Chinese Journal of Chemistry, **2004**, *22*, 845—848

Article

Enantiomeric Separation of Epinephrine and Salbutamol by Micellar Electrokinetic Chromatography Using β-Cyclodextrin as Chiral Additive

ZHENG, Yan-Peng*,[a](郑妍鹏) MO, Jin-Yuan[b](莫金垣)

[a] *School of Science, Beijing Jiaotong University, Beijing 100044, China*
[b] *School of Chemistry and Chemical Engineering, Zhongshan University, Guangzhou, Guangdong 510275, China*

Enantiomeric separations of epinephrine and salbutamol, by means of micellar electrokinetic chromatography (MEKC) employing β-cyclodextrin as chiral additive in ammonium chloride-ammonia solution were investigated. In this system, the analytes migrated with the micellar phase towards the anode and were detected by electrochemistry using gold microelectrode at +0.65 V vs. SCE. The success of the chiral separations is strongly dependent on the concentration of β-CD and SDS, and the optimal concentration is 8 mmol•L^{-1} and 15 mmol•L^{-1} respectively. The effects of detection potential, pH value of electrolyte and applied voltage were discussed also. Using the proposed method, baseline separation of the enantiomers could be accomplished in 6 min. Further, an attempt was made to elucidate the plausible mechanism of the chiral recognition.

Keywords micellar electrokinetic chromatography, electrochemical detection, enantiomer separation, epinephrine, salbutamol

Introduction

The development of chiral substances, especially in the pharmaceutical field, places increasing demands on analytical methods for the separation of these kinds of isomers and the chiral purity control of drugs in pharmacokinetic studies. As the enantiomers of epinephrine and salbutamol have different pharmacological and toxicological characteristics, separation and quantitation of the single enantiomers are required.

Analytical methods used so far for the enantiomer separation include high performance liquid chromatography (HPLC),[1-3] thin-layer chromatography (TCL),[4] gas chromatography (GC),[5] mass spectrometry (MS)[6] and capillary electrophoresis (CE).[6,7]

CE has become a powerful tool for enantiomer separation, particularly because of the relatively short analysis time. Direct methods of enantioseparation can be performed by simply adding a chiral selector to the background electrolyte. And buffer additives such as chelator-metal complexes, proteins, bile salts, cyclic oligosaccharides, especially cyclodextrins (CD) and their derivatives, *etc.*, are widely used as chiral selectors.[8]

Native CDs (α, β and γ) and their derivatives have been mainly used in capillary zone electrophoresis (CZE) and several instances of the combination of CD and micellar electrokinetic chromatography (CD-MEKC) allowed the enantiomeric resolution of neutral compounds. In this work, a method for enantiomeric separations of epinephrine and salbutamol was investigated based on the β-cyclodextrin (β-CD)-sodium dodecyl sulfate (SDS)-micellar electrokinetic chromatography (MEKC) separation mode. In this system, the analytes migrate with the micellar phase towards the anode and are detected by electrochemistry using gold microelectrode at +0.65 V versus SCE reference electrode. The success of the chiral separations is strongly dependent on the concentration of β-CD and SDS (The optimal concentration is 8 mmol•L^{-1} and 15 mmol•L^{-1} respectively). The effects of detection potential, pH value of electrolyte and applied voltage were studied also. Using the proposed method, baseline separation of the enantiomers could be accomplished in 6 min. The plausible mechanism of the chrial recognition was discussed.

Experimental

Chemicals

Epinephrine and salbutamol were purchased from Sigma (St. Louis, MO, USA). β-CD was purchased from Boao biology science and technology Corp. (Shanghai, China). SDS, ammonium chloride, ammonia and sodium hydroxide were purchased from the Chemical Reagent Factory of Guangzhou (Guangzhou, China). Other reagents used were of analytical grade and doubly distilled water was used to prepare all solu-

* E-mail: yuati@sohu.com
Received November 24, 2003; revised and accepted April 6, 2004.
Project supported by the National Natural Science Foundation of China (No. 29675033), the Natural Science Foundation of Guangdong Province (No. 001237) and Pandeng Foundation of Beijing Jiaotong University (No. PD245).

tions.

