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Capillary electrophoresis with amperometric detection of curcumin in Chinese herbal medicine pretreated by solid-phase extraction

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Abstract

In the present study, curcumin from Chinese herbal medicine turmeric was determined by capillary electrophoresis with amperometric detection (CE–AD) pretreated by a self-designed, simple, inexpensive solid-phase extraction (SPE) cartridge based on the material of tributyl phosphate resin. An average concentration factor of 9 with the recovery of >80% was achieved when applied to the analysis of curcumin in extracts of turmeric. Under the optimized CE–AD conditions: a running buffer composed of 15 m*M* phosphate buffer at a pH 9.7, separation voltage at 16 kV, injection for 6 s at 9 kV and detection at 1.20 V, CE–AD with SPE exhibited low detection limit as $3\cdot10^{-8}$ mol/l ($S/N = 3$), high efficiency of $1.0\cdot10^{5}$ N, linear range of $7\cdot10^{-4}$–$3\cdot10^{-6}$ mol/l ($r = 0.9986$) for curcumin extracted from light petroleum. The method developed resulted in enhancement of the detection sensitivity and reduction of interference from sample matrix in complicated samples and exhibited the potential application for routine analysis, especially in food, because a relatively complete process of sample treatment and analysis was described.

Keywords: Turmeric; Pharmaceutical analysis; Curcumin

1. Introduction

Turmeric has been used for centuries in Chinese traditional system of medicine, as a spice and a natural food color. Curcumin (Fig. 1), the biologically active component of turmeric, has been reported to possess anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-bacterial, and anti-tumor features [1–3].

It fights free radical formations in blood and body tissues and helps to prevent various cardio-vascular, viral and other chronic diseases. These features were clinically proved.

The use of herbal products as alternative medicines is becoming more popular. However, the analysis of their active gradients present in herbal products encounters major difficulties due to the trace amount of pharmacologically active compounds and to the complexity of the matrix. In view of increasing demands for solving quality-related problems of herbal products, the development of simple and reliable methods for the purification and/or the sensitive and selective determination of active components in herbal products is essential [4].

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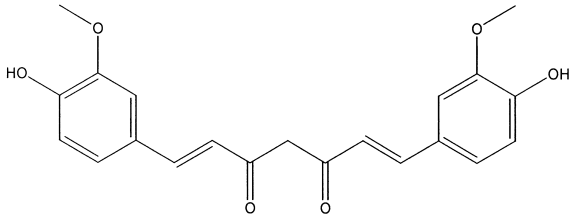

Fig. 1. Molecular structure of curcumin.

Paper or thin-layer chromatography followed by spectrophotometric analysis was described for determining the curcuminoids pigment. But the procedure was time-consuming and lacking in precision [5,6]. Two high-performance liquid chromatographic methods were reported that offered a more convenient and accurate means of separating and estimating individual curcuminoids [7,8], and gas chromatography with supercritical fluid extraction was also used [9]. But these methods were performed in organic solvents, band broadening was obvious and column efficiency was unsatisfactory.

In this paper, aqueous capillary electrophoresis (CE)–amperometric detection (AD) was used. As is well known that CE–AD integrated the high separation efficiency of CE with high sensitivity, high selectivity of AD [10,11], and it has been shown to be a very powerful and efficient tool for the analysis of a wide variety of analytes [12–14]. In parallel, efforts have been made to establish techniques for sample enrichment and pre-purification compatible with CE [15]. For the sample preparation, solid-phase extraction (SPE) currently seems to be the most common tactic because only simple procedures are needed and a small amount of solvent is used [16–19], and a large number of commercially available SPE cartridges were used in chromatographic separation, such as high-performance liquid chromatography, gas chromatography, thin-layer chromatography and were tested to be effective for one kind or several kinds of analytes [20,21]. But for CE analysis, the analyte containing extracts obtained by SPE is normally evaporated to dryness in a post extraction step and reconstituted in a suitable medium in order to preconcentrate the analytes of interest and to ensure direct compatibility with the CE system. The former aspect is of high importance owing to the limited detectability of CE, while the latter point is connected to the problems of injecting organic solvents in CE [22]. In our experiment, turmeric was extracted with light petroleum, but the aqueous CE–AD was incompatible with complex organic solution which might absorb onto the capillary column and foul the electrode at the end of the capillary. Furthermore, the complexity of the sample matrix made the separation and detection difficult. In our work, tributyl phosphate (TBP) resin was utilized as the adsorbent for extraction of a phenolic-analogue structure of curcumin. The feasibility of employing TBP resin as a new SPE material for the extraction of curcumin from organic phase in its molecular form to the aqueous phase in its ionic form was investigated, and the conditions of separation and detection were discussed.

