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Short communication

Identification and quantitative determination of uric acid in human urine and plasma by capillary electrophoresis with amperometric detection

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Abstract

Application of capillary zone electrophoresis with electrochemical detection to the identification and quantitative determination of uric acid in human urine as well as plasma is described. This work was carried out in a 30 cm×25 μm I.D. fused-silica capillary with tricine buffer and a carbon fiber bundle was employed as a working electrode, the working voltage in amperometric detection was set at +0.80 V (vs. SCE). The sample constituent is identified by stopped flow-linear sweep voltammetry. Under optimal conditions, a lower detection limit of 0.48 fmol was obtained for uric acid. © 1997 Elsevier Science B.V.

Keywords: Detection, amperometric; Uric acid

1. Introduction

Capillary electrophoresis (CE) is a highly efficient separation technique that affords the separation of charged analytes in a short period of time. Recently, the application of CE methodology to the analysis of biological fluids has shown great promise for its use as a clinical tool [1]. The integration of electrochemical detection with capillary electrophoresis (CE–ED) results in a highly efficient and sensitive system capable of analyzing ultrasmall sample volumes. CE–ED has successfully been employed to analyze catecholamines [2], carbohydrates [3], amino acids [4] and purine bases [5]. However, there are still few reports [6] concerning CE–ED employed in clinical analysis. To illustrate the utility of CE–ED for the determination of low-molecular-mass metabolites in human biological fluids, we report here the use of CE–ED to examine uric acid (UA) in urine and plasma.

UA has great clinical significance in the etiology of gout and has also been hypothesized to provide an antioxidant defense [7]. Though the analysis of UA by CE with UV detection has been reported [8], the preparation procedure, including lyophilization and reconstitution remained tedious. In addition, peak identification by electrophoretic mobility was still difficult due to matrix effects. Micellar electrokinetic capillary chromatography (MECC) with UV or fast-scanning detection has also been applied to the determination of UA [9,10] and it allows direct introduction of plasma samples. Although it was shown to be an attractive method for therapeutic drug monitoring, there remained some difficulties in

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the direct determination of many endogenous compounds. Moreover, the resolution of UA needed longer retention time because its peak is adjacent to that of plasma proteins.

In this paper, we present a simple and rapid method for UA analysis in biological fluids using an CE–ED system. It was also revealed that the ionic strength of the sample introduced to CE considerably influences the separation efficiency and that the extent differs according to CE running buffers. In addition, the novel aspect of this work is that stopped-flow linear sweep voltammetry (SF-LSV) has been developed to identify peaks in human biological fluid samples. By this method UA in urine and plasma could be quantitated and satisfactory results were obtained.

2. Experimental

2.1. Chemicals and reagents

UA was purchased from Sigma. A UA stock solution was prepared by dissolving 10 mg of crystals in 100 ml of 10 m*M* NaOH. All other reagents were of analytical reagent grade, doubly distilled water was used for the preparation of all solutions. The urine and plasma samples were obtained from healthy adult male subjects.

2.2. CE–ED system

Electrophoresis was driven by a high voltage supply (Shanghai Third Analytical Instrument Factory, Shanghai, China). Polyimide-coated fused-silica capillaries (330 μm O.D., 25 μm I.D.) were obtained from Yongnian Optical Fiber Factory (Hebei, China), and capillary of 30-cm length was used. The basic CE–ED system and the construction of the electrochemical detection cell have been described elsewhere [11]. Briefly, the disk working electrode was constructed with a bundle of 6-μm carbon fibers and the disk diameter was about 300 μm. In order to enhance sensitivity, electrode pretreatment was accomplished by oxidation of the carbon fibers at a potential of +2.0 V (vs. SCE) for 90 s while the operating buffer was flowing past the detector. The working electrode was vertically aligned with the outlet of the capillary in the detection cell based on wall-jet approach. The electrochemical detector was set at +800 mV (vs. SCE). Samples were introduced by gravity at a height of 8 cm for a duration of 15 s. The resulting injection volume was approximately 0.4 nl calculated by the reported equation [12].

2.3. Stopped-flow linear sweep voltammetry (SF-LSV)

SF-LSV was carried out by immediately sweeping in the potential range from 0.0 to 1.0 V just after shutting down the high-voltage of the electrophoretic system while UA peak was approaching maximum. The sweep rate used was 50 $mV\,s^{-1}$ in all the experiments.

