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Rapid and sensitive determination of anthraquinones in Chinese herb using 1-butyl-3-methylimidazolium-based ionic liquid with β-cyclodextrin as modifier in capillary zone electrophoresis

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Abstract

The present study reported the ionic liquid (IL) used as running electrolyte in capillary zone electrophoresis (CZE) with β-cyclodextrin (β-CD) as modifier for the separation of anthraquinones extract of Chinese herb *Paedicalyx attopevensis* Pierre ex Pitard. The optimum running electrolyte was 60 mM 1-butyl-3-methylimizolium tetrafluoroborate (1B-3MI-TFB) solution with 4.0 mM β-CD. The pH was 10.00 and the applied voltage was 20 kV with detection at 254 nm. The present method was compared with others and the effect of Joule heating was discussed as well. More significantly, this method is the development of the ionic liquids application to the capillary electrophoresis.

Keywords: Capillary zone electrophoresis; Ionic liquids; 1-Butyl-3-methylimizolium tetrafluoroborate; β-Cyclodextrin; Anthraquinones

1. Introduction

Ionic liquids (IL), sometimes called molten salts, are liquids at ambient temperatures and consist entirely ionic species. In the past, they were mainly of interest to electrochemists. However, recently they have become apparent that a wide range of chemical reactions can be conducted using this class of solvents [1], such as liquid–liquid extraction [2,3], organic synthesis [4–8], electrochemistry [9,10], catalysis for clean technology [11,12], ultralow volatility liquid matrixes for matrix-assisted desorption/ionization mass spectrometry [13], and separations [14–20]. Ionic liquids that consist of a bulky pyridinium or imidazolium cation paired with a variety of anions [9,21] have lots of properties of conventional organic solvents, such as excellent solvation qualities, a low viscosity, and a wide temperature range [22–24]. But, they have two characteristics of nonvolatility and high electrical conductivities that conventional organic solvents have not [4,22,23,25]. Ionic liquids are environmentally benign, nonvolatile, and nonflammable with a high thermal stability [25]. The application of ionic liquids for the separation of various compounds has been recently recognized. Yanes et al. reported the development of a fairly robust capillary electrophoresis method for the separation of polyphenols found in grape seed extracts in which the ionic liquids were used as the only background electrolyte [15]. And recently, Yanes et al. developed a CE method for the same analysis using l-alkyl-3-methylimidazolium-based ionic liquids as the background electrolyte [16]. Qin et al. reported that 1,3-dialkylimidazolium-based room temperature ionic liquids as background electrolyte and coating material in aqueous CE [19]. Mwongela et al. reported the use of ionic liquids as modifier in the separation of the achiral and chiral analytes in micellar electrokinetic chromatography [20].

In CE, cyclodextrins (CDs) used as chiral and enantiomeric selectors for separation has been reviewed many times, CDs are ideally suitable due to their well-documented ability to include in their cavity proper guest molecules. The unusual properties of CDs originate in their unique structure.

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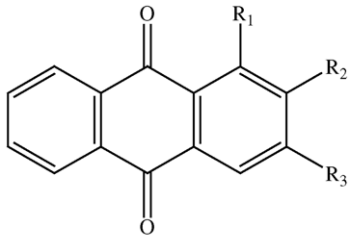
CDs consist of a family of oligosaccharides, composed of glucose units connected to each other through a 1,4-glucoside bond. Despite a hydrophilic surface (the outside region due to the presence of hydroxyl groups-position 2, 3 and 6 of glucopyranose), CDs contain a hydrophobic cavity. It is the presence of this cavity that enables CDs to entrap analytes without formation of formal chemical bonds [26–28]. CDs are now widely used as run buffer additives for CE analyses. It has been shown that the selectivity of CE is enhanced by using CDs as chiral selectors due to their ability to include a wide variety of water-insoluble molecules into their hydrophobic cavity [29]. The effect of CDs on the spectral properties of guest molecules has led to their use as reagents in various spectrometric analyses, including UV–visible spectrophotometric analysis [30], fluorescence and chemiluminescence methods [31,32], and nuclear magnetic resonance spectroscopy [33]. In this work, β-CD was firstly used in the IL as running electrolyte, and its effect was discussed.