2. Experimental

2.1. Chemicals

Curcumin was purchased from Sigma (St. Louis, MO, USA), sodium dihydrogenphosphate, disodium hydrogenphosphate, sodium hydroxide and light petroleum (b.p. 30–60 °C) of analytical grade were purchased from Beijing Chemical Reagent Factory. Tributyl phosphate resin (TBP) (65–70 mesh) was purchased from Beijing Nucleic Energy Institute (Beijing, China) and three kinds of turmeric collected in different areas of China were obtained from local drug stores and nonwoven fabric was laboratory made. All water used was double-distilled and all solutions were filtered through 0.22 μm membranes. Stock solutions consisted of 1 m*M* curcumin in 0.01 *M* NaOH and $5 \cdot 10^{-5}$ mol/l curcumin in light petroleum were wrapped with black paper and stored at 4 °C in the refrigerator. Fresh stock solution was made each day, because curcumin was light sensitive and unstable in neutral or alkaline pH. The pH of the Na_2HPO_4–NaH_2PO_4 buffer solution was adjusted by adding NaOH solution with the same concentration and pH was measured by a Φ-60 pH meter (Beckman, USA), Moreover, buffer solutions were degassed under sonication before electrophoresis.

2.2. CE–AD system

Electrophoresis was driven by a high-voltage power supply (9323-HVPS, Beijing, China). Cyclic voltammetry and amperometric detection were carried on a CHI 832 potentiostat (CH Instruments, USA) connected with a detection cell based on a wall-jet configuration. A conventional three-electrode system was used including a 33-μm diameter carbon fiber disk electrode, an Ag/AgCl reference electrode and a Pt wire counter electrode. The detailed procedures for the preparation of the working electrode and detection cell were described elsewhere [23,24]. To minimize the interference of external electric noise, the electrochemical cell was housed in a laboratory-made Faraday cage, and the outlet end of the capillary was always maintained grounded through a stainless steel tube. Uncoated fused-silica capillaries with a 25-μm inner diameter and 360 μm outer diameter cut to 32 cm in length were used. A new capillary was washed with 0.1 *M* NaOH overnight. In order to obtain good reproducibility, a reactivation process between runs was done by flushing the capillary with double-distilled water, 0.1 *M* NaOH, double-distilled water and buffer solution for 90, 90, 90 and 180 s, respectively, then maintaining the detection potential for 2 min.

2.3. Sample preparation and extraction equipment

An amount of about 10 g turmeric was ground into fine particles, then 8.8237 g weighed accurately fine particles were extracted with 100 ml light petroleum under continuous stirring for 20 h. The collected extracts were filtered and diluted to 250 ml and stored at 4 °C and marked as sample 1, sample 2 and sample 3. A simple extraction device for SPE was illustrated in Fig. 2. A 1-cm I.D. medical syringe was employed as the extraction cartridge filled with 0.6 g tributyl phosphate resin. Two pieces of nonwoven fabric with 1 cm diameter were served as a filtration membrane, which allowed organic and inorganic reagents to pass while simultaneously avoiding TBP resin leakage and large dead volume. When applied to extract standard curcumin solution prepared in light petroleum, the cartridge can be used at least ten times without significant deactivation. But for extraction of curcumin from turmeric in light petroleum, only three times can be used, because some hard to evaporate oils from Turmeric retained in the cartridge, which affected the extraction recovery.