2.4. Sample preparation

Urine samples for the analysis were diluted 50 fold with 80 m*M* tricine buffer (pH 8.0). The samples were vortex-mixed for 15 s and filtered through a 0.45-μm filter. The filtrate was used for CE analysis.

Aliquots of plasma (0.2 ml) were mixed with 0.2 ml of 12% trichloroacetic acid (TCA) for deproteinization. The solution was vortexed for 30 s. After centrifugation for 5 min (3000 *g*), the supernatants were filtered and diluted three fold with the tricine buffer.

3. Results and discussion

3.1. Optimization of CE–ED conditions

Compared with universal UV absorption spectrometry, amperometry is a selective detection method which is employed to monitor electroactive constituents. As a consequence, amperometric detectors could not only afford higher detection sensitivity for CE but also reduce separation difficulties. In this CE–ED system, the electroactive endogenous compounds with greater concentration in urine or plasma are uric acid and ascorbic acid. In our experiments phosphate buffer (pH range 6.0–8.0) as the com-

monly used buffer, was initially tried in the separation of the analytes. It was found that ascorbic acid and UA have the same mobility when the pH of the buffers is around 6.7. In order to avoid this potential interference, buffer of pH 8.0 was chosen so that a satisfactory resolution for the standard solution was obtained. The results are shown in Fig. 1A. When this buffer was employed to analyze the plasma sample, however, it was found that the peak of UA markedly deteriorated (Fig. 1B). This could be caused by the difference in sample preparation. When 80 m*M* tricine buffer (pH 8.0) was used, this influence was dramatically reduced and a sharp peak of UA appeared on the electropherogram. Fig. 1C and D show the typical electropherograms of the urine and plasma sample using tricine buffer, respectively.

The direct determination of UA in human plasma by MECC was previously reported [9,10]. In order to solubilize plasma proteins and reduce undesirable adsorption on the inner wall of the capillary, the surfactant SDS had to be added to the CE buffer. In fact, the adjacent plasma proteins peaks could still influence the separation of UA and the capillary had to be sequentially washed by several solutions between the runs to shorten retention time and regenerate the surface condition of the capillary wall. In another approach, serum samples had to be lyophrilized and then reconstituted for the CE analysis in order to reduce the influence of matrix effects [8]. In this work, plasma was prepared using TCA to deproteinize the sample and extract analytes into the aqueous phase. As a result, the samples became homogenous by extraction and the procedure reduced the sample matrix effects and the interference of plasma proteins. On the other hand, the procedure no doubt increased the ionic strength of the sample. To further observe if the high ionic strength of the sample introduced to CE affects the peak profile of UA, samples including different concentrations of NaCl were separated with phosphate buffer. Fig. 2 compares the electropherograms of UA prepared in various concentrations of NaCl solutions. It was thus confirmed that increasing the ionic strength of the sample causes serious peak broadening. This phenomenon could be attributed to the diffusion effect. When tricine buffer was employed as the electrophoretic medium, this influence was dramatically reduced and a sharp peak of UA appeared in the electropherogram. Tricine is a weak electrolyte and