Paedicalyx attopevensis Pierre ex Pitard (Rubiaceae) [34] has been used as folk medicine in China for the treatment of icterus hepatitis. To exploit this herb, we investigated the chemical constituents of it and found that it contains anthraquinones such as l,3-dihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-*O*-β-D-xylosyl(1-6)-β-D-glucoside (**1**), 1-hydroxy-2-methoxy-3-hydroxymethyl-9,10-anthraquinone-1-*O*-β-D-glucoside (**2**), 1-methoxy-2-methyl-3-hydroxy-9,10-anthraquinones(rubiadin-l-methylether) (**3**), 1-methoxy-2-formyl-3-hydroxy-9,10-anthraquinone (**4**), and other compounds. The structures of **1**–**4** were shown in Fig. 1. Anthraquinones are best known as antioxidant activity and antipyretic agents [35], anti-tumor promoters, Epstein–Barr virus activation [36], anti-human cytomegalovirus activity [37]. In our laboratory, it was found that **4** has good cytotoxic activity to the human hepatocellar carcinoma cell approved by SRB assay (Sulforhordamine B) (IC_{50} = 16). They are also widely distributed in *Rubiaceae* plants. So *Paedicalyx attopevensis* Pierre ex Pitard may be a resource for extracting these active compounds. They had been analyzed by many techniques, such as colorimetry [38], thin-layer chromatography (TLC) [39,40] and high-performance liquid chromatography (HPLC) [41,42]. However, the method of TLC cannot be applied to simultaneously determine several components in single crude herb or in a medicinal preparation [43–45]; HPLC suffers from limitation such as consumption of materials and time and the number of prior steps often required to obtain the species of interest from the sample matrix. Up to now, there is no report on simultaneous determination of these four mainly anthraquiones compounds in *Paedicalyx attopevensis* Pierre ex Pitard. Thus, it is necessary to develop a simple, economical and efficient method for the simultaneous determination of these compounds in this herb.



1, R_1 = OH R_2 = CH_2OH R_3 = O-Glu-Xyl

2, R_1 = O-Glc R_2 = OCH_3 R_3 = CH_2OH

3, R_1 = OCH_3 R_2 = CH_3 R_3 = OH

4, R_1 = OCH_3 R_2 = CHO R_3 = OH

Fig. 1. The structures of the analytes (Glu, glucose; Xyl, xylose; Glc, galactose); (**1**) 1,3-dihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-*O*-β-D-xylosyl(1-6)-β-D-glucoside, (**2**) 1-hydroxy-2-methoxy-3-hydroxymethyl-9,10-anthraquinone-1-*O*-β-D-glucoside, (**3**) 1-methoxy-2-methyl-3-hydroxy-9,10-anthraquinones(rubiadin-1-methylether), (**4**) l-methoxy-2-formyl-3-hydroxy-9,10-anthraquinone.

In the present work, a simple and sensitive method based IL was developed. The IL used in this study was 1-butyl-3-methylimidazolium tetrafluoroborate (1B-3MI-TFB) because of its high conductivity and good solvating properties, and which was shown to improve the resolution of the analytes. The purpose of this study was to demonstrate that capillary zone electrophoresis with UV absorption detection is capable of analysis anthraquinones in Chinese herb using IL as main running electrolyte and β-CD as modifier. The concentration of IL, β-CD, pH of running electrolyte and applied voltage were investigated in order to achieve satisfactory separation and good sensitivity.

2. Experimental

2.1. Apparatus

A Waters Quanta 4000 Capillary system (Millipore, Waters Chromatography Division of Milford, MA, USA) was used. Capillary electrophoresis was performed using a 50.0 cm (42.4 cm to the detector) × 75 μm I.D. fused-silica capillary (Yongnian Photoconductive Fibre Factory, Hebei Province, China). The data acquisition was carried out with a Maxima 820 Chromatography Workstation. Sample was introduced from the end of the capillary by hydrodynamic injection where the sample vial was raised by 10.0 cm for 5 s. Direct UV detection was employed at a wavelength of 254 nm. The capillary was prior to its first use by consecutively flushing with 0.5 M NaOH for 10 min, deionized water for 10 min and the electrophoresis buffer for 10 min. All operation were controlled at 25.5 ± 0.5 °C. A PHS-10A acidity meter (Xiaoshan Science Instrumentation Factory, Zhejiang, China) was used for the pH measurement.

2.2. Materials

Most of 1,3-dialkyimidazolimum-based ionic liquids are water, air stable and have the good characterization of

separation for CE, the application of ionic liquids in CE separation was focused on the several kinds of them frequently, 1-butyl-3-methylimidazoium tetrafluoroborate is also water, air stable and especially it has the good characterization of separation for CE. In this study, 1-butyl-3-methylimidazoium tetrafluoroborate (1B-3MI-TFB) used as running electrolyte for capillary electrophoresis system was synthesized from 1-butyl-3-methylimidazolium chloride according to literature [16].

The four anthraquinones used as standards were gifts to the authors and their characterization by NMR, IR, MS were obtained from the state key laboratory of OSSO, Lanzhou Institute of Chemical Physics (see Fig. 1 for the structures), Lanzhou, China. *Paedicalyx attopevensis* Pierre ex Pitard was collected from Hekou city, Yunnan province, China, and identified by Prof. Wang Wenjiu, Southwest Forest College, Kunming, China. β-CD was purchased from China Medicine Group, Shanghai Chemical Reagent Company, Shanghai, China.

Hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol, dimethylsulphoxide (DMSO) were of analytical regent grade. Deionized water was used throughout.

2.3. *Paedicalyx attopevensis Pierre ex Pitard sample preparation*

A portion (1.0000 g) of the *Paedicalyx attopevensis* Pierre ex Pitard powder was weighed into a 25-ml sample vial. Ethanol (20 ml) then was added into it. The sample vial was put in an ultrasonic bath and extracted for 1 h. Extraction was repeated three times. The extraction solutions were combined together and the organic solvent was evaporated. Then, the residue was dissolved with ethanol–dimethylsulphoxide (4:1, v/v) to 10 ml. All the solutions were passed through a 0.45 μm cellulose acetate filter (Shanghai Xinya Purification Apparatus Factory, Shanghai, China) before being injected into the capillary electrophoresis system.