Apparatus and electrophoretic conditions

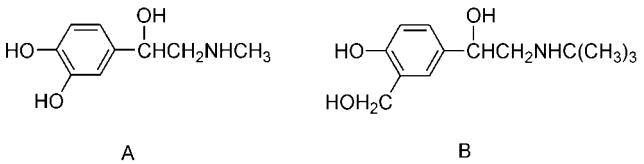
Experiments were carried out using the laboratory-made capillary electrophoresis system (School of Chemistry and Chemical Engineering of Zhongshan University, Guangzhou, China) equipped with an ampere detector.[9] An untreated fused-silica capillary was obtained from Yongnian Optical Fiber Factory, 50 cm× 25 μm I.D. (Hebei, China) and the separations were carried out under the condition of a constant temperature (25 ℃) and a constant humidity (relative humidity 65%). The gravity sample introduction method was used, with the sampling height being 20 cm, the sampling time 15 s and the applied voltage 10 kV. The background electrolyte (BGE) consisted of a solution of 15 mmol•L^{-1} ammonium chloride at pH=8.6 (adjusted with ammonia solution), 8 mmol•L^{-1} β-CD and 15 mmol•L^{-1} SDS.

Results and discussion

Effect of detection potential on detection

In this paper, epinephrine and salbutamol were selected for the electrophoretic separation and their structures are shown in Scheme 1. Hydrodynamic voltammograms for epinephrine and salbutamol are shown in Figure 1. Current intensity of the analytes was increased with the increase of detection potential in the capillary electrophoresis-electrochemistry detection. However, along with the increase of detection potential, especially when detection potential was over +0.8 V, the baseline noise was increased and the stability of the electrode was decreased. So with the consideration of sensitivity, baseline noise and stablility, +0.65 V was chosen as the detection potential for epinephrine and salbutamol respectively.

Scheme 1 The chemical structures of epinephrine (A) and salbutamol (B)



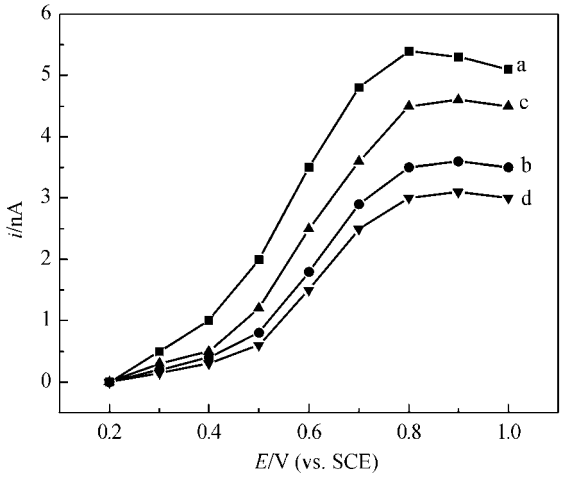


Figure 1 The hydrodynamic voltammograms for epinephrine and salbutamol. a, *R*-epinephrine; b, *S*-epinephrine; c, *R*-salbutamol; d, *S*-salbutamol.

Effect of β-CD concentration on selectivity and chiral recognition

The racemic analytes can be combined with micellar phase, and also can be combined with β-CD. But in most cases, only when analytes are combined with β-CD, the enantioseparation can be obtained. Though the concentration of SDS can affect the enantiomeric separation, the difference in effective mobility of the host-guest complexation is the key factor. The chiral selector has an optimal concentration which is in inverse proportion to the the host-guest complexation constants.[10] It will lead to a general decrease in resolution whether the concentration of β-CD is too low or too high. The mechanism can be explained by the following equation:

$$\Delta\mu=\frac{(\mu_1-\mu_2)(K_1-K_2)c}{1+(K_1+K_2)c+K_1K_2c^2} \quad (1)$$

where $\Delta\mu$ is the difference in effective mobility, K_1 and K_2 are the stability constants of the complexes formed and subscripts 1 and 2 represent the two enantiomers, c is the concentration of chiral additive. It could be seen from the equation, when the concentration of β-CD is zero or very high, $\Delta\mu$ is near zero and leads to a decrease in resolution.