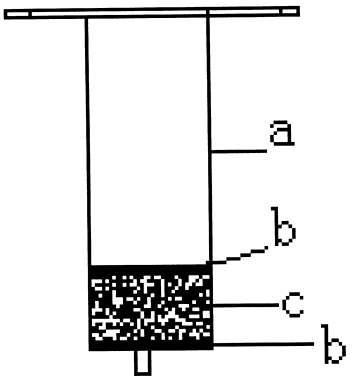

Fig. 2. Basic principle of SPE device. a = medical syringe; b = nonwoven fabric; c = TBP resin.

3. Results and discussion

3.1. Optimum conditions of CE–AD

Amperometric detection of curcumin was based on the electrochemical reaction of analytes on the carbon fiber electrode surface. From cyclic voltammograms in Fig. 3, curcumin can be oxidized above +0.8 V although no significant oxidation peak was observed. In order to determine the exact optimal potential for amperometric detection, hydrodynamic voltammograms were acquired by repeating several injections of the same solution into CE system and recording the peak current among varying applied potentials. As shown in Fig. 4, typically the anodic current starts at 0.8 V and rises rapidly and eventually a value of 1.2 V was selected as a comprise of high sensitivity and low background current because higher potential usually leads to large background currents.

The amount of the sample introduced into the capillary by electromigration depends on the EOF (electroosmotic flow) and the electrophoretic mobility of the solute [25]. In the present work, the effect

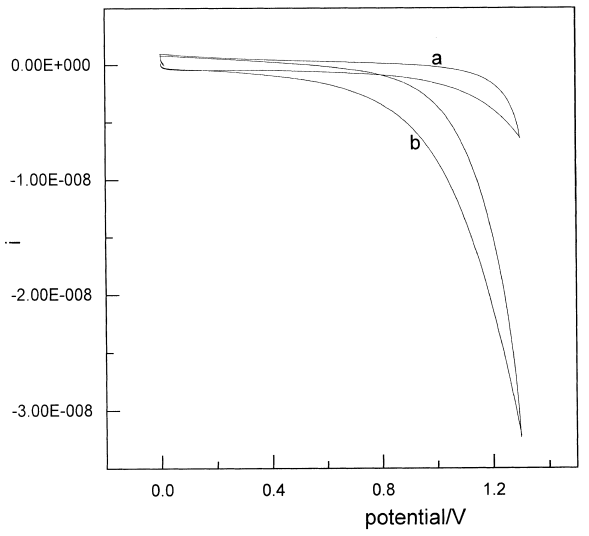

Fig. 3. Cyclic voltammograms of a: 10 m*M* NaH_2PO_4–Na_2HPO_4 buffer, pH 7.20, b: with 10^{-4} mol/l curcumin. carbon fiber electrode, 33 μm diameter; separation capillary, 32 cm×25 μm I.D., 360 μm O.D.; scan rate: 50 mV/s.

of the injection time and injection voltage on peak current (i_p) and number of theoretical plates (N) was studied. N was calculated according to the following equation:

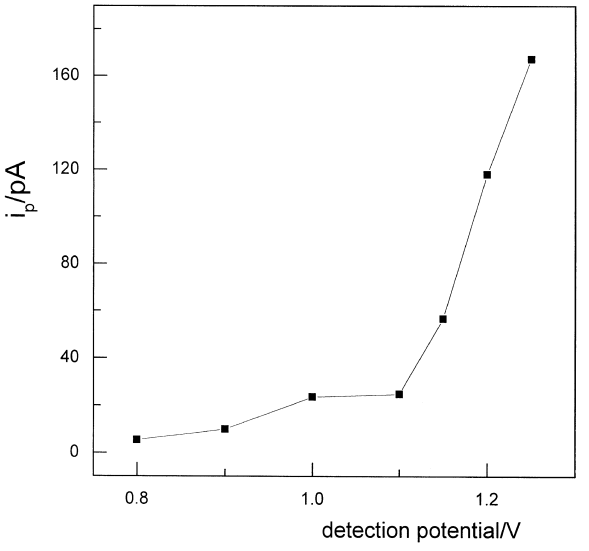

Fig. 4. Hydrodynamic voltammogram of 10^{-4} mol/l curcumin. NaH_2PO_4–Na_2HPO_4 buffer, 10 m*M*, pH 7.20; sample injection, 3 s at 10 kV; separation voltage, 15 kV.

