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Determination of aminopyrine and its metabolite by capillary electrophoresis–electrochemical detection

Weihong Zhou, Jun Liu, Erkang Wang*

Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

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Abstract

An electrochemical pretreatment regime for a cylindrical carbon fibre microelectrode was optimized for the determination of aminopyrine (AM) and its metabolite 4-aminoantipyrine (AAN) by capillary electrophoresis (CE)–electrochemical detection (ED). Under optimized conditions, a response of high sensitivity and stability was obtained for AM and AAN at a detection voltage as low as 0.9 V following CE–ED, by which AM and AAN were separated satisfactorily. The calibration graph was linear over three orders of magnitude and the limits of detection for AM and AAN were in the femtomole range.

1. Introduction

Aminopyrine has been widely used as an analgesic and antipyretic drug, and one of its major metabolites in human fluids is 4-aminoantipyrine. Several methods have been developed for the determination of AM and AAN [1–7], such as spectrophotometry [1], thin-layer chromatography [2], gas chromatography–mass spectrometry [3] and high-performance liquid chromatography [4–7].

Since its introduction over a decade ago, capillary electrophoresis (CE) has been shown to be a powerful tool for the separation of a wide range of analytes, and has become a very important technique in the area of liquid-phase separations. To make full use of its advantages, sensitive detection systems are required. Although much of the research in CE has been carried out with UV detection, these detectors lack sensitivity because of their path-length-dependent response. Laser-based fluorescence detectors can provide more sensitive detection but are limited to analytes with fluorescence. Originally described by Wallingford and Ewing [8], electrochemical detection (ED) has an advantage over these methods in that the response is not dependent on pathlength, so both sensitivity and selectivity can be provided.

The carbon fibre microelectrode has been widely used in CE–ED, but the surface of the electrode changes with time owing to adsorption of species from solution. These changes often result in variations in sensitivity or reversibility and sometimes lead to complete inhibition of charge transfer. In order to observe reproducible and well defined electrochemical behaviour, pretreatment of carbon fibre microelectrodes is required.

A variety of methods have been devised for

* Corresponding author.

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pretreating solid electrodes, including polishing, electrochemical treatment, chemical treatment, flaming, vacuum heat treatment, radiofrequency plasma treatment and laser treatment. Electrochemical pretreatment has been used successfully for the activation of carbon fibre microelectrodes [9,10]. For CE–ED it is very convenient to use electrochemical pretreatment because it can be performed while the microelectrode is inserted in the capillary column with the buffer flowing past its surface.

The purpose of the work reported here was to investigate various parameters concerning the electrochemical pretreatment of carbon fibre microelectrodes for the determination of AM and AAN. Electrochemical activation was evaluated with respect to duration of applied potential, potential range and solution conditions.

2. Experimental

2.1. Apparatus

The power supply (Beijing Strong Biological and Electronic) provided a 30 kV d.c. high voltage. An uncoated fused-silica capillary of 50 μm I.D. was obtained from Yongnian Optical Fibre Factory (Hebei, China). The CE–ED system has been described previously [11]. The electrochemical detector is isolated from the applied electrical field by using a Nafion joint as described by O'Shea et al. [12]. This joint is positioned in the cathodic buffer reservoir and permits ion movement but not bulk electrolyte flow. Both the Nafion joint and electrochemical cell were shielded in an aluminium box to reduce external noise. An El-400 dual microelectrode potentiostat (Ensman Instrumentation) was used as an amperometric detector. A Model 056 recorder (Hitachi) was used.

The electrochemical cell is similar to that described by Wallingford and Ewing [13]. Electrochemical detection was performed with 30 μm diameter carbon fibres protruding 1–2 mm from drawn glass capillaries as the working electrodes. The microelectrode was mounted on an X–Y–Z micromanipulator (laboratory-built) and positioned in the electrochemical detection cell. With the aid of an optical microscope, the microelectrode was aligned and inserted into the capillary column. The cell was operated in a two-electrode configuration, with an Ag–AgCl reference electrode. UV–Vis detection was carried out on a CV^4 capillary absorbance detector (ISCO).

Sample introduction was accomplished using an electromigration system and the volume injected was calculated in the continuous fill mode by recording the time required for the sample to reach the detector.

2.2. Cyclic voltammetry

Cyclic voltammetric experiments were performed in a three-electrode system cell. A platinum wire was employed as the auxiliary electrode. Cyclic voltammetry was used to evaluate the effect of various electrochemical pretreatments on the chemical nature and electrochemical reversibility of electrochemically treated carbon fibre surfaces.

