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Simultaneous determination of polycarboxylic acids by capillary electrophoresis with a copper electrode

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Abstract

The simultaneous determination of polycarboxylic acids including oxalic acid, citric acid, malonic acid, malic acid, tartaric acid, aspartic acid and glutamic acid was achieved by capillary electrophoresis with a copper disk electrode ($d=200$ μm). In the system, 0.2 mmol/l cetylpridinium bromide (CPB) was used as an electroosmotic flow (EOF) modifier to reverse the direction of EOF. The effects of the solution pH and CPB concentration on separation were evaluated to achieve the optimum separation conditions. At the working potential of +0.14 V (vs. saturated calomel electrode), the calibration curves for all polycarboxylic acids studied were linear with 2~3-orders of magnitude and all the detection limits ($S/N=3$) were below 15 fmol except malonic acid. Furthermore, the oxalic and citric acids in urine were successfully separated and determined with high sensitivity.

Keywords: Electrochemical detection; Detection, electrophoresis; Polycarboxylic acids; Carboxylic acids

1. Introduction

The separation and detection of polycarboxylic acids is an interesting and challenging topic in analytical biochemistry because these compounds are amply present in foods, beverages, body fluids and so on, and are involved in many biochemical, physiological or pathological processes. Oxalic and citric acids in urine, e.g., are indicators for various bodily disorders, of which the formation of renal stone is the most widely known [1]. In the past, chromatographic techniques, such as gas chromatography (GC) [2], ion chromatography (IC) [3] or high-performance liquid chromatography (HPLC) [4], have been mainly implemented for the separation and determination of polycarboxylic acids.

As a modern analytical technique, capillary electrophoresis (CE) has become an attractive method with promising features, such as high separation efficiency, short analysis time, simple analytical procedure and micro-quantity consumption of samples. Electrochemical detection (ED), which is typically operated in the amperometric mode, was described as the tailor-made technique for CE because of its many features superior to UV detection [5]. The design of detection cell is simple and can be miniaturized with little or no loss in sensitivity. Moreover, through proper control of the detection potential and/or careful selection of the working electrode, the selectivity and sensitivity are tunable according to the different analytical aims. Recently, the applications of CE–ED to the determination of a

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wide range of analytes have been reviewed by Holland and Lunte [6].

Although CE with UV detection [7–13] has been investigated for the determination of polycarboxylic acids, the potential of CE–ED has been little documented in this field. In this paper, we describe the analytical procedure for the determination of polycarboxylic acids commonly present in biological samples by CE–ED with a small copper electrode. It was demonstrated that this new method is rapid, sensitive and quantitative, and especially, it is considered suitable for the selective determination of oxalic and citric acids in urine with simple procedure and high sensitivity.

2. Experimental

2.1. Reagents

The polycarboxylic acids standards were purchased from Sigma (St. Louis, MO, USA). All other chemicals were analytical grade. All chemicals were used as received without further purification. The separation electrolyte, phosphate buffer solution containing cetylpridinium bromide (CPB) was prepared with double distilled water and adjusted to proper pH with sodium hydroxide using a pH meter. 0.1 mol/l stock solutions of standards, stored at 4°C, were diluted serially using the separation electrolyte just before CE analyses. Urine samples were from health male volunteers and stored at 4°C for no more than 4 h before use. Prior to CE analyses, all samples were filtered with a 0.25-μm filter. Urine samples were diluted properly in the separation electrolyte.

2.2. Apparatus

The CE–ED system was assembled in the laboratory. Electrophoresis was driven by a 30-kV high-voltage power supply (Third Analytical Instrument Factory of Shanghai, China). The uncoated fused-silica capillary (60 cm×50 μm I.D.) was obtained from Yongnian Optical Fiber Factory (Hebei, China). The detailed construction of the wall-jet electrochemical detection cell has been described elsewhere [14,15]. The outlet end of the capillary was always maintained at ground. The electrochemical cell was shielded in a copper box to reduce external disturbance. A conventional three-electrode mode was used. The copper working electrode was constructed with a 200 μm diameter copper wire as described previously [16]. Before the initial utilization, the electrode surface was wet polished with 0.05 μm alumina powder, rinsed with a stream of deionized water and sonicated for 3 min. A saturated calomel electrode (SCE) and a platinum wire were used as a reference electrode and a counter electrode, respectively. The electrodes were connected to an amperometric detection (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China), which provided the applied constant potential and measured the resulting current.