2.4. *Electrophoretic procedure*

New capillaries were conditioned by rinsing with 0.5 M sodium hydroxide for 10 min, deionized water for 10 min, and then the running electrolyte for 10 min. Between each run, the capillary was rinsed with deionized water for 2 min, 0.5 M sodium hydroxide for 2 min, deionized water for 2 min, and then the running electrolyte for 2 min, successively.

Running electrolytes for the electrophoretic runs included 1B-3-MI-TFB [20–80 mM] and β-CD [1–6 mM] were prepared in deionized water.

3. Results and discussion

To achieve satisfactory separation using 1B-3MI-TFB as running electrolyte, the combined effects of 1B-3MI-TFB concentration, β-CD concentration, the pH of running electrolyte and applied voltage on the migration time and selectivity of anthraquinones were investigated.

3.1. *Mechanism of separation using 1B-3MI-TFB and β-CD*

In general, the presence of ionic liquids as running electrolytes provides an acidic environment, the imidazolium ions coat the capillary walls thus engendering anodic electroosmotic flow (EOF). In this study, the anthraquinones migrated after the neutral solvent. Current theories of CE hold that the EOF emanates from the migration of the loosely hold counterions (in this case, the tetrafluoroborate anion) in the outermost layer of the electrical double layer and that the magnitude of this EOF is primarily a function of the electrophoretic mobility of this ion. It was reported that the mobility of the ions responsible for the EOF in a given system is also subject to the ion association constants with the immobilized counterions in the system [46]. In this investigation, the weaker association between the ionic liquid cation and its counterion is responsible for the dissolution in CZE, and which provided the possibility that 1B-3-MI-TFB and β-CD affect together to the separation. As illustrated in Fig. 2, the anthraquinones may associate with the imidazolium ions or with the β-CDs, they may be entirely or partly embedded in the cavity of the β-CDs, so the association with the free imidazolium ions in the bulk solution is weaken. This association could be partially driven by the hydrophobic, hydrogen bonding, or by the ion-dipole/ion-induced-dipole interactions between the anthraquinones and the 1B-3MI-TFB. And those analytes, which was not embedded in the cavity of the β-CDs have rather stronger association with the imidazolium ions in the system. Therefore, the association between the analytes and the imidazolium ions was different. Consequently, this different association or embedding makes the mixture of anthraquinones to be separated excellently.

Joule heating is a critical factor for efficient separation with HPCE. It is caused mainly by the conductivity of the electrolyte and the voltage applied across the capillary. And, the conductivity directly affects the current. It is depended mainly on the charges of the electrolyte. In the experiment, it was observed that the current was smaller compared to the traditional borax buffer (Table 1), which can decrease the effect of Joule heating. The ionic liquids have good electrical

Table 1
The current (μA) of the 1B-3MI-TFB solution and the borax buffer[a]

	20 mM	40 mM	60 mM	20 mM (pH 10.00)	20 mM + 4 mM β-CD (pH 10.00)
Borax buffer	60.0	152.0	270.0	120.0	135.1
1B-3MI-TFB	37.1	78.2	124.0	62.1	45.4

[a] The applied voltage was 20 kV.

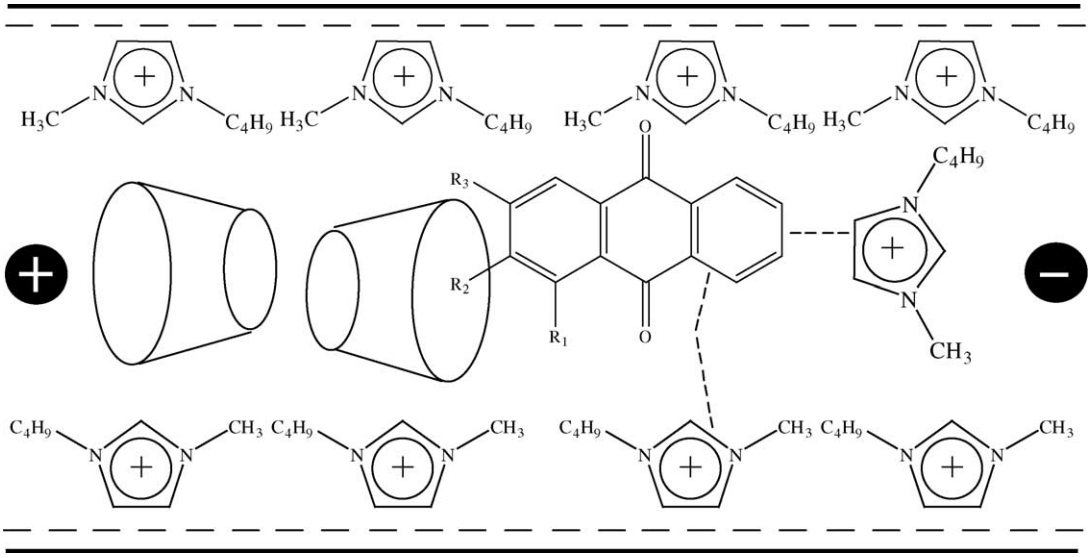


Fig. 2. Mechanism of the anthraquinones' separation using 1-butyl-3-methylimidazolium-based ionic liquid and β-CD.