$$N = 5.54(t_m / W_{1/2})^2 \quad (1)$$

where t_m is the migration time and $W_{1/2}$ is the width at half height of the electrophoretic peak. It was expected that low voltage for a long time was superior to high voltage for a short time [26]. As is shown in Fig. 5A, 6 s at 10 kV showed the maximum i_p and N, shorter time or longer time led to smaller N and i_p. Then the injection time was fixed at 6 s, the effect of injection voltage (Fig. 5B) showed the similar trend as in Fig. 5A, 9 kV was obviously the suitable injection voltage. Thus, by electrokinetic injection for 6 s at 9 kV it produced the best result.

Buffer concentration (C_b) was optimized by varying from 5 to 40 m*M*. Data listed in Table 1 show that with increasing C_b, t_m and N increased. But the maximum value of i_p was obtained at 15 m*M*. This result was a complex of many factors, mainly related to the change of double-layer thickness and the differences of ionic strength between buffer and sample zone, and then the ζ potential and the migration velocity [27], because higher C_b results in thinner double layer and lower ζ potential, hence lower electroosmotic flow (EOF). Considering that the noise and migration time increase with increasing C_b, 15 m*M* phosphate buffer was used in the following experiment because of largest i_p and better N.

The influence of buffer pH is of capital importance since when the pH varies, so does the EOF, the state of ionization of analytes in the sample zone and their effective charges change too. Therefore their apparent mobility and all the parameters affecting the efficiency and selectivity of the separation will be modified [28]. A wide range of pH from 7.20 to 10.50 was studied. The experimental dependence was illustrated in Fig. 6, the best N and higher i_p were obtained at pH 9.7. This result may reflect the change of electroactivity due to the deprotonation of curcumin, agreed with Fadhil and Fatima [29]. Since curcumin was light sensitive and easily degraded in alkaline solutions, pH 9.7 was acceptable.

Another important parameter affecting separation and detection is separation voltage, which was selected as 16 kV. Experimental result after data treatment (not listed) agreed well with Jorgenson [30].