2.3. Electrophoretic procedure

Prior to the first use, a new capillary was washed successively with 0.1 mol/l NaOH, deionized water and the running electrolyte, each for 5 min, and then equilibrated with the running electrolyte under the separation voltage for about 2 h until the migration times of analytes did not change significantly. When the running electrolyte was altered, the above steps should be repeated. After every running, the capillary was washed with the running electrolyte for 5 min. The buffer in the anodic and cathodic reservoirs was renewed every five runs in order to keep the same pH between the two reservoirs.

Because the electrophoretic mobility of polycarboxylic acids is larger than that of the electroosmotic flow (EOF), a negative-voltage power supply was used and the detection was changed to be at the anode. At the same time, the cationic surfactant as an EOF modifier was used to reverse the direction of the EOF by reversing the charge on the inner wall of capillary. Sample introduction was performed hydrostatically at the cathodic side by elevating the sample reservoir in which the capillary was immersed to a constant height of 10 cm for 10s. The volume injected was about 2.5 nl.

2.4. Cyclic voltammetry

Cyclic voltammetry (CV) experiments were performed with CHI660 Electrochemistry Working Station (CH Instruments, USA) with a three-electrode

system as described above. Cyclic voltammograms were obtained in 15 mmol/l phosphate buffer using a scan rate of 10 mV/s.

3. Results and discussion

3.1. Electrode response

The detection mode with a copper electrode has two different mechanisms in flowing system: an electrocatalytic oxidation mechanism in strong alkaline medium and a complexation mechanism in neutral medium [17]. The latter mode has been used to determine amino acids in CE [18] and HPLC [19–22] and amino-alcohols in flowing system [23]. In order to identify the mechanism of polycarboxylic acids on copper electrode, CV experiments were carried out firstly. The CV results for a copper electrode in phosphate buffer (pH 7.0) with or without citric acid are shown in Fig. 1. The CV obtained in blank phosphate buffer exhibited a broad anodic wave at about +0.15 V and a sharp cathodic peak at −0.02 V (Fig. 1a), corresponding to the formation of copper (II) oxide and its reduction, respectively. These results were similar to what was reported in the literature [19,22]. Upon addition of citric acid, the CV showed an increased anodic current and a decreased cathodic current (Fig. 1b). The former, we assumed, could be attributed to the dissociation of the oxide film because of the complexation of citric acid with Cu(II) on the electrode surface and thereby the further oxidation of the electrode surface. The latter could be assumed that the soluted copper (II) oxide partly entered into the solution in the form of a complex with citrate and left the electrode surface, and hence, could not be reduced. The assumption was supported by the experimental result that cathode charge ($1.2\cdot10^{-6}$ C) is less than anode charge ($2.3\cdot10^{-6}$ C) for 1 mmol/l citric acid. Furthermore the buildup of Cu(II) in solution was confirmed by atomic absorption measurements when Ye and Baldwin studied the response of amino acids on a copper electrode [18]. In addition, the anodic current increased with the increasing of oxalic acid (Fig. 1c), which was the basis of amperometric detection for polycarboxylic acids. Oxalic acid, malonic acid, malic acid, tartaric acid, aspartic acid and glutamic acid had similar electrochemical behaviors (not shown in Fig. 1).