For the study of enantiomer resolution, the analytes were injected by using a BGE (15 mmol•L^{-1} ammonium chloride-ammonia solution, 15 mmol•L^{-1} SDS) at pH=8.6 supplemented with different amounts of β-CD in the range of 0—20 mmol•L^{-1}. The experimental results are shown in Figure 2 and Table 1. They show that when the analytes were run in the BGE at pH=8.6 without chiral additive, no enantiomeric separation was obtained. The resolution increased with increasing concentration of β-CD for epinephrine and salbutamol, whereas the resolution increased up to 2.02, 1.82, respectively and then decreased. We also found that with the increasing concentration of β-CD, the peak height of *R*, *S*-isomer of epinephrine and salbutamol decreased. And the peak height of *R*-isomer was higher than that of the *S*-isomer. So 8 mmol•L^{-1} β-CD was chosen as the optimal concentration when resolution, analyzing time and peak height were considered.

Effect of SDS concentration on selectivity and chrial recognition

The effect of SDS concentration on selectivity and chiral recognition was investigated by using a BGE (15 mmol•L^{-1} ammonium chloride-ammonia solution, 8 mmol•L^{-1} β-CD) at pH=8.6 supplemented with diffe-

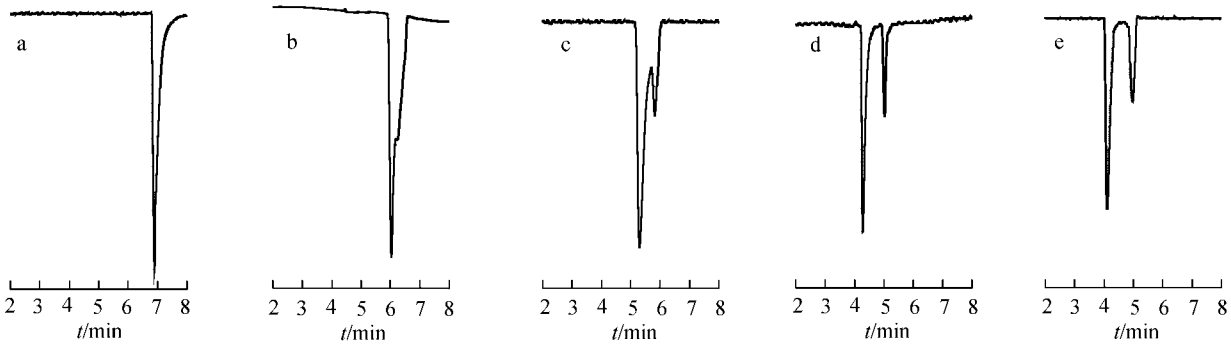


Figure 2 Effect of β-CD concentration on the enantiomeric separation of epinephrine. a. 0; b. 2; c. 4; d. 8; e. 10 mmol•L^{-1}.

Table 1 Effect of β-CD concentration on migration time and resolution[a]

	c (β-CD)/(mmol•L^{-1})	0	2	4	6	8	10
	t_1/min	6.78	5.89	5.13	4.62	4.20	3.98
Epinephrine	t_2/min	6.78	6.18	5.70	5.28	4.94	4.80
	R_S	0	0.21	0.56	1.24	2.02	1.98
	t_1/min	6.49	5.64	4.92	4.46	4.05	3.90
Salbutamol	t_2/min	6.49	5.91	5.43	5.05	4.72	4.64
	R_S	0	0.18	0.49	1.11	1.82	1.76

[a] t_1, t_2: migration time; R_S: resolution.

