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Determination of hydroxyanthraquinoids in Rhubarb by cyclodextrin-modified micellar electrokinetic chromatography using a mixed micellar system of sodium dodecyl sulfate and sodium cholate

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Abstract

A cyclodextrin-modified micellar electrokinetic chromatographic (CD-MEKC) method was established to determine five hydroxyanthraquinoids in Rhubarb. The five components were successfully separated by using the mixed micellar system consisting of 20 mmol/l sodium dodecyl sulfate (SDS) and 20 mmol/l sodium cholate (SC) with 10 mmol/l β-cyclodextrin in phosphate buffer (pH 10.4). The separation was optimized by adjusting buffer pH, concentrations of β-cyclodextrin and SC and applied voltage. The proposed method was validated and applied to the determination of two commercial Rhubarb samples. The results obtained were satisfactory.
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Keywords: Rhubarb; Anthraquinone derivative; Cyclodextrin-modified micellar electrokinetic chromatography; Determination

1. Introduction

Rhubarb, an ancient Chinese medicinal herb, has various pharmacological actions, such as purgation, antibacterial, curing mental and renal disorders, antitumor and antimutagenicity [1,2]. Besides its pharmacological values, Rhubarb can also be made as nourishing food. The pharmaceutically relevant components of Rhubarb are hydroxyanthraquinoids, including chrysophanol, emodin, physcion, aloe-emodin, rhein and their glucosides.

TLC [3] and HPLC [4] have been the commonly used methods for the separation and determination of active constituents in Rhubarb. But few of these methods are entirely adequate because of either poor accuracy, low resolution or the requirement of tedious pretreatment. High performance capillary electrophoresis (HPCE) has proved to be a highly efficient separation technique in pharmaceutical industry. Sheu et al. [5] adopted micellar electrokinetic chromatography (MEKC) for the assay of three anthraquinones

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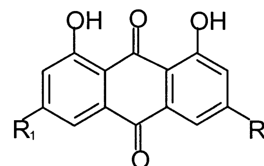
and two sennosides in Rhubarb using sodium dodecyl sulfate (SDS) micelle. Zong et al. [6] also used SDS micelle to separate the five anthraquinones in Rhubarb in a different buffer system. Chai et al. [7] employed an alternative sodium deoxycholate (SDC) micelle to separate and determine the same five components in Rhubarb. However, cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) has not been reported for the determination of anthraquinone derivatives in Rhubarb. A relatively new area in CD-MEKC is the use of mixed micelle systems. Although most of the reported methods of CD-MEKC have involved the use of achiral surfactant SDS, chiral bile salt micelles have also been used for difficult separations in CE applications. In this paper, we propose a β-CD modified micellar electrokinetic chromatographic method, which employs a mixed micellar system of SDS and bile salt sodium cholate (SC) for the determination of five hydroxyanthraquinoids in Rhubarb. The analytical performance of the method was examined in terms of linearity response, precision and recovery. Two commercial Rhubarb samples were analyzed with the proposed method.

2. Experimental

2.1. Chemicals

Chrysophanol, emodin, aloe-emodin, physcion and rhein were purchased from the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. The chemical structures of these five anthraquinoids are drawn in Fig. 1. β-CD was bought from the Development Center of Special Chemical Reagents (Tianjin, China). SDS was purchased from Academy of Military Medical Science, Beijing, China. SC was purchased from Chemical Reagent Factory, Shanghai, China. *O*-phthalic acid was bought from Beijing Chemical Factory, Beijing, China. Anhydrous ethanol was of analytical grade. Commercial Rhubarb samples were bought from local Tong Ren Tang Drug Store in Anguo, Hebei province, China. Double distilled water was used for preparing solutions.



Physcion	R_1=OCH_3	R_2=CH_3
Emodin	R_1=OH	R_2=CH_3
Aloe-emodin	R_1=H	R_2=CH_3OH
Rhein	R_1=H	R_2=COOH
Chrysophanol	R_1=H	R_2=CH_3

Fig. 1. Structures of the five compounds.