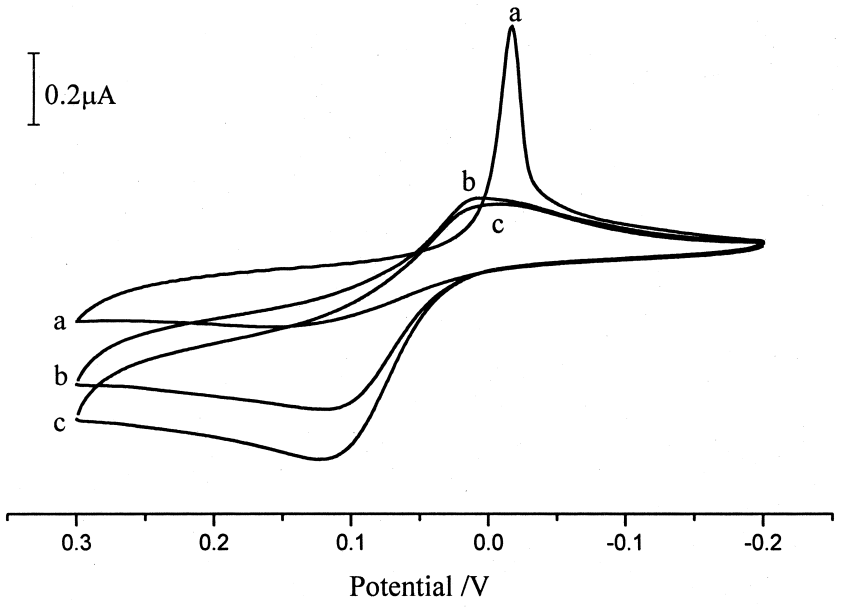

Fig. 1. Cyclic voltammograms on a copper electrode in 15 mmol/l phosphate buffer, pH 7.0. Analyte: (a) blank, (b) 1 mmol/l citric acid, (c) 1.5 mmol/l citric acid. Scan rate, 10 mV/s.

However, many other carboxylic acids occurring in bio-samples, in our experiments, have no response at all. They are acetic acid, adipic acid, glutaric acid, lactic acid, maleic acid, pyruvic acid, succinic acid and α-ketoglutaric acid. This interesting phenomenon showed the high selectivity of a copper electrode to polycarboxylic acids, which was favorable for determining selectively polycarboxylic acids in bio-samples.

3.2. Optimization of separation conditions

It is well known that the migration of analytes in CE depends on their charge-to-mass ratios. In conventional CE, sample introduction is carried out at the anode and detection at the cathode for the EOF generally surpasses the rate of migration of analytes (toward the cathode). However, polycarboxylic acids are typically low-molecular-mass species with the relatively large charge-to-mass ratios. Therefore, they migrate toward the anode proceeding the EOF. To circumvent this problem, the detection is often changed to be at the anode and cationic surfactants are added to neutralize the negative surface charge on the inner wall of bare fused-silica capillary. It will reduce the EOF or even reverse the direction of the EOF. Cationic surfactants, such as tetradecyltrimethylammonium bromide (TTAB) [7–13], were used extensively in CE with UV detection. Our preliminary experiments indicated that CPB was the best one of all cationic surfactants investigated, including CPB, cetyltrimethylammonium bromide (CTAB) and TTAB. The effect of the concentrations of CPB from 0.1 to 0.5 mmol/l on the migration time of the analytes interest is shown in Fig. 2. It was apparent that the relatively satisfactory separation for all analytes was obtained with 0.2 mmol/l CPB. When the concentration of CPB was above 0.4 mmol/l, the separation became poor. We finally chose 0.2 mmol/l CPB in the following experiments.

Since the net electric charge of a polycarboxylic acid is pH dependent, the charge-to-mass ratio, accordingly, is dependent on pH. Therefore, the electrophoretic mobility of a polycarboxylic acid is strongly affected by the buffer pH. Unfortunately, the optimization of the running buffer pH is subjected to the detection because the response of a copper electrode to complex reagents is not available when the solution pH is below ca. 6 [23] (according to our results, this pH limit was 5.8). The effect of the buffer pH from 6.0 to 8.5 on the migration time of the polycarboxylic acids was investigated, as shown

