curve 3). This is because AA had been oxidized during the sample treatment due to its strong reduction ability. The concentrations of Cys, GSH and UA in the human blood sample obtained by the standard calibration method are 3.78×10^{-5} mol/l for Cys, 3.08×10^{-4} mol/l for GSH and 7.67×10^{-4} mol/l for UA, which are very close to the values (3.70×10^{-5} mol/l for Cys, 4.24×10^{-4} mol/l for GSH and 4.17×10^{-4} mol/l for UA) reported in the literature [18]. In order to prove the reliability of the method, certain amounts of their standards with the concentration C_{add} were added to the human blood sample with the concentration C_{sample}. Then, the sample after adding the standards was measured, and its concentration was found to be C_{found}. Thus, we can obtain the recovery defined by the following equation:

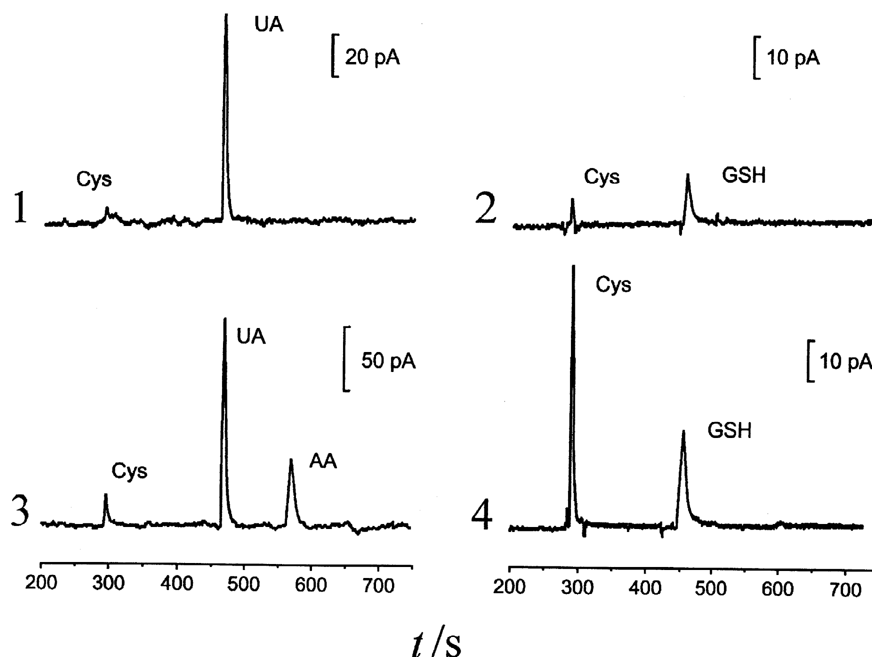

Fig. 3. Electropherograms of the human blood sample (1,2) without and (3,4) with the standard solution containing 5.00×10^{-5} mol/l Cys, GSH and AA, and 8.00×10^{-5} mol/l UA. (1,3) Using carbon fiber bundle electrode; (2,4) using Au–Hg electrode. Conditions as in Fig. 2.

$$\text{Recovery (\%)} = [(C_{\text{found}} - C_{\text{sample}})/C_{\text{add}}] \times 100\% \quad (1)$$

The recoveries of the method for the three compounds (Cys, GSH and UA) are between 94 and 102% for the blood sample.

4. Concluding remarks

Capillary electrophoresis with electrochemical detection of dual-electrodes is a valuable technique for the analysis of biochemical substances that are oxidized or reduced at different kinds of electrode material. The carbon fiber bundle–Au–Hg dual electrode constructed in here is a good example. In this scheme, different potentials are applied to the two electrodes of the dual electrode. Cys, GSH, UA and AA can be identified and quantified simultaneously by the dual electrode in one run.

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