conductivities [47,48] and excellent solvation qualities in water. The 1B-3-MI-TFB ionic liquid exists as the form of positively charged imidazolium groups and free $[PF_4]^-$ in water, and in the capillary some positively charged imidazolium groups may associate with capillary wall and the analytes. Because the borax exists as absolute $[Na]^+$, $[B_4O_7]^{2-}$ or $[H_2BO_3]^-$ ion, the charge of 1B-3-MI-TFB was smaller than that of borax buffer at the same concentration. Therefore, in the same applied voltage, the current of ionic liquid electrolyte is smaller than that of borax buffer. In addition, from Table 1, it was also found that the addition of β-cyclodextrin resulted in an increase in current for borax buffer but a decrease in current for the ionic liquid. This is the same principle as mentioned above. The current was smaller, accordingly the joule heating was decreased and efficient separation was obtained.

3.2. Effect of 1B-3MI-TFB concentration

To examine the imidazolium–anthraquinones association, the migration time of the EOF and the anthraquinones were plotted versus the concentration of the 1B-3MI-TFB electrolyte solution, respectively (shown in Fig. 3A). From Fig. 3A, it can be seen that the migration time of the EOF and the analytes decrease when the concentration of 1B-3MI-TFB increased from 20 to 80 mM. In order to achieve good sensitivity, we also investigated the effect of 1B-3MI-TFB concentration on peak height and peak area (shown in Fig. 3B and C, respectively). Fig. 3B and C indicated that the peak height and peak area increased with the increasing of 1B-3MI-TFB concentration from 20 to 60 mM and then decreased with further increasing of 1B-3MI-TFB concentration from 60 to 80 mM. In order to obtain good sensitivity, and short analysis time, 60 mM 1B-3MI-TFB was considered to be a good comprise between sensitivity and migration time.

3.3. Effect of β-CD concentration

In CE, the additions of organic modifier to the buffer contribute to the alteration of selectivity and improvement of resolution. In this study, the peaks of anthraquinones in sample overlapped with other components in the absence of β-CD. In the first stage of our work, the β-CD was not used for the separation, the concentration of the 1B-3MI-TFB and the pH of the running electrolyte were investigated. Under those analytical conditions, analytes were only partially separated. Using a solution that containing 40 mM 1B-3MI-TFB as running elctrolyte and at pH 10.00, a typical electropherogram of the real sample was shown in Fig. 4. The lack of resolution maybe attributed to the fact that ratio of mass to size of all analytes are very similar. Further experiments were performed by changing the pH and the concentration of 1B-3MI-TFB. However, the results did not provide an acceptable separation.

So, in this work, we added β-CD to the running electrolyte and hope the resolution can be improved. Fig. 5 showed the effect of the β-CD on the migration time and peak areas of the analytes. It is apparent that the migration time of the four analytes decreased when the β-CD was added. From the plot of migration time versus β-CD concentration, it was found that the migration time was slightly changed when the β-CD concentration was changed from 1 to 5 mM; and at 6 mM, the migration time was longer. As it can be seen from the plot of peak area versus β-CD concentration, the peak areas were larger while the β-CD concentration increased from 2 to 4 mM. When β-CD was added to the buffer, clear separation of the real sample was achieved (Fig. 6B). Using a solution that composed of 60 mM 1B-3MI-TFB (pH 10.00) and 4 mM β-CD as running electrolyte a base line separation was accomplished. This can be attributed to the influence of the hydrophobic cavity of