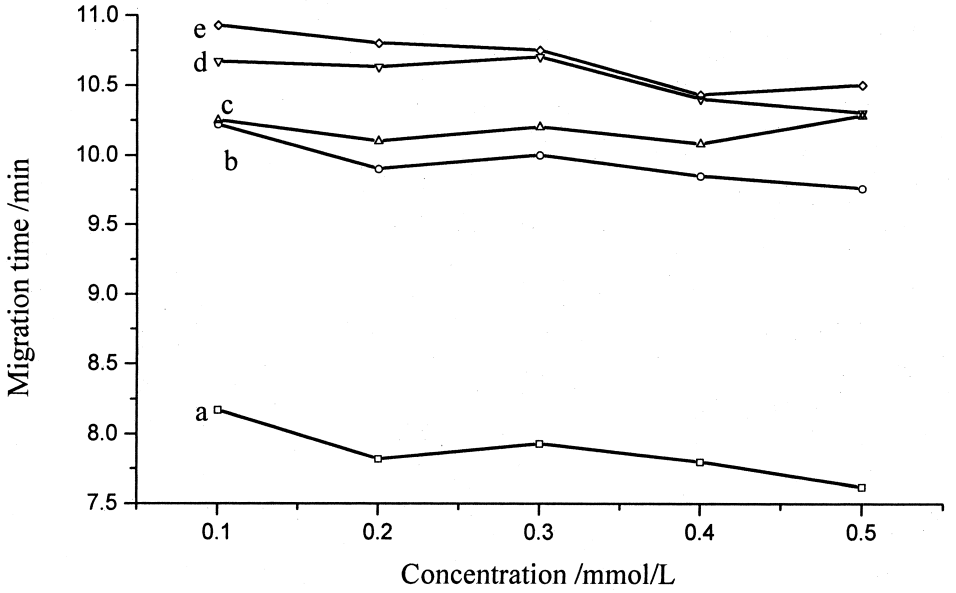

Fig. 2. Effect of CPB concentration on the migration times of polycarboxylic acids. Separation voltage, −15 kV; running buffer, 15 mmol/l phosphate, pH 7.0; detection potential, +0.14 V vs. SCE. (a) Oxalic acid, (b) malonic acid, (c) citric acid, (d) malic acid, (e) tartaric acid.

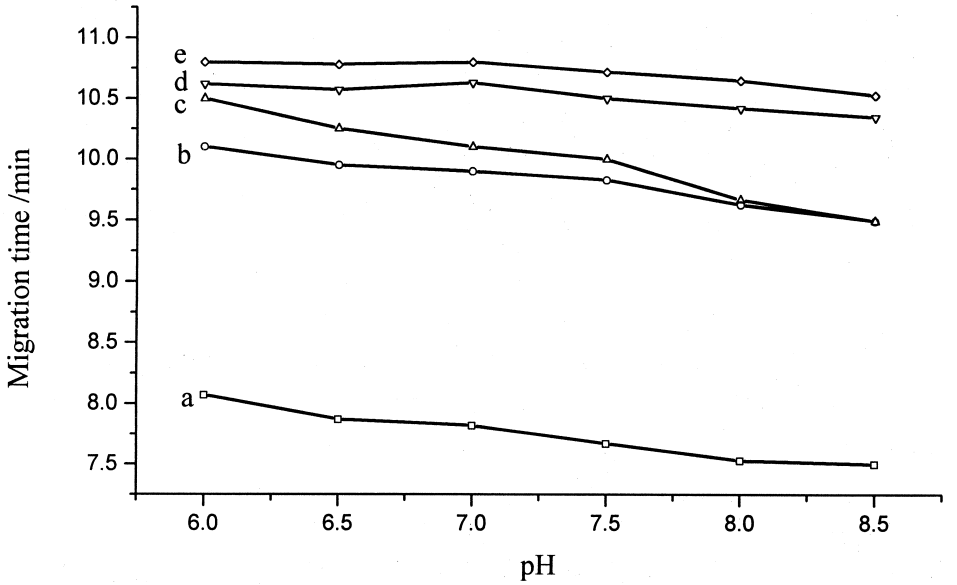

Fig. 3. Effect of the pH of the running buffer on the migration times of the polycarboxylic acids. Separation voltage, −15 kV; running buffer, 15 mmol/l phosphate+0.2 mmol/l CPB. Detection potential, +0.14 V vs. SCE. (a) Oxalic acid, (b) malonic acid, (c) citric acid, (d) malic acid, (e) tartaric acid.

in Fig. 3. The migration time of all analytes investigated decreased as the buffer pH increased, but malonic acid and citric acid were affected more significantly for their relatively high pK_a. When the buffer pH was 8.5, malonic and citric acid could not be separated completely. As far as the satisfactory separation and sensitive detection were concerned, the optimum pH was fixed at 7.0.