2.2. Instrumentation

Experiments were carried out on a 1229 type HPCE Analyser system (Manufactured by Beijing Institute of New Technology and Application, Beijing, China). Detection was followed by direct UV absorptiometric measurement at 254 nm. An uncoated fused silica capillary (50 μm ID) (Hebei Yong Nian Optical Fibre Factory, Hebei, China) with effective length of 45 cm was used. The temperature was kept at 25 ± 1 °C. The applied voltage was 14 kV and the current level was 38 μA. Electrokinetic injection was at 10 kV for 5 s.

2.3. Procedures

Phosphate buffer was prepared by mixing 30 mmol/l Na_2HPO_4 and 30 mmol/l KH_2PO_4 solution and the desired pH values were adjusted with 0.1 mol/l NaOH. Internal standard (I.S.) solution was prepared by dissolving 0.1 mg *O*-phthalic acid with 10 ml water. A 0.5 g sample of Rhubarb powder was extracted in Soxhlet extractor with 15 ml of 2 mol/l H_2SO_4 and 70 ml anhydrous ethanol. The extraction was continued until the aqueous phase was almost colorless. Then the extract was evaporated to dryness on a water bath. The residue was dissolved by anhydrous ethanol. After addition of 30 μl I.S. solution (*O*-phthalic acid), the extract was diluted to 50 ml with separating buffer. The solution was filtered through a 0.45 μm membrane before injection. Fixed amounts of pure aloe-emodin, rhein and emodin were added to Raw Rhubarb of known contents, respectively, and the mixtures were extracted according to the

above-mentioned method. The extracts were used for recovery studies. Prior to experiment, the capillary was washed firstly with 0.1 mol/l NaOH for 20 min, and then with distilled water for 20 min, lastly with buffer solution for 10 min.

3. Results and discussion

3.1. Separation by MEKC with SC or SDS

MEKC with SC solution alone was applied to separate the five anthraquinones. During the experiment, methanol was used as the electroosmotic flow (EOF) marker, and Sudan III was used as micelle marker. By varying SC concentrations (10–30 mmol/l) and pH value (7.2–10.4), only four peaks showed up. Moreover, chrysophanol, physcion and emodin could not be baseline-separated, and the peak shapes were not satisfactory. SDS in run buffer alone was also attempted to the separation, but the result obtained was not successful. Changes of SDS concentration, pH value and applied voltage didn't improve the situation. The five analytes were coeluted with the micelle. These are shown in Fig. 2a and b, respectively.

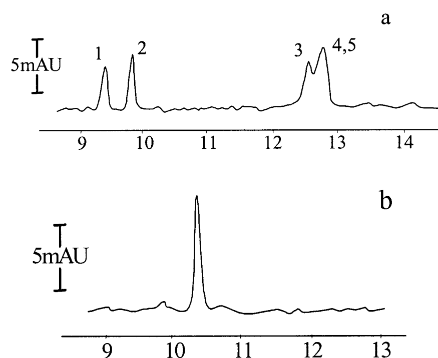


Fig. 2. Electropherograms of MEKC of five compounds. Conditions: a, phosphate buffer (pH 9.6) with 10 mol/l SC; b, phosphate buffer (pH 9.6) with 20 mmol/lSDS; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

3.2. Separation by CD-MEKC with single micelle

Cyclodextrins (CDs) are well known for their potential to discriminate between positional isomers, homologues and enantiomers. In addition, CD promotes the aqueous solubility of hydrophobic compounds. β-CD, a very useful chiral selector, has become extensively used in separation science. Initially, CD modified capillary zone electrophoresis was attempted to separate the anthraquinones in 30 mmol/l phosphate and 10 mmol/l β-CD solution. But serious band broadening and poor resolutions of the five components were observed.

It has been reported [8] that the use of CD in MEKC is effective for the separation of lipophilic compounds. Consequently, β-CD modified single micelle systems with SDS or SC were tried in the separation. In SC system, the five components were separated on baseline, while band tailing and broadening was observed. When SDS was used in CD-MEKC, only four peaks of five components appeared. The peaks of aloe-emodin and rhein overlapped together. The electropherograms are drawn in Fig. 3a and b, respectively.