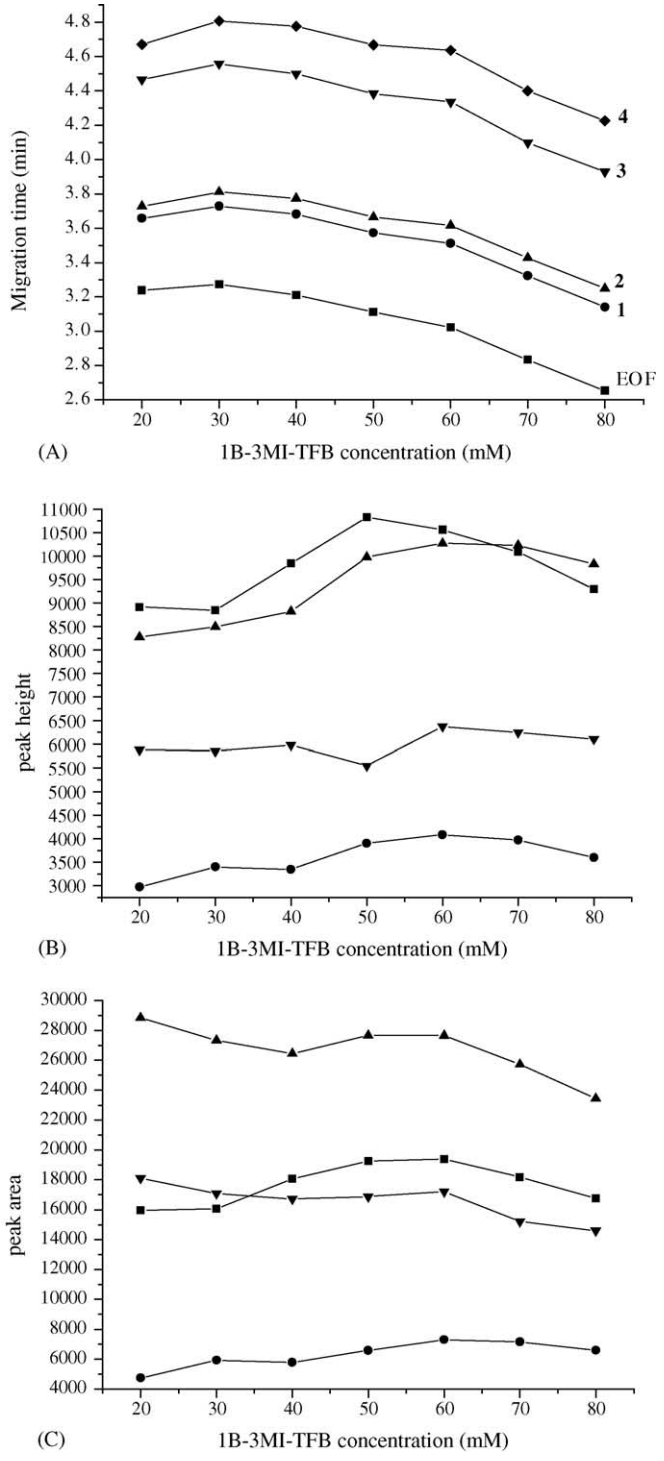


Fig. 3. Effect of ionic liquid concentration: 20–80 mM 1-butyl-3-methylimidazolium (pH 10.00). Capillary: 50 cm (42.4 cm to detector) × 75 μm I.D. Applied voltage: 20.0 kV. Cartridge temperature: 25.5.0 ± 0.5 °C. Detection: 254 nm.

the β-CDs and the 1B-3-MI-TFB. The analytes may partition into or out of the cavity, and the migration velocities of the analytes can be affected as well. When the analytes partition into the cavities, their velocities are retarded. The differences of polarity, size and structure of the analytes molecules cause the differences in their partitioning behavior, which then results in differences in the migration velocities of the analytes and improvement of separation efficiency.

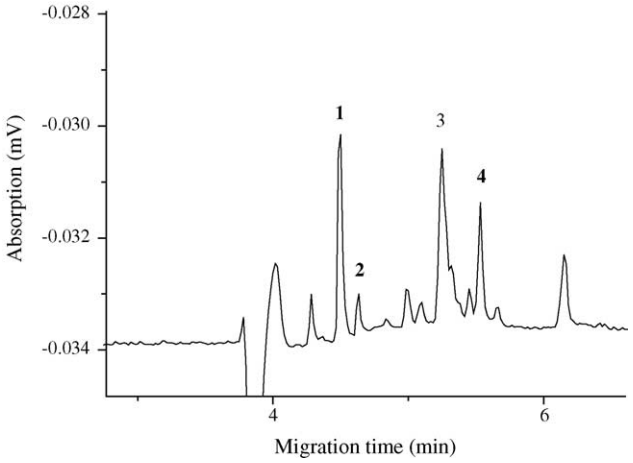


Fig. 4. The electropherogram of the real sample, 40 mM 1-butyl-3-methylimidazolium (pH 10.00), applied voltage: 15 kV. Other conditions as in Fig. 3.