2.3. Reagents

AM and AAN were obtained from Beijing Chemical. All reagents were of analytical-reagent grade and were used as received. All solutions were prepared with doubly distilled water and passed through a membrane filter (0.45 μm) before use. Phosphate solution (4 mM) was used as the separation buffer and was adjusted to the appropriate pH with phosphoric acid.

2.4. Sample preparation

Human urine samples were diluted immediately in 4 mM buffer (1:5) and filtered with a 2-μm pore size filter. This solution was injected directly on to the capillary column.

3. Results and discussion

3.1. Effect of pretreatment parameters

Electrochemical pretreatment methods involve anodization, anodization followed by cathodization, alternating potential applying a triangular or square wave form to the microelectrode, etc. However, simply holding the microelectrode at positive potentials can give good results. Fig. 1 shows cyclic voltammograms of (A) AM and (B) AAN at (a) untreated and (b) anodized carbon fibre microelectrodes. It can be seen that the peak obtained with the pretreated electrode was much higher than that with the untreated electrode. AM yields irreversible oxidation waves at ca. 0.7 and 1.3 V and AAN at 0.5 and 1.1 V on the pretreated electrode, revealing a significant improvement in magnitude and sharpness of the anodic peaks. Several workers have noted that anodic pretreatment of glassy carbon electrodes introduces oxygen functionalities, including hydroxyl, carbonyl and carboxyl groups, on the surface [14,15]. The work of Rice et al. [16] on carbon paste electrodes [16] indicated that anodic pretreatment made the graphite surface more hydrophilic, thus removing some of the adherent organic layer. The activation of the pretreated carbon fibre electrode in our work may also be as a result of the introduction of oxygen functionalities and the removal of passivating layers that hinder electron transfer.

The dependence of the peak current of both AM and AAN on the pH of the buffer solution illustrates that the peak current decreased with increase in pH. This may be the reason why anodic treatments in acidic media can produce a greater increase in carbon–oxygen bonds than that in neutral or basic pH buffers. Because a low pH of the solution leads to comparatively long migration times, pH 5 was selected in the CE separation.

Apart from pH, other treatment parameters had few effects on AM. The effect of the treatment conditions on AAN is illustrated in Fig. 2. The data points on the graphs were obtained with the same microelectrode; once inserted into the outlet of the capillary and positioned in the direction cell, the same electrode was used in CE–ED without replacement. The duration of pretreatment was evaluated for periods ranging from 15 to 60 s. As shown in Fig. 2A, after 30 s there was no improvement in sensitivity and actually a decline in the peak

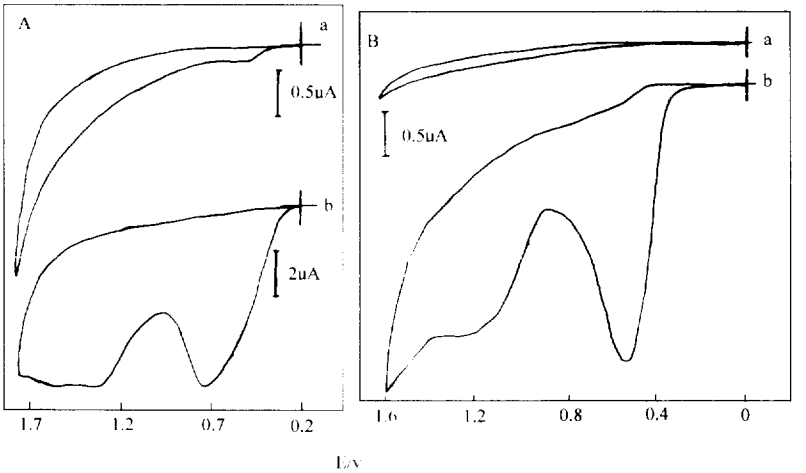

Fig. 1. Cyclic voltammograms of (A) 10^{-3} *M* AM and (B) 10^{-3} *M* AAN obtained at carbon fibre microelectrodes (a) untreated and (b) treated at 1.5 V for 30 s. Supporting electrolyte, 4 m*M* phosphate buffer (pH 4.82); scan rate, 100 mV s^{-1}; electrode diameter, 30 μm. *E* (V) vs. Ag–AgCl.