3.3. Separation by CD-MEKC with mixed micelle

CD-MEKC of single micelle systems with SDS or SC revealed neither good peak shape nor high resolution, therefore, in order to improve separation selectivity and resolution, mixed micelle system was adopted. Incorporation of chiral surfactant SC into β-CD and SDS created a three pseudostationary phases system. Two types of

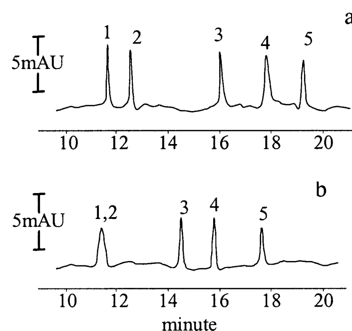


Fig. 3. Electropherograms of CD-MEKC of single micelle. a: phosphate buffer (pH 9.6) with 10 mol/l SC and 10 mmol/l β-CD; b: phosphate buffer (pH 9.6) with 20 mmol/lSDS and 10 mmol/l β-CD; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

anionic micelles with different properties migrated towards the anode, and neutral chiral β-CD, a third phase, moved in the direction of cathode with the EOF. The five components can be partitioned among these three phases. Compared with a conventional CD-MEKC including two phases, the separation mechanisms of the system investigated are more complex. The effect of concentrations of two chiral selectors SC/β-CD, buffer pH value and applied voltage on separation selectivity was studied one by one in the following text.

In the range of 10–30 mmol/l, influence of SC concentration on the separation was investigated while keeping SDS and β-CD concentration constant. Increase of migration time with increasing SC concentration was observed in Fig. 4. The five analytes are hydrophobic compounds, especially chrysophanol and physcion. Large SC concentration increased the solubilization of hydrophobic compounds in the micelle. The difference of capacity factors of the analytes became large, because the five compounds had different hydrophobicity to the micelle. Therefore, increase of SC concentration was helpful to improve peak resolutions. Rhein possessing one carboxylic group was completely ionized at pH 10.38, so the migration time of rhein was mainly affected by the electrophoretic mobility itself. At 20 mmol/l, good resolutions of the five components with shorter migration times were obtained.

In the buffer solution of 30 mmol/l phosphate containing 20 mmol/l SDS, 20 mmol/l SC and 10 mmol/l β-CD, effect of pH value on the retention was studied. Fig. 5 shows the variation results of migration times of five analytes with pH values. Known from the figure, migration times decreased with increasing pH value. Since the five compounds are weak acid, in the range of pH value 7.9–10.5, the five components would be deprotonated to a different extent, so they moved toward the anode (against the detector). Maybe increase of pH value caused the increase of EOF. The increase of EOF shortened migration time window, resulting in the reduction of migration times of the five analytes. At pH 10.4, good resolutions with the shortest migration times were achieved.

Existence of β-CD in the mixed micelles is very important for improving the separation selectivity. With the increase of β-CD concentration, the migration times of the five components decreased and the migration order changed slightly. The variation of migration time with β-CD concentration is shown in Fig. 6. The change can be

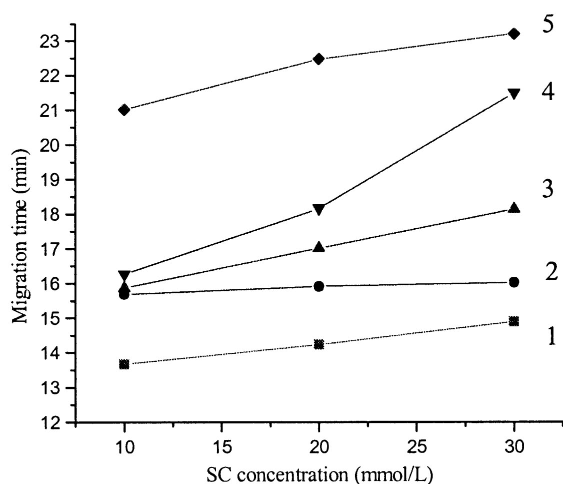


Fig. 4. Influence of SC concentration on migration time. Conditions: 30 mmol/l phosphate buffer (pH 10.4) with 20 nmol/l SDS; applied voltage 14 kV; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