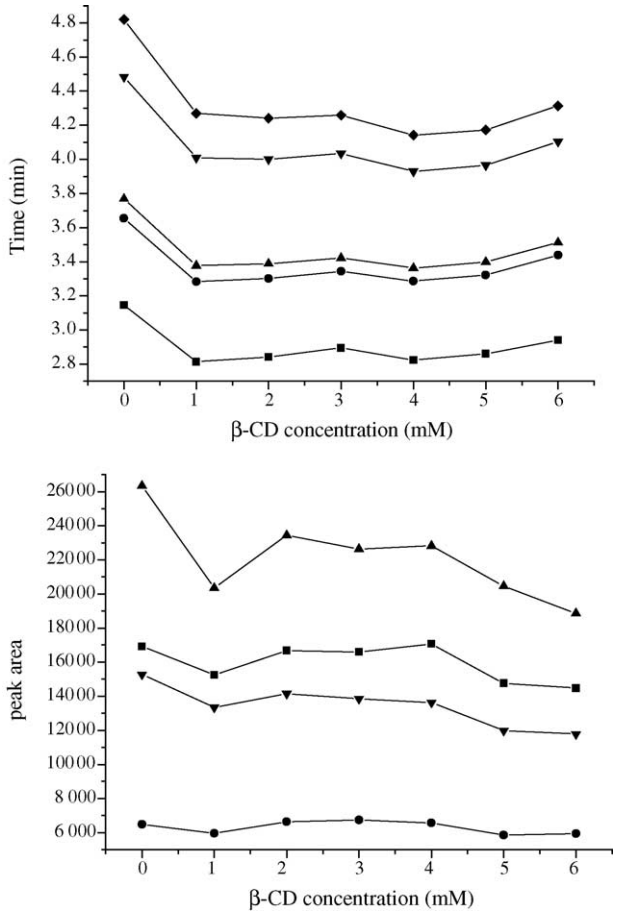


Fig. 5. Effect of β-CD concentration: 60 mM 1-butyl-3-methylimidazolium (pH 10.00) 0–6 mM β-CD. Other conditions as in Fig. 3.

Table 2
Quantitative results of the anthraquinones[a]

Compound	Detection limit (μg/ml)	Linear range (μg/ml)	Linearity[b]	Correlation coefficient
1	0.56	4.0–500	$y = 501.80 + 93.14x$	0.9997
2	3.75	15.8–500	$y = -43.82 + 13.93x$	0.9992
3	0.31	2.0–500	$y = 889.39 + 168.85x$	0.9998
4	0.19	4.0–500	$y = 760.33 + 274.63x$	0.9996

[a] The applied condition were described in Section 3.5.
[b] x denotes concentration (μg/ml), y denotes peak area of the analyte.

3.4. Effect of the running electrolyte pH

The pH of the electrolyte is a governing factor in separation. To verify the effect of running electrolyte pH on migration behavior, experiments were performed with pH ranging from 9.00 to 10.50. It was found that the four anthraquinones were not separated well when the pH was lower than 9.50, the current was larger when the pH exceeded 10.50 and the effect of joule heat on the separation was great. The results at a narrow pH of the buffer demonstrated a significant role of the pH in the separation. With respect to the baseline separation, symmetrical peaks and the effective mobility (migration time) of the analyte, 10.00 was chosen as the optimal running electrolyte pH for the sample separation.

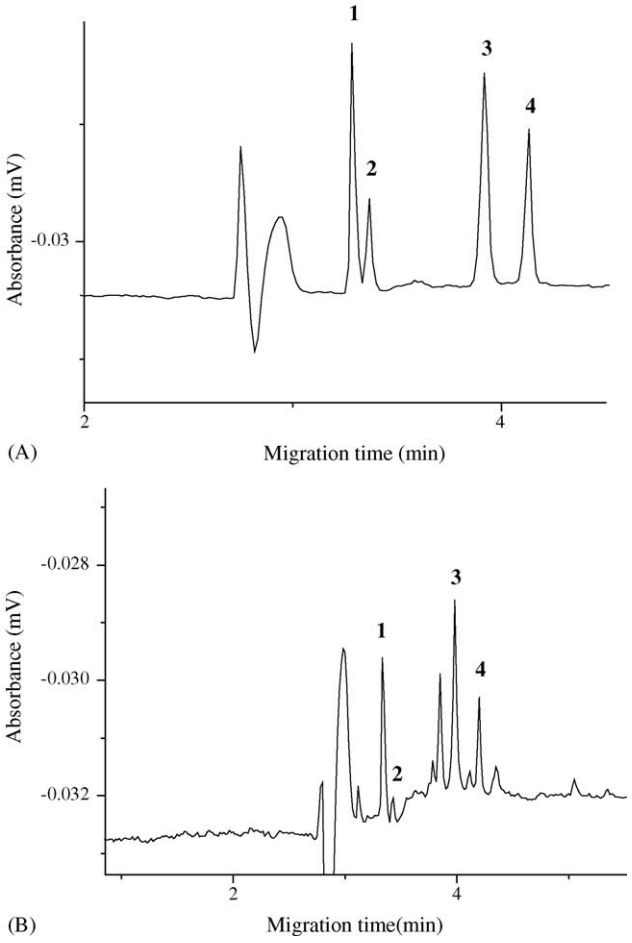


Fig. 6. The electropherogram of the standards mixture solution (A) and real sample (B), the concentrations of the standards were 200 μg/ml for **1**, 500 μg/ml for **2**, 125 μg/ml for **3**, and 62.5μg/ml for **4**, respectively. Buffer conditions: 60 mM 1-butyl-3-methylimidazolium (pH 10.00). Applied voltage: 20.0 kV. Other conditions as in Fig. 3.

3.5. Effect of the applied voltage

The separation voltage determines the migration time directly and influences the resolution. So attempts were made to optimize the separation conditions by using different applied voltage from 15.00 to 25.50 kV. With increasing voltage migration time become shorter but the resolution and the areas of the peaks of the four analytes decreased. Based on these experiments, 20.00 kV was selected as the optimum voltage to accomplish a good compromise.

According to the factors mentioned above, the best resolution was obtained with running electrolyte containing 60 mM 1B-3MI-TFB, 4 mM β-CD, at pH 10.00 and 20.00 kV applied voltage. All the four analytes in real sample were well separated within 4.5 min.