3.3. Separation of polycarboxylic acids

Fig. 4 shows the electropherogram recorded under the optimum conditions for a laboratory-prepared mixture containing seven polycarboxylic acids. The migration time of the analytes increased in the following order: (1) oxalic acid, (2) malonic acid, (3) citric acid, (4) malic acid, (5) tartaric acid, (6) aspartic acid, (7) glutamic acid. This order of the migration time is in accordance with the order of their charge-to-mass ratios. The larger one's charge-to-mass ratio is, the faster it elutes out. For example, aspartic and glutamic acids, which are dicarboxylic amino acids, trail far behind the others. It is because in neutral medium they exist in the form of zwitterions, $^{-}OOCCH_2CH(NH_3^+)COO^-$ and $^{-}OOCCH_2CH_2CH(NH_3^+)COO^-$, respectively, and there is only one net negative charge in their molecules. So their charge-to-mass ratios are lower than those of the other dicarboxylic acids with the similar mass to them, and their migration rates toward the anode (the detection end in our experiments) were slower than those of the other polycarboxylic acids. For this reason, separating them from the others is easy and they were not included in the above discussion to the optimization of separation of polycarboxylic acids.

3.4. Quantitative response of CE–ED

In order to examine the capabilities of the present CE–ED system to measure polycarboxylic acids, a series of related experiments were carried out. The initial study involved investigating the hydrodynamic voltammograms (HDVs) to assess the effect of the working potential on the current response in flowing system. The applied working potential was changed from the negative to the positive. The novel HDVs for polycarboxylic acids were obtained at the copper electrode under CE conditions with wall-jet amperometric detection and are shown in Fig. 5. All