Under the optimized conditions of CE–AD, the

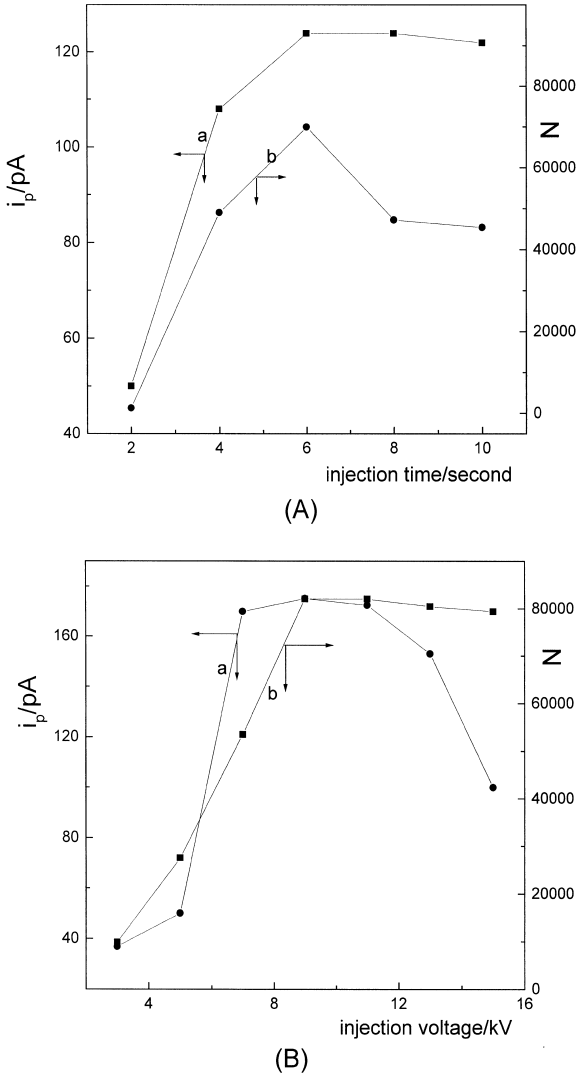

Fig. 5. (A) Effect of injection time on i_p and N; a: i_p of 10^{-4} mol/l curcumin, b: N of 10^{-4} mol/l curcumin; injection voltage, 10 kV; NaH_2PO_4–Na_2HPO_4 buffer, 10 mM, pH 7.20; detection potential, 1.20 V; separation voltage, 15 kV. (B) Effect of injection voltage on i_p and N; a: i_p of 10^{-4} mol/l curcumin, b: N of 10^{-4} mol/l curcumin; injection time, 6 s; NaH_2PO_4–Na_2HPO_4 buffer, 10 mM, pH 7.20; detection potential, 1.20 V; separation voltage, 15 kV.

limit of detection of 10^{-6} mol/l ($S/N = 3$) was obtained, which was relatively high compared with other CE–AD applications. Thus it greatly diminished the applicability in other sample matrix.

Table 1
Effect of buffer concentration (C_b) on migration time (t_m), i_p and N

C_b/mM	t_m/s	i_p/pA	N
5	180	89.5	8921
10	207	73.7	10 432
15	223	90	15 789
20	236	71.7	18 572
25	247	72	19 375
30	251	63	21 452
35	257	57	22 753
40	262	32	23 520

Conditions: NaH_2PO_4/Na_2HPO_4 buffer, pH = 7.20; electrokinetic injection, 6 s at 9 kV; detection potential, 1.20 V; separation voltage, 15 kV.

3.2. SPE procedure

As mentioned in Section 3.1, the limit of detection was not sufficient for a critically analytical task, due to the presence of some oil which was difficult to evaporate and carbon fiber microelectrode was easily fouled. Moreover, negative peak and an unstable baseline were observed because of organic solvents used in CE. The SPE method proposed in the present work can overcome these disadvantages. In our work, 3 ml $1 \cdot 10^{-6}$ mol/l curcumin in light petro-

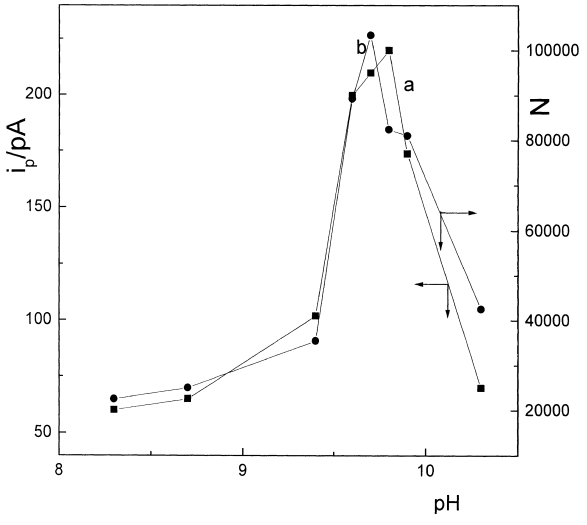

Fig. 6. Effect of pH on i_p and N. a: i_p of 10^{-4} mol/l curcumin, b: N of 10^{-4} mol/l curcumin. NaH_2PO_4–Na_2HPO_4 buffer, 15 mM; detection potential, 1.20 V; electrokinetic injection, 6 s at 9 kV; separation voltage, 15 kV.