3.6. Separation and detection of four anthraquinones in *Paedicalyx attopevensis Pierre ex Pitard*

3.6.1. Linearity, detection limit and reproducibility

The linear relationship between the concentration of the four analytes and the corresponding peak areas were ob-

Table 3
Reproducibility of the four analytes in CE[a]

Compound	R.S.D. (%)			
	Integrated area		Retention time	
	Intra-day	Inter-day	Intra-day	Inter-day
1	0.97	1.36	0.76	1.01
2	1.12	1.53	0.95	1.25
3	0.91	1.21	0.69	0.81
4	0.83	1.02	0.67	0.73

[a] The applied condition were described in Section 3.5.

Table 4
Analysis results of four anthraquinones in *Paedicalyx attopevensis* Pierre ex Pitard

Analyte (sample)	Content (mg/g)	R.S.D. (%)
1	0.57	3.7
2	0.57	0.6
3	0.31	5.3
4	0.056	4.6

Table 5
The comparison of the present method to others

	Capillary	Buffer conditions	Detection (nm)/ detection limit (μg/ml)	Analysis time (min)	Analytes/application	The results of quantitative analysis (mg/g)
CE [49]	90 cm × 75 μm I.D. fused-silica capillary tube	30 mM sodium borate (pH10.56) and acetonitrile (9:1, v/v)	260/1.76–4.56	39	Anthraquinones/determination of the components in the unpretreated extract of Rhei Rhizoma	0.78–2.04
CE [50]	94 cm × 74 μm I.D. fused-silica capillary tube	10 mM sodium dodecyl sulfate, 12.5 mM sodium dihydrogenphosphate and 15 mM sodium borate and acetonitrile (3:1)	254/unreported	30	Rhein, emodin, aloe-emodin, sennoside A and sennoside B/determination of the anthraquinoids in Chinese herbal preparations	0.186–10.053
Present method	50 cm × 75 μm I.D. fused-silica capillary tube	60 mM 1B-3MI-TFB, 4.0 mM β-CD (pH 10.00)	254/0.19–3.75	4.5	Anthraquinones/separation and determination the real sample of Chinese herb *Paedicalyx attopevensis* Pierre ex Pitard	0.056–0.57

tained. The detection limit, linear range, correlation coefficient, and linearity were shown in Table 2. The reproducibility of the migration time and peak area of four components in the experiment was determined by repeated ($n = 6$) injection of a standard mixture solution under optimum conditions. The results were shown in Table 3.

3.6.2. Determination of the compounds in Paedicalyx attopevensis Pierre ex Pitard

The sample solution of Chinese herb was analyzed by the present method under the optimized conditions. The peaks were identified by comparing the migration times and spiking the standards to the sample solution. The typical electropherogram was shown in Fig. 6B.

Table 4 listed the quantities of four analytes in the Chinese herb and their relative standard deviations. The relatively large R.S.D. of real sample were probably due to the complexity of the Chinese herb preparation and the heterogeneity of the concentrated powder. The recovery of the four anthraquinones was determined with the standard addition and with the results of 94–105% for **1**, 90–99% for **2**, 93–107% for **3** and 87–98% for **4**.

Compared the present method to others [49,50] (Table 5), it was observed that the present method was applicable in the analysis of anthraquinones and was easy to use for the analysis of the herb. Especially, it is very simple and sensitive. The reproducibility and detection limit were better, and the analysis time was shorter.

4. Conclusions

In this work, the CZE based IL-β-CD method was reported firstly and its application was discussed. Successful separation and identification of four anthraquinones of *Paedicalyx attopevensis* Pierre ex Pitard extracts has been achieved using 1-butyl-3-methylimidazolium-based ionic liquid as the main running electrolyte solutions with β-CD as modifier. The method can be used to identify the purification of the anthraquinones, monitor some anthraquinones in the isolation of natural products, chemical reactions, etc. It is especially significant that the method has developed greatly the ionic liquids method in CE.

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References

[1] M. Freemantle, Chem. Eng. News 78 (2000) 37.
[2] M.S. Selvan, M.D. Mckinley, R.H. Dubois, J.L. Atwood, J. Chem. Eng. Data 45 (2000) 841.