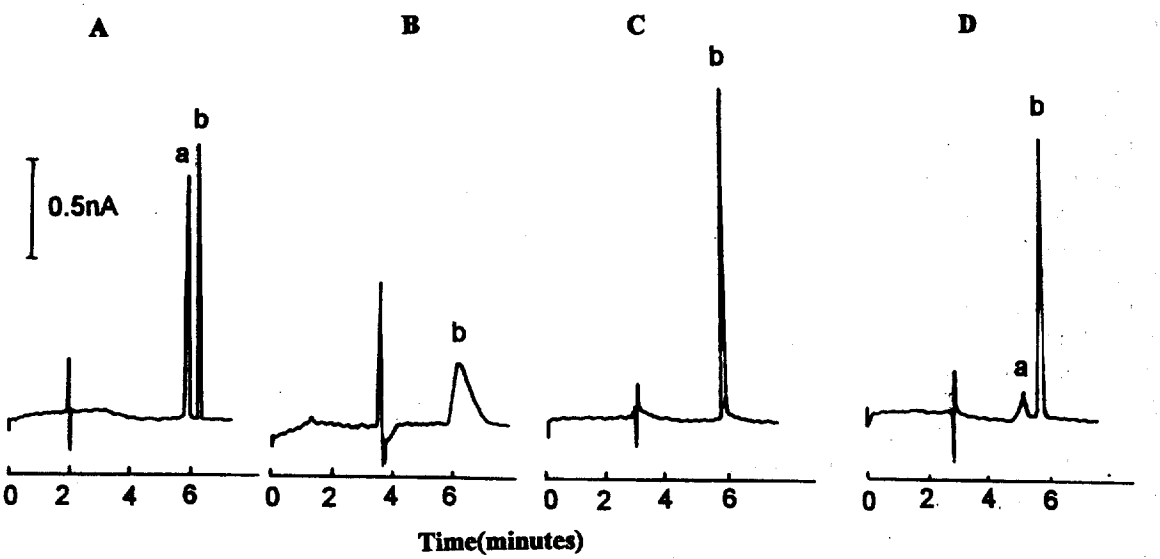

Fig. 1. Electropherograms of (a) ascorbic acid and (b) UA in (A) a standard solution, (B) and (D) plasma samples, (C) urine samples. CE buffers: (A) and (B), 20 m*M* phosphate buffer (pH 8.0); (C) and (D), 80 m*M* tricine buffer (pH 8.0). Separation voltage, 12 kV.

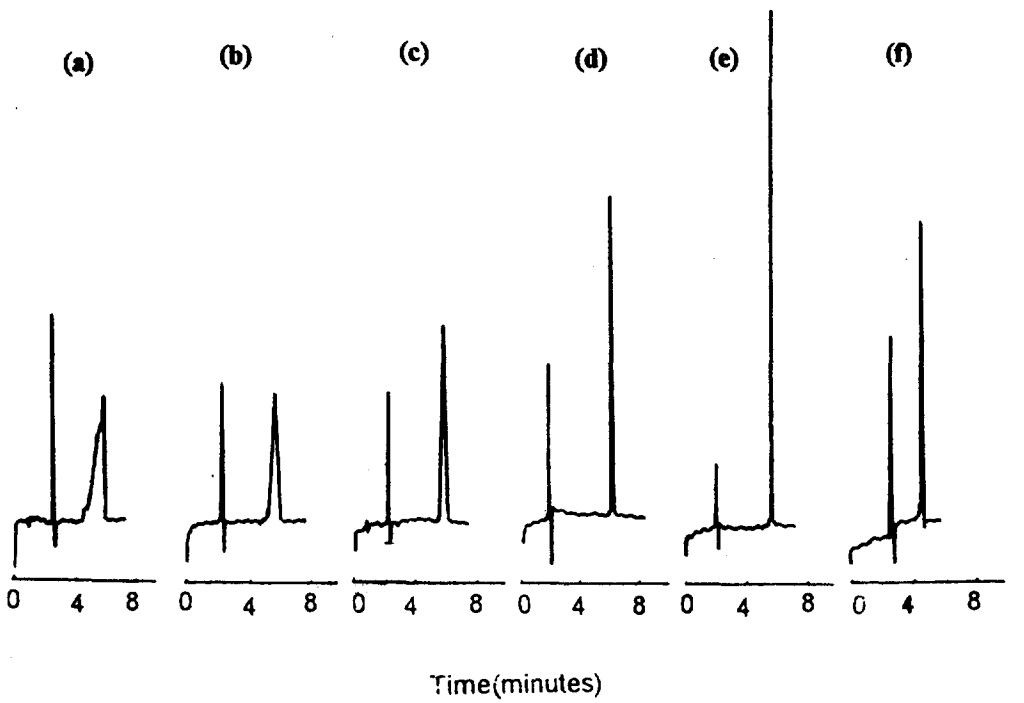

Fig. 2. Electropherograms of UA prepared in various NaCl concentrations at 12.0 kV. (a–e) 40 m*M* phosphate buffer (pH 8.0), (f) 80 m*M* tricine buffer (pH 8.0). NaCl concentrations of the samples introduced to the CE are (a) 10%, (b) 5%, (c) 3%, (d) 1%, (e) 0%, and (f) 10%, respectively.

has a low mobility value. Thus, its low migration velocity partly alleviates the diffusion effect. It could be concluded that the UA peak broadening in the plasma sample resulted from increased ionic strength of the sample rather than from acidity caused by the addition of TCA. Tricine buffer (pH 8.0) was appropriate for analysis of urine and plasma samples and thus was used for subsequent sample analysis.

The optimal detection potential for CE was determined by measuring hydrodynamic voltammograms in the CE–ED system (Fig. 3). With increasing the applied voltage the peak current of UA consistently increased and approached a plateau at +0.80 V. On the other hand, a noise current was also observed at voltages higher than +0.60 V. Thus, the maximum of signal-to-noise ratios was obtained at +0.80 V and this potential was selected for subsequent sample analysis. At potentials higher than +0.90 V, the baseline became quite unstable.