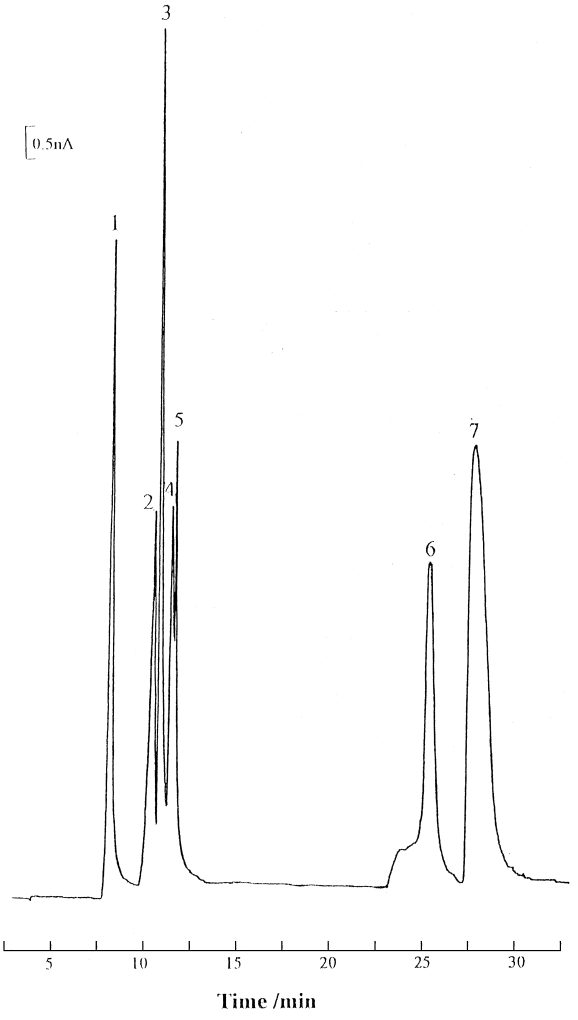

Fig. 4. Electropherogram of (1) oxalic acid (80 μmol/l), (2) malonic acid (800 μmol/l), (3) citric acid (50 μmol/l), (4) malic acid (160 μmol/l), (5) tartaric acid (160 μmol/l), (6) aspartic acid (80 μmol/l), (7) glutamic acid (80 μmol/l). Electrophoresis medium: 15 mmol/l phosphate buffer, pH 7.0+0.2 mmol/l CPB. Other conditions as in Fig. 2.

polycarboxylic acids investigated showed almost the same trends in response except the peak amplitudes. They were different from the S-shaped ones obtained by Stulik et al. [22] on a copper electrode for amino acids in neutral buffer solution, but similar somewhat to those reported by Zhou and Wang [24] on a copper-based chemically modified electrode for carboxylic acids in neutral phosphate buffer. When detection potentials were below 0.00 V, all polycarboxylic acids exhibited cathodic currents, which increased slowly as the potential shifted positively. In the voltage range above +0.02 V, anodic currents appeared and increased slightly with more positive potentials, exhibiting maximum currents at +0.14 V. The operating potential of +0.14 V was selected for CE–ED with the best stable baseline and the biggest current response.

The quantitative response of CE–ED to polycarboxylic acids was investigated and the results are summarized in Table 1. The linearity of CE–ED was evaluated by analyzing the standard solution with respect to the peak current. The peak currents varied linearly over 2~3-orders of magnitude of concentrations with correlation coefficients (r) of at least 0.99. The detection limits, which were calculated when $S/N=3$, were observed to be a wide variation. As far as citric acid was concerned, the detection limit was as low as 1.3 fmol or 0.5 μmol/l concentration. To our knowledge, such a low limiting detection has not yet been reported in literature. On the other hand, the present method is not so sensitive for other polycarboxylic acids, such as malonic acid with a detection limit of 97.5 fmol, and even has no response to some other organic acids as mentioned above.

Finally, the stability of the detection system was examined by injecting 80 μmol/l oxalic acid. The short-term stability was studied by 30 successive injections over a period of approximately 4 h. The relative standard deviation (RSD) of the current response to oxalic acid on the same electrode initially pretreated was 1.6%. After above experiments, the long-term stability of the detection system was checked by 10 successive injections of oxalic acid every day for four days running. During this period, the electrode was kept in the detection cell all time without any treatment. The responses obtained for oxalic acid slowly decreased day after day. Compared with the first day, the average response in the fifth day still remained 81%. Additionally, the variation of the baseline was quite small. Almost the same background current could always be obtained during the process of the above experiments after the electrode had been subjected to the working potential for about 15 min. These experimental results showed that the stability of the CE–ED system with a Cu electrode, based on a complexing mechanism, could