leum was taken as the standard sample throughout the SPE investigation experiment. Typically, the cartridge was activated and conditioned first with 2 ml 0.01 *M* NaOH and double-distilled water until the final eluent was neutral, and then 3 ml $1\cdot10^{-6}$ mol/l curcumin prepared in light petroleum was introduced into the cartridge, after seconds of adsorption process, diluted NaOH solution was injected by a microinjector, the NaOH eluent was collected and injected directly into CE without further treatment. The effect of eluent pH on the recovery was investigated. The recovery was calculated by comparing the amount of curcumin extracted from standard sample with that of the original standard sample. As is shown in Fig. 7, the recovery was independent of the eluent pH, higher pH corresponds to higher recovery, but higher pH means higher concentration of NaOH, which resulted in large background noise under a detection potential of 1.20 V, finally, pH 9.5 was used because it produced a relatively higher recovery with a lower pH. The effect of the eluent volume on the recovery and concentration factor was evaluated by varying the eluent volume from 0.1 to 0.55 ml. The result is shown in Table 2, the recovery increased with the increase of the eluent volume, in contrast, the concentration factor decreased. As a comprise of high recovery and high concentration factor, the eluent volume of 0.3 ml was chosen, under the optimized desorption conditions, the recovery of the extraction of curcumin in light petroleum was above 85%.

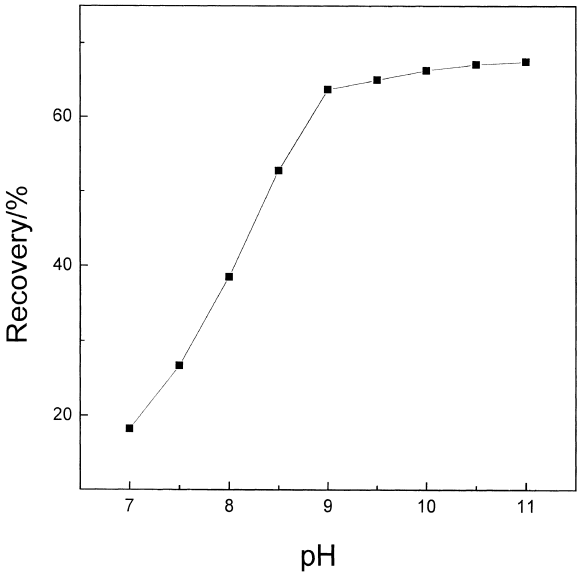

Fig. 7. Effect of eluent pH on extraction recovery. Extraction sample: 3 ml light petroleum spiked with 10^{-6} mol/l curcumin, eluent volume: 0.2 ml, separation voltage at 16 kV, injection 6 s at 9 kV, detection at 1.20 V, 15 m*M* phosphate buffer at pH 9.7.

Table 2
Effect of eluent volume on the extraction recovery and preconcentration factor

Eluent volume (ml) [a]	Recovery (%)[b] $n=6$	Concentration factor[c] $n=6$
0.1	38	11.4
0.15	51	10.3
0.20	67	10.5
0.25	78	9.4
0.30	85	8.5
0.35	85	7.4
0.40	86	6.5
0.45	87	5.8
0.50	89	5.3
0.55	91	4.8

CE–AD conditions: 15 m*M* NaH_2PO_4/Na_2HPO_4 buffer, pH = 9.7; electrokinetic injection, 6 s at 9 kV; detection potential, 1.20 V; separation voltage, 16 kV.

[a] Eluent pH was fixed on 9.5.

[b] Recovery was calculated by comparing the amount of the extract from standard curcumin solution in petroleum ether with that of the 3-ml 10^{-6} mol/l curcumin in petroleum ether.

[c] Concentration factor was calculated by comparing the peak current of the extract of the standard curcumin solution in petroleum ether with that of the 10^{-6} mol/l standard curcumin solution in NaOH solution.