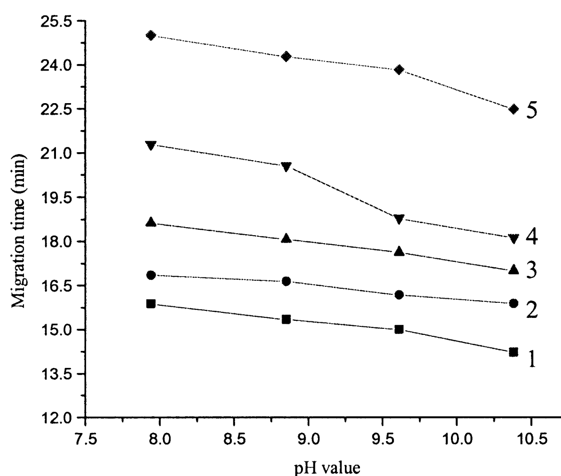


Fig. 5. Effect of pH value on migration time. Conditions: 30 mmol/l phosphate buffer with 20 nmol/l SDS, 20 mmol/l SC and 10 mmol/l β-CD; applied voltage 14 kV; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

explained by an inclusion-complex formation. Wang et al. [9] proved that anthraquinones in Rhubarb could get into the inside cavity of β-CD, hence the inclusion-complexes were formed. After formation of the inclusion-complexes, volumes of the solutes became larger than before and net charge of complexes changed, perhaps which brought about slower migration of them toward the anode. Stability of inclusion-complex formation was different among the solutes, hence migration order was altered slightly. In the range of 10–20 mmol/l of β-CD concentration, the five analytes had good peak shape and reolution, further increase of the concentration deteriorated resolution.

In the CD-MEKC system investigated, there existed hydrophobic interactions, hydrogen bonding effects between the solutes and the micelles, and inclusion-complex interactions between the solutes and β-CD. Linear solvation energy relationship (LSER) [10] study of mixed micellar system showed that mixed SDS-bile salt system had properties that were more similar to bile salt system than SDS system. The main source of selectivity variations between SDS and SC was due to hydrogen bonding effects. As stated-above, it was assumed that inclusion-complex stability of solutes with β-CD seemed to be the predominant interaction contrasted to those of solutes with two kinds of micelles in the course of separation.

Higher voltage shortened analysis time, coupling with excessive Joule heating, while lower voltage would increase the analysis time. In the range of 13–14 kV, high separation efficiency was obtained, therefore, 14 kV was the suitable voltage. In order to prevent excessive Joule heating, buffer concentration of 30 mmol/l phosphate with relatively low ionic strength was used in the experiments throughout.

Typical electropherogram of the five analytes was demonstrated in Fig. 7a under the selected conditions: 30 mmol/l phosphate (pH 10.4); 20 mmol/l SDS; 20 mmol/l SC and 10 mmol/l β-CD; applied voltage 14 kV.

3.4. Validation of the method

The linearity of the five components in standard solutions was investigated at five concentration levels in the range of 3.86–32 μg/ml for aloe-emodin, 4.0–36.6 μg/ml for rhein, 4.6–56.6 μg/ml

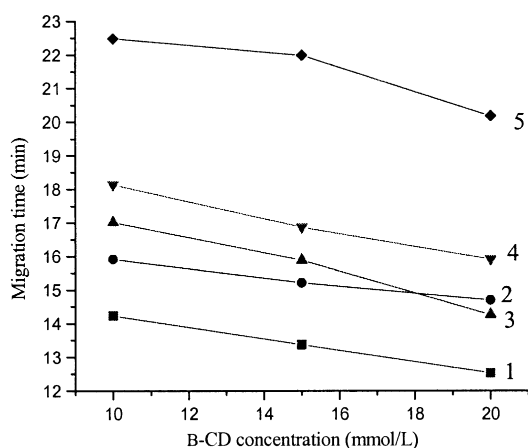


Fig. 6. Influence of β-CD concentration on migration time. Conditions: 30 mmol/l phosphate buffer (pH 10.4) with 20 nmol/l SDS and 20 mmol/l SC; applied voltage 14 kV; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