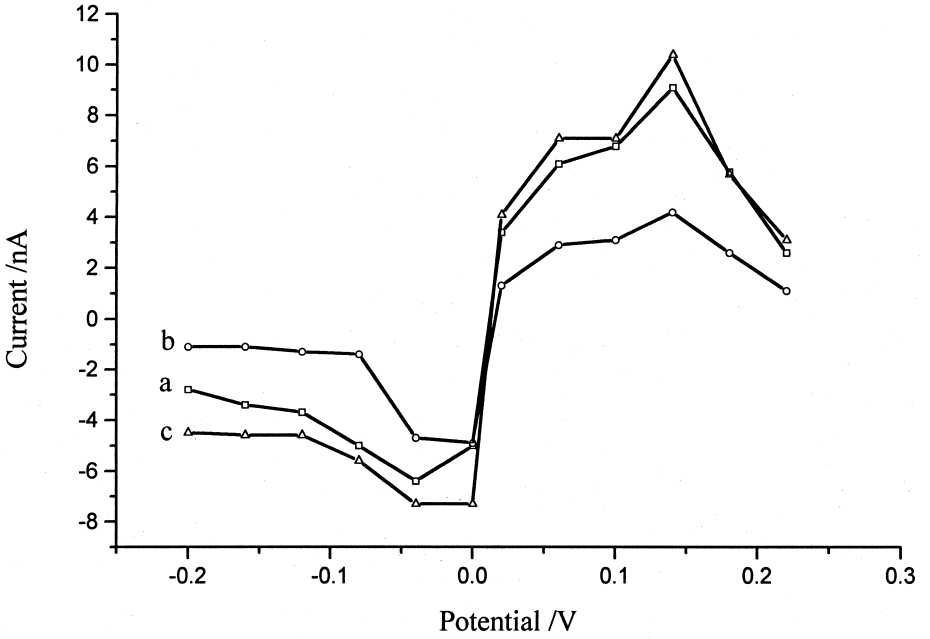

Fig. 5. Hydrodynamic voltammograms of (a) oxalic acid (80 μmol/l), (b) malonic acid (800 μmol/l), (c) citric acid (50 μmol/l). Other conditions as in Fig. 4.

be compared favorably with that of the reported CE–ED system, based on an electrocatalytic oxidation mechanism in strongly alkaline medium [16,18].

3.5. Determination of oxalic and citric acids in urine

Fig. 6 shows the electropherograms of the two different urine samples at pH 7.0. Peaks 1 and 2 in the electropherograms correspond to oxalic and citric acid, respectively, which could be identified by the following facts. At first, the heights of peaks 1 and 2 were enhanced prominently and no new peaks appeared after standard oxalic and citric acids were added in the samples as shown in (a′) and (b′). Secondly, the above results were also the same when the pH of the running medium was changed (the electropherograms are not given). If the other constituents except oxalic acid or citric acid in urine

Table 1
Quantitative responses of CE–ED to polycarboxylic acids[a]

Compound	Linear range (mol/l)	r^b	Detection limit[c] (fmol)
Oxalic acid	$2.5\cdot10^{-6}$~$4.0\cdot10^{-3}$	0.999	2.6
Malonic acid	$5.0\cdot10^{-5}$~$2.0\cdot10^{-2}$	0.994	97.5
Citric acid	$1.0\cdot10^{-6}$~$4.0\cdot10^{-3}$	0.999	1.3
Malic acid	$8.0\cdot10^{-6}$~$1.0\cdot10^{-3}$	0.995	15.0
Tartaric acid	$8.0\cdot10^{-6}$~$1.0\cdot10^{-3}$	0.992	13.8
Aspartic acid	$4.0\cdot10^{-6}$~$8.0\cdot10^{-4}$	0.998	7.5
Glutamic acid	$4.0\cdot10^{-6}$~$8.0\cdot10^{-4}$	0.998	7.0

[a] Experimental conditions as in Fig. 4.
[b] The number of points for linear regression calculation was seven.
[c] The injection volume was estimated to be 2.5 nl.