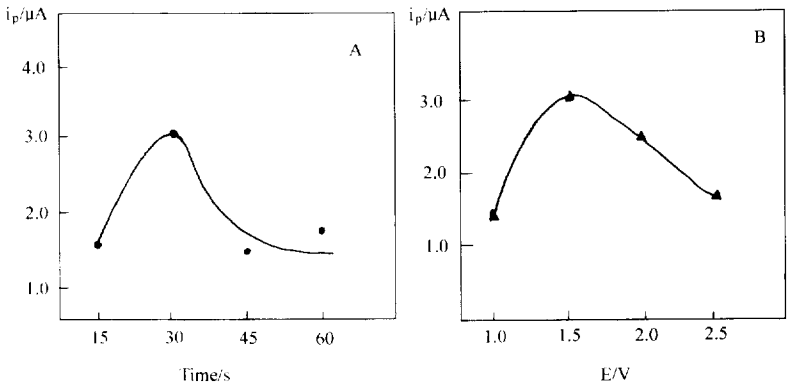

Fig. 2. Effect of AAN treatment conditions: (A) pretreatment time and (B) upper potential limit. Supporting electrolyte, 4 m*M* phosphate buffer (pH 4.82); analyte concentration, 1 m*M*; electrode diameter, 30 μm.

current occurred. Such a process has also been observed on carbon fibre microelectrodes by other workers [9,10]. We assumed that with the increase in oxygen functionalities on the microelectrode by anodization, the electrode surface was cleaned and possessed more active sites than before, but with the increasing time, more and more oxygen functionalities were introduced and thus might have formed a passivating film coating on the surface, which would deactivate the electrode. A maximum point was also observed when we investigated the effect of potential limits (Fig. 2B). An increase in the voltammetric response was obtained on changing the potential limit from 0 to 1.5 V, followed by a gradual decrease in current.

In order to be practical for routine applications in capillary electrophoresis, the initial catalytic activity should be maintained over a long period of time. After pretreatment, the electrode was kept in the buffer for 3–20 min. An equilibration period is necessary for a more stable response. The electrode showed a steady response after being kept in buffer for over 5 min and retained its catalytic activity for 15 min.

The pretreatment conditions selected as optimum were a potential of 1.5 V for 30 s in pH 5 buffer and maintaining the treated electrode in buffer for 5 min or longer. Using the optimum pretreatment between each scan, the response was constant with the precision, calculated as the relative standard deviation, being better than 1.8% for AAN.

3.2. CZE with electrochemical detection

Insufficient stability and reproducibility are often drawbacks of electrochemical detectors. Fig. 3 shows the stability of the electrochemically treated electrode after one pretreatment. The

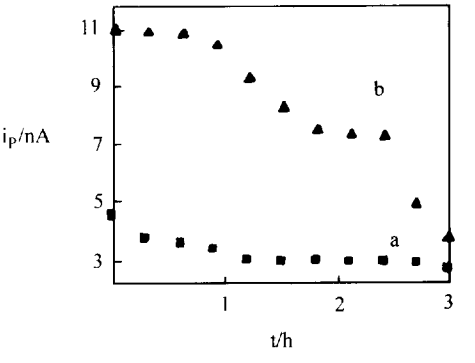

Fig. 3. CE–ED peak current change for (a) 0.5 m*M* AM and (b) 1 m*M* AAN at an electrochemically pretreated carbon fibre microelectrode. 50 μm I.D. capillary; 33 cm long separation capillary; 1.7 cm detection capillary; injection by electromigration, 10 s at 12 kV; separation voltage, 12 kV; 4 m*M* phosphate buffer (pH 4.82).

activity of treated electrodes used in the CE–ED system was observed to decrease slightly with time for AM within 3 h after eleven successive injections, but for AAN it changed quickly. It is recommended that pretreatment be performed between each run. The detector response was also found to be very reproducible when the electrode was pretreated between injections.

The effect of the CZE flow conditions on the anodic current response as a function of the applied potential was examined by means of hydrodynamic voltammograms (HDVs) as shown in Fig. 4. The HDVs obtained under CZE conditions for AM and AAN were peak-shaped and reached their maximum current at 0.91 and 1.2 V, respectively. Because high potential give rise to a higher background current, 0.9 V was selected for the simultaneous determination of AM and AAN. Under the optimized conditions, a response of high sensitivity and stability was obtained for AM and AAN at a detective voltage as low as 0.9 V following CE–ED, by which AM and AAN were separated satisfactorily in short capillary length and eluted within 5 min as shown in Fig. 5. Regression analysis for AM