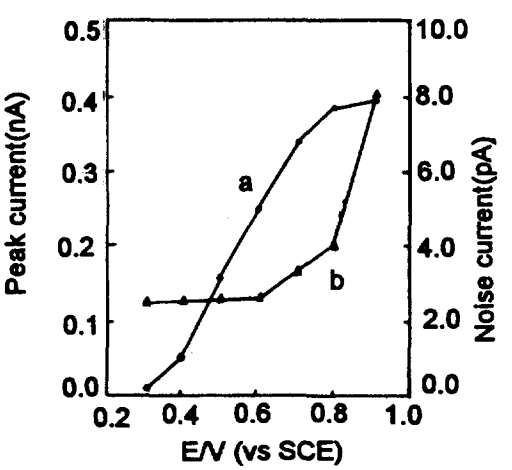

Fig. 3. Hydrodynamic voltammogram. (a) Peak current, (b) noise current. Conditions as in Fig. 1C.

3.2. Identification of peaks

Peak identifications in urine and plasma separations were assigned by matching the electrophoretic mobility with that of the standard compound. However, sample matrix effects and small deviations of pH can result in significant influences on the mobility of UA. In order to further identify the peak, SF-LSV was developed as a new approach to identification. Fig. 4 shows voltammograms of UA standard compound and authentic samples obtained by SF-LSV technique. Fig. 4 shows that either the standard compound or authentic samples produced similar oxidation peaks at +0.31 V, which corresponded to Ep of UA. Owing to the matrix effect the profile of the UA oxidation peak in the plasma sample is slightly different from that in the standard sample, although the anodic potentials of both peaks are still identical. Because the sample introduced into the CE–ED system was very small (50 fmol), the oxidation peaks of UA were relatively small in comparison with the corresponding background currents. In addition, a suitable sweep rate (50 mV s^{-1}) is required to minimize the analyte's diffusion from the surface of the working electrode. It was found in the experiments that a faster sweep rate (e.g. 100 mV s^{-1} or 200 mV s^{-1}) led to bigger background currents and masking of the analyte peak. Therefore, a sweep rate of 50 mV s^{-1} was experimentally chosen to assign peaks. This method of SF-LSV analysis could provide more details for peak assignment.

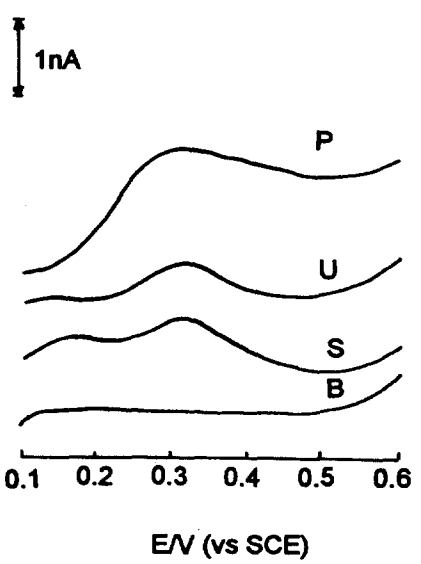

Fig. 4. Stopped flow-linear sweep voltammograms: B, background; S, the standard solution of UA; U, urine sample; P, plasma sample.

3.3. Analytical characterization

In order to evaluate the characteristics of this CE–ED system, the linearity, reproducibility and limit of detection for UA were determined. The calibration curve in the range 2.0–20.0 μg ml^{-1} was $y=0.200x+0.347$ ($r=0.9995$). The relative standard deviations of the peak height were 7.7% (5.0 μg ml^{-1}) and 6.0% (10.0 μg ml^{-1}). The limit of detection for UA was 0.48 fmol (0.20 μg ml^{-1}). Three urine samples were analyzed and the concentrations of UA were 210, 623 and 836 μg ml^{-1}, respectively. The recovery of UA from plasma was determined at three different concentrations (5.0, 6.7, 10.0 μg ml^{-1}) and the average recovery ranged from 88.5 to 100.3%. Two plasma samples were prepared and analyzed by this CE–ED system, the concentrations of UA were 69.0 and 43.2 μg ml^{-1}, respectively. These results are in agreement with those reported in the literature [8]. These data suggest that electrochemical detection could provide a lower limit of detection. In the proposed CE–ED system manipulation of the working electrode did not need the aid of a microscope, and the disk electrode was easily polished and replaced. These characteristics would routinely promote the development of ED for CE.

Acknowledgments

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