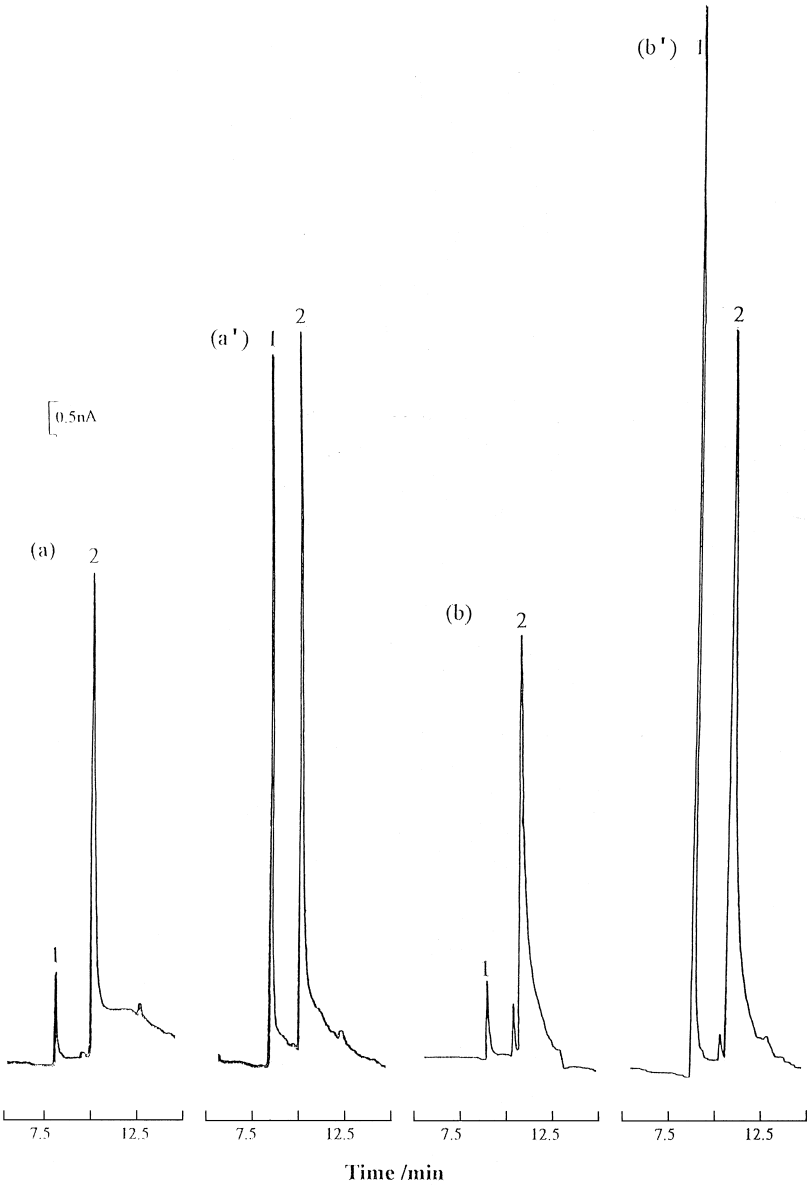

Fig. 6. Electropherograms of diluted urine samples with and without the standard solutions. (a) Urine sample A, a 10-fold dilution; (a′) (a)+8.0 μmol/l oxalic acid+15.0 μmol/l citric acid; (b) urine sample B, a 20-fold dilution; (b′) (b)+125.0 μmol/l oxalic acid+20.0 μmol/l citric acid. Running medium, 15 mmol/l phosphate buffer, pH 7.0. Other conditions as in Fig. 4.

were contained in peaks 1 or 2, they ought to be separated from each other because the analytes show different electrophoresis behaviors at the different pH. As soon as the peaks of oxalic and citric acids in the urinary electropherograms were confirmed, the quantitative analyses of urinary oxalic and citric acids were carried out easily by the calibration. The results are shown in Table 2. The concentrations determined in urine are in agreement with the normal values reported in Ref. [25].

Table 2
The concentrations and recoveries of oxalic and citric acids from three urinary samples[a]

No.	Oxalic acid (μmol/l)				Citric acid (μmol/l)			
	Concentration	Added	Found	Recovery (%)	Concentration	Added	Found	Recovery (%)
1	110.3	80.0	74.5	93	302.8	15.0	14.2	95
2	187.5	125.0	120.3	96	525.6	20.0	19.4	97
3	204.1	65.0	61.1	94	712.3	15.0	15.3	102

[a] Experimental conditions as in Fig. 6. Nos. 1, 2 and 3 were diluted 10-fold, 20-fold and 20-fold, respectively. Concentration was calculated from the determined value times the diluting fold.

4. Conclusions

Polycarboxylic acids, including oxalic acid, malonic acid, citric acid, malic acid, tartaric acid, aspartic acid and glutamic acid can be simultaneously determined by coupling CE with wall-jet amperometric detection as mentioned in this paper. This method has been proved to give a wide linear response range and low detection limits for these compounds. In particular, we have documented that the method is very suitable for the determination of oxalic and citric acids in urine with high sensitivity and little interference.

Acknowledgements

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