[3] S. Dai, Y.H. Ju, C.E. Barnes, J. Chem. Soc., Dalton Trans. 8 (1999) 1201.
[4] J.S. Wilkes, M.J. Zaworotko, J. Chem. Soc., Chem. Commun. 13 (1992) 965.
[5] M.J. Earle, P.B. McCormac, K.R. Seddon, Chem. Commun. 20 (1998) 2245.
[6] N.E. Leadbeater, H.M. Torenius, J. Org. Chem. 67 (2002) 3145.
[7] T. Welton, Chem. Rev. 99 (1999) 2071.
[8] S.J. Nara, J.R. Harjani, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 2979.
[9] J.R. Sanders, E.H. Ward, C.L. Hussey, J. Electrochem. Soc. 133 (1986) 325.
[10] V.E. Dickinson, M.E. Willaims, S.M. Hendrickson, H. Masui, R.W. Murray, J. Am. Chem. Soc. 121 (1999) 613.
[11] C.J. Adams, M.J. Earle, K.R. Seddon, Chem. Coummun. (1996) 1625.
[12] C.P. Mehnert, R.A. Cook, N.C. Dispenziere, M. Afeworki, J. Am. Chem. Soc. 124 (2002) 12932.
[13] D.W. Armstrong, L.K. Zhang, L. He, M.L. Gross, Anal. Chem. 73 (2001) 3679.
[14] J.E.L. Dullius, P.A.Z. Suarez, S. Einloft, R.F. de Souza, J. Dupont, J. Fischer, A. DeCian, Organometallics 17 (1998) 815.
[15] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robison, A.M. Stalcup, Analyst 125 (2000) 1919.
[16] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robison, A.M. Stalcup, Anal. Chem. 73 (2001) 3838.
[17] D.W. Armstrong, L. He, Y.S. Liu, Anal. Chem. 71 (1999) 3873.
[18] M. Vaher, M. Koel, M. Kalijurand, J. Chromatogr. A 979 (2002) 27.
[19] W.D. Qin, H.P. Wei, S.F.Y. Li, J. Chromatogr. A 985 (2003) 447.
[20] S.M. Mwongela, A. Numan, N.L. Gill, R.A. Agbaria, I.M. Warner, Anal. Chem. 75 (2003) 6089.
[21] J.S. Wilkes, J.A. Levisky, R.A. Wilson, C.L. Hussey, Inorg. Chem. 21 (1982) 1263.
[22] A.A. Fannin, A.D. Floreani, A.L. King, S.J. Landers, J.B. Piersma, J.D. Stech, L.R. Vaughn, S.J. Wilkes, L.J. Williarm, J. Phys. Chem. 88 (1984) 2614.
[23] C.L. Hussey, Pure Appl. Chem. 60 (1988) 1763.
[24] C.L. Adams, M.J. Earle, K.R. Seddon, Green Chem. (2000) 21.
[25] J.L. Anderson, J. Ding, T. Welton, D.W. Armstrong, J. Am. Chem. Soc. 124 (2002) 14247.
[26] E. Schneiderman, A. Stalcup, J. Chromatogr. B 745 (2000) 83.
[27] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[28] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[29] J. Wang, I. Warner, J. Chromatogr. A 711 (1995) 297.
[30] M. Macchia, G. Manetto, C. Mori, C. Papi, N. Di Pietro, V. Salotti, F. Bortolotti, F. Tagliaro, J. Chromatogr. A 924 (2001) 499.
[31] Z. Gong, Y. Zhang, H. Zhang, J. Cheng, J. Chromatogr. A 855 (1999) 329.
[32] J.P. Alarie, T. Vo Dinh, Talanta 38 (1991) 529.
[33] D. Greatbanks, R. Pickford, Magn. Reson. Chem. 25 (1987) 208.
[34] The Illustrated Handbook of Chinese Advanced Plant (IV) Institute of Botany, Chinese Academy Sciences, Science Press, Beijing, 1994, p. 210.
[35] G.C. Yen, P.D. Duh, D.Y. Chuang, Food Chem. 70 (2000) 437.
[36] J. Koyama, I. Morita, K. Tagahara, M. Ogata, T. Mukainaka, H. Tokuda, H. Nishino, Cancer Lett. 170 (2001) 15.
[37] D.L. Barnard, D.W. Fairbairn, K.L. O'Neill, T.L. Gage, R.W. Sidwell, Antiviral Res. 28 (1995) 317.
[38] J. Chen, Y. Fang, Y.H. Diao, J. Pharm. Pract. 20 (2002) 297.
[39] N.N. Que, Y.J. Ren, S.L. Li, Y.P. Wang, H. Li, Pharm, J. Chin. PLA 17 (2001) 211.
[40] R.Z. Zhao, R.M. Ou, Primary Chin. Mater. Med. 16 (2001) 20.
[41] R.Z. Zhao, R.M. Ou, A.Q. Li, Chin. Pharm. 14 (2003) 74.
[42] C. Yu, H.M. Lan, Y. Wang, Primary Chin. Mater. Med. 16 (2002) 9.
[43] L.Y. He, S.R. Luo, Acta Pharm. Sin. 15 (1980) 555.
[44] S. Kitanaka, et al., Chem. Pharm. Bull. 33 (1985) 1274.
[45] S. Kitanaka, M. Takido, Chem. Pharm. Bull. 32 (1984) 860.
[46] M.T. Glaceran, L. Puignou, M.J. Diez, J. Chromatogr. A 732 (1996) 167.
[47] J.L. Anderson, J. Ding, T. Welton, D.W. Armstrong, J. Am. Chem. Soc. 124 (47) (2002) 14247.
[48] C.L. Hussey, Pure Appl. Chem. 60 (1988) 1763.
[49] W.C. Weng, S.J. Sheu, J. High Resolut. Chromatogr. 23 (2) (2000) 143.
[50] S.J. Sheu, H.R. Chen, Anal. Chim. Acta 309 (1995) 361.