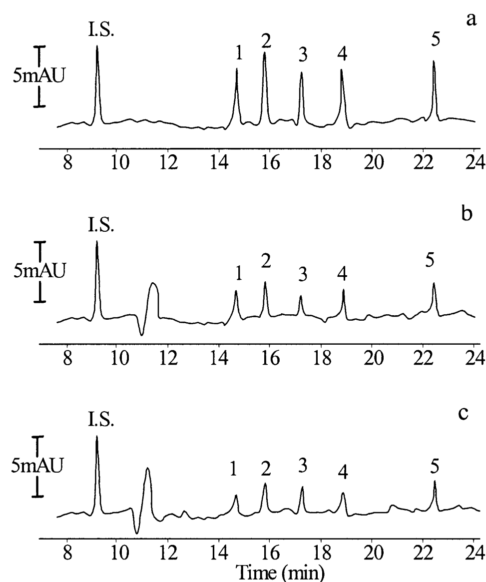


Fig. 7. Typical electropherograms of CD-MEKC with mixed micelles of five anthraquinones. a: standard samples; b: raw rhubarb; c: Mongolian rhubarb; 1 aloe-emodin; 2 rhein; 3 chrysophanol; 4 physcion; 5 emodin.

for emodin, 5–80 μg/ml for chrysophanol and 6–85 μg/ml for physcion. Each solution was analyzed three times. Calibration graphs were constructed by concentration (y, μg/ml) against peak-area ratio (x). The regression equations of curves and their correlation coefficients were calculated and listed as follows: aloe-emodin, $y = 14.51x - 0.3201$ ($r = 0.9998$); rhein, $y = 10.53x - 0.0026$ ($r = 0.9996$); emodin, $y = 12.13x - 0.0092$ ($r = 0.9998$); chrysophanol, $y = 10.05x + 0.16$ ($r = 0.9985$); physcion, $y = 10.63x + 0.11$ ($r = 0.9982$).

The intra- and inter-day reproducibility of this method was determined on the basis of peak-area (relative to I.S.) by measuring ten and nine replicate injections, respectively. For each ingredient, the intra-, inter-day relative standard deviation (RSD) values, detection limits and quantitation limits were listed in Table 1. Detection limits were estimated as a peak height with a signal-to-noise ratio of 3. Quantitation limits were calculated by the peak height and comparing it with ten times the level of noise observed on the baseline of the electropherogram.

The results of standard addition recovery of three ingredients from sample composites of Rhubarb were calculated (see Table 1).

3.5. *Determination of anthraquinone derivatives in Rhubarb samples*

Under the optimized conditions, two samples of Raw Rhubarb and Mongolian Rhubarb were analyzed by this proposed method. The electropherograms are given in Fig. 7b and c, respectively, and the calculated contents of the anthraquinone derivatives are listed in Table 2. No interference was observed at the migration time of the components in the two samples. Since peak-areas of chrysophanol and physcion had no good reproducibility, their contents were not determined.

4. Conclusion

The proposed CD-MEKC method of mixed micelle system demonstrates good separation selectivity and high resolution for separation and determination of five hydroxyanthraquinoids in Rhubarb. An inclusion-complex mechanism is indicated in this ternary system. The selectivity and resolution of the method was found to be dependent on buffer pH and concentrations of β-CD, chiral and achiral micelles. The results of quantitative determination of anthraquinoids in

Table 2
Contents of anthraquinone derivatives in Raw Rhubarb and Mongolian Rhubarb

Sample	Content (mg/g) ($n = 3$)		
	Aloe-emodin	Rhein	Emodin
Raw Rhubarb	1.096	1.320	0.968
Mongolian Rhubarb	0.853	0.983	1.142

Table 1
Results of recovery and reproducibility of the method

Compound	Concentration (μg/ml)	Recovery (%) ($n = 3$)	RSD (%)		Detection limit (S/N = 3)	Quantitation on limit (S/N = 10)
			Intra-day	Inter-day		
Aloe-emodin	22.62	98.2	1.51	1.67	0.75	2.41
Rhein	16.51	96.5	1.32	2.01	0.89	2.85
Emodin	33.87	100.08	1.12	1.59	0.96	2.36
Chrysophanol	38.52	91.1	1.89	2.13	1.07	3.18
Physcion	41.19	92.3	1.65	2.11	1.15	3.42

two commercial Rhubarb samples show that anthraquinoids amounts of various samples are different from each other, so the quality control of Rhubarb is needed. The established method is not only suitable to the determination of Rhubarb sample, but can be applied to the analysis of other herbal medicines and pharmaceutical preparations.

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