During the extraction experiment, we tried to keep the eluent of H_2O, NaOH, light petroleum to pass the cartridge by gravity with a slow flow-rate to ensure a complete adsorption–desorption process. But it was difficult since light petroleum is not miscible with water, and a soft pressure was usually exerted to push down a small quantity of the eluent until it could drop by gravity. Channeling of the resin during the extraction process was inevitable and it was the important reason to exchange a new cartridge after several extractions.

3.3. Validation of SPE applied to CE–AD

Based on the fundamental work and optimization reported above, curcumin in turmeric from different drug stores with different producing area was quantified. Electropherograms of sample a:10^{-5} mol/l standard curcumin solution prepared in NaOH, b: the

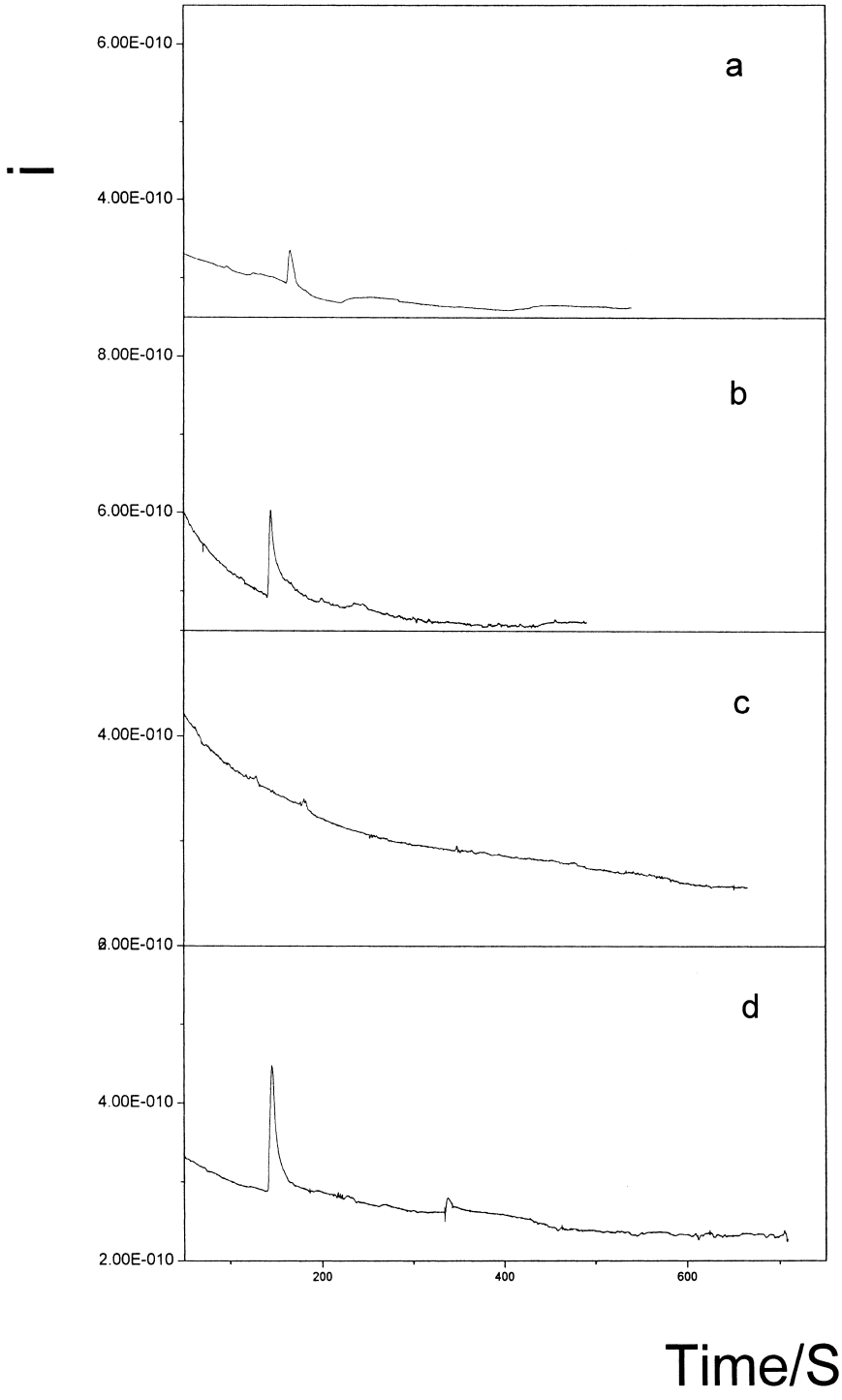

Fig. 8. Electropherograms of samples (a) 10^{-5} mol/l standard curcumin solution prepared in NaOH, (b) the extract of sample 1 defined in Section 2.3, (c) light petroleum after extracted of curcumin of sample 1, (d) the extract of sample 1 spiked with 10^{-6} mol/l curcumin. Extraction condition: 0.3 ml NaOH solution with pH 9.5, CE–AD conditions: separation voltage at 16 kV, injection 6 s at 9 kV, detection at 1.20 V, 15 m*M* phosphate buffer at pH 9.7.

Table 3
Validation result[a] for SPE-CE–AD of curcumin in different turmerics

	RSD of concentration $n=6$	Recovery (%)[b] $n=6$	Concentration[c] ($\times 10^{-7}$mol/l) $n=6$	Curcumin content (mg/g) $n=6$
Sample 1	6.3	85.5	2.42	0.32
Sample 2	5.7	80.6	4.79	0.63
Sample 3	4.5	81.0	3.80	0.50

CE–AD conditions: 15 m*M* NaH_2PO_4/Na_2HPO_4 buffer, pH=9.7; electrokinetic injection, 6 s at 9 kV; detection potential, 1.20 V; separation voltage, 16 kV.

[a] Extraction conditions: 0.3 ml NaOH solution with pH 9.5.

[b] Recovery was calculated by comparing the measured amount of the curcumin added into the petroleum ether solution of turmeric with the known amount of the curcumin added.

[c] Concentration means the original concentration of curcumin in 250 ml petroleum ether.