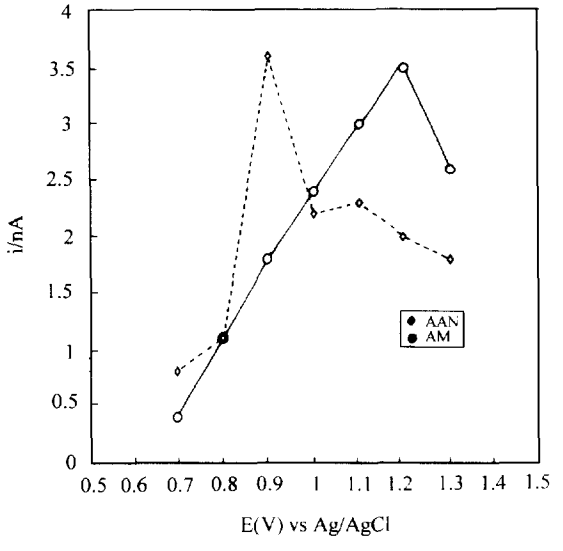

Fig. 4. Hydrodynamic voltammograms of 0.01 *M* each of AM and AAN at a carbon fibre microelectrode. Injection by electromigration, 5 s at 12 kV; other conditions as Fig. 3.

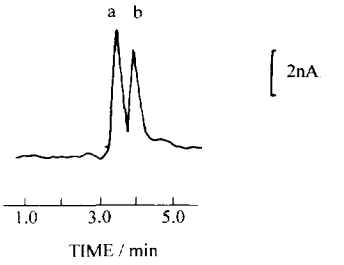

Fig. 5. Electropherogram of (a) AM ($1 \cdot 10^{-3}$ *M*) and (b) AAN ($1 \cdot 10^{-3}$ *M*). Other conditions as in Fig. 4.

over the concentration range $5 \cdot 10^{-5}$–$1 \cdot 10^{-2}$ *M* (based on an injection volume of 14.4 nl, corresponding to 144–144 pmol) resulted in a correlation coefficient of 0.984 ($n = 6$). Regression analysis for AAN over the range of 430 fmol–172 pmol resulted in a correlation coefficient of 0.995 ($n = 6$). The slope of the curves obtained was 8.24 nA/m*M* for AM and 7.15 nA/m*M* for AAN. Detection limits of 0.40 pg for AM and 1.46 pg for AAN were estimated (signal-to-noise ratio = 3).

To compare the sensitivity of ED with UV–Vis absorbance detection, an on-column UV detector (234 nm) was used under the same separation conditions as in CE–ED. The detection limit with the UV–Vis method was $1 \cdot 10^{-4}$ *M*, corresponding to 142 pg for AM and 111 pg for AAN. The mass detection limits with CE–ED are also more sensitive than those obtained by LC–ED in previous studies with an unmodified electrode (10 ng or $4.3 \cdot 10^{-6}$ *M* for AM [6]) and a glassy carbon electrode dispersed with α-alumina particles (1.4 ng or $3.0 \cdot 10^{-7}$ for AM and 0.8 ng or $2.0 \cdot 10^{-7}$ *M* for AAN [7]).

To evaluate the performance of the system for the analysis of real samples, the detection of AM and AAN added to human urine sample was studied. Fig. 6A illustrates a typical electropherogram obtained at a carbon fibre electrode for a diluted (1:5) urine sample from a healthy female volunteer. Also shown for comparison (Fig. 6B) is the electropherogram of a urine sample with $6 \cdot 10^{-5}$ *M* AM and AAN added. Good sensitivity and selectivity can be achieved

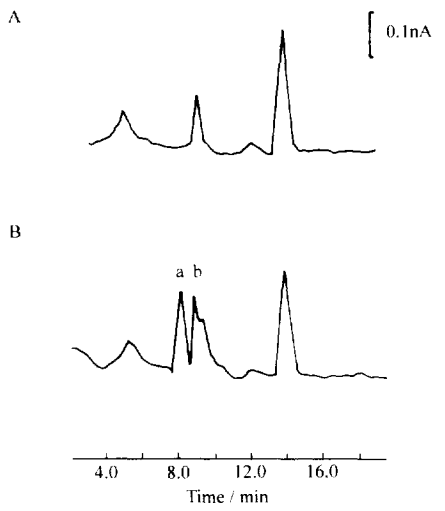

Fig. 6. Electropherogram of (A) diluted (1:5) urine sample and (B) urine sample with (a) $6 \cdot 10^{-5}$ *M* AM and (b) $6 \cdot 10^{-5}$ *M* AAN added. 52 cm long separation capillary; 1.5 cm detection capillary; other conditions as in Fig. 3.

with application of the proposed method to the determination of the drug in urine.

4. Conclusion

This is the first report dealing with the detection of AM and AAN using CE–ED. The method has proved to have good reproducibility, a wide linear response range and low mass detection limits at a lower detection voltage compared with LC–ED [6,7].

Acknowledgement

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