extract of sample 1 defined in Section 2.3, c: light petroleum after extracted of curcumin of sample 1, d: the extract of sample 1 spiked with 10^{-6}mol/l curcumin were shown in Fig. 8. From Fig. 8, we can conclude that an absolute amount of curcumin retained in the cartridge, since there is no obvious peak observed in electropherogram c. And the recovery calculated by comparing electropherograms a, b and c seems to be acceptable relative to the extraction recovery of the standard curcumin sample prepared in light petroleum described in Table 2. The analytical results of three kinds of turmeric were listed in Table 3, repetitive extraction and CE varied between 4.5 and 6.3% RSD of concentration and 80.6–85.5% recovery ($n=6$), and the recovery seemed to be lower than that of the extraction of the standard curcumin sample prepared in light petroleum. Under the optimized conditions: separation voltage at 16 kV, injection 6 s at 9 kV, detection at 1.20 V, 15 m*M* phosphate buffer at pH 9.7, capillary and electrode were dealt with as described above, samples from light petroleum were preconcentrated in the TBP resin cartridge with a concentration factor average of 9, the validation result was shown below: the limit of detection was $3\cdot10^{-8}$ mol/l ($S/N=3$), the standard curve was linear between $7\cdot10^{-4}$ and $3\cdot10^{-6}$ mol/l, the calibration equations and regression coefficients for curcumin were $y=0.2074x-0.9090$, $R^2=0.9986$, the within-day variations of peak current and migration time after 10 replicate extractions and electrophoresis were 5.1 and 3.2%, respectively.

4. Conclusions

The present study demonstrated a simple and sensitive method to determine curcumin in tumeric by CE–AD with a simple, inexpensive and self-made extraction cartridge loaded with tributyl phosphate resin. Due to high sensitivity and selectivity of this method, the method could be used to detect trace amounts of curcumin in more complex sample matrix, such as curry powder, herbal products or body fluids. It would exhibit great promise in pharmaceutical, food and clinical analysis.

Acknowledgements

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