Table 2 Effect of SDS concentration on migration time and resolution[a]

	c (SDS)/(mmol•L^{-1})	0	5	10	15	20	25
	t_1/min	3.74	3.86	4.01	4.20	4.42	4.67
Epinephrine	t_2/min	3.96	4.13	4.62	4.94	5.12	5.68
	R_S	0.39	0.58	1.66	2.02	1.91	1.67
	t_1/min	3.71	3.82	3.93	4.05	4.20	4.41
Salbutamol	t_2/min	3.87	4.02	4.47	4.72	4.82	4.99
	R_S	0.43	0.53	1.47	1.82	1.68	1.58

[a] t_1, t_2: migration time; R_S: resolution.

rent amounts of SDS in the range of 0—30 mmol•L^{-1} and the experiment results are shown in Table 2. Though SDS has no chiral recognition, it is essential for the enantiomeric separations of epinephrine and salbutamol. SDS is charged negatively and its migration direction is opposite to that of electroosmotic flow (EOF). Because the velocity of EOF is higher than that of SDS, SDS moves towards the cathode. Increasing the concentration of SDS leads to a general increase in the migration time for epinephrine and salbutamo. This causes increasing of the complexation reaction time, and as a result, it is good for the enantiomeric separation. However, with the increase of the concentration of SDS, current intensity increases, which results in the increase of the broadness of zone and the decrease of the resolution. Taking these factors into consideration, we chose 15 mmol•L^{-1} SDS as the optimal concentration.

Effect of pH and concentration of BGE on resolution

The effect of the pH of BGE on the resolution was investigated by using different buffers at pH in the range of 7.0—10.0 containing 8 mmol•L^{-1} β-CD, 15 mmol•L^{-1} SDS. Figure 3 shows the effect of the pH of the BGE on the resolution of the epinephrine and salbutamol. The results showed a maximum of the resolution at pH 8.6 for epinephrine and salbutamol respectively with the increase of buffer pH. One explanation for this is probably that the increase in buffer pH may cause a stronger complexation reaction by stereoselective bonds (hydrogen) between the amino groups of the analytes and hydroxyl groups of the β-CD. On the other hand,

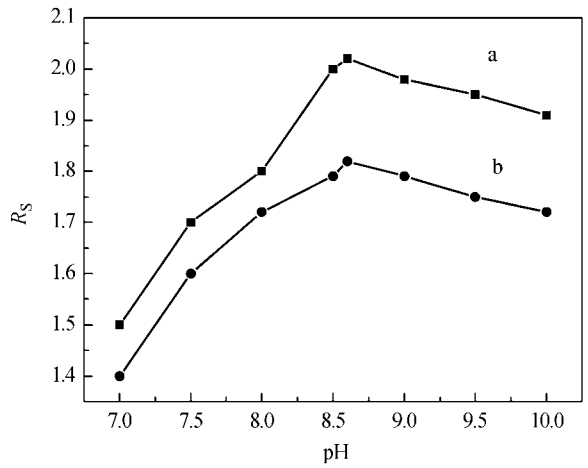


Figure 3 Effect of the pH of the BGE on resolution a, epinephrine; b, salbutamol.

with the increase of buffer pH, the negative charge of the Si-OH of fused-silica wall becomes denser, the zeta potential becomes high and the EOF increases, thus causing shorter migration time and lower resolution. So pH=8.6 was chosen as the optimal buffer pH.

The effect of buffer concentration on resolution was carried out by using different buffer in the range of 6—20 mmol•L^{-1} ammonium chloride at pH=8.6 containing 8 mmol•L^{-1} β-CD, 15 mmol•L^{-1} SDS. An increase in the buffer concentration caused an increase in the migration time of the analytes owing to the influence of the higher ionic strength of the electrophoretic media, thus making the resolution increase. However, a

too high concentration of the buffer solution would lead to appreciable Joule heat, which can strongly influence the efficiency of the separation. So taking these two factors into consideration, we chose 15 mmol•L^{-1} as ammonium chloride concentration.

Effect of applied voltage on resolution

The experiments for the study of the effect of applied voltage on resolution were carried out in the BGE of 15 mmol•L^{-1} ammonium chloride-ammonia solution at pH 8.6, containing 8 mmol•L^{-1} β-CD and 15 mmol•L^{-1} SDS. The results are shown in Figure 4. The applied voltage was another important factor that influences separation. The increase of the voltage could enhance the separation efficiency and reduce the analysis time. However, with the increase of the voltage, the Joule heat increases, leading to the increase of the current intensity and the broadness of zone, thus can make the resolution decrease. In this paper, +10 kV was chosen as the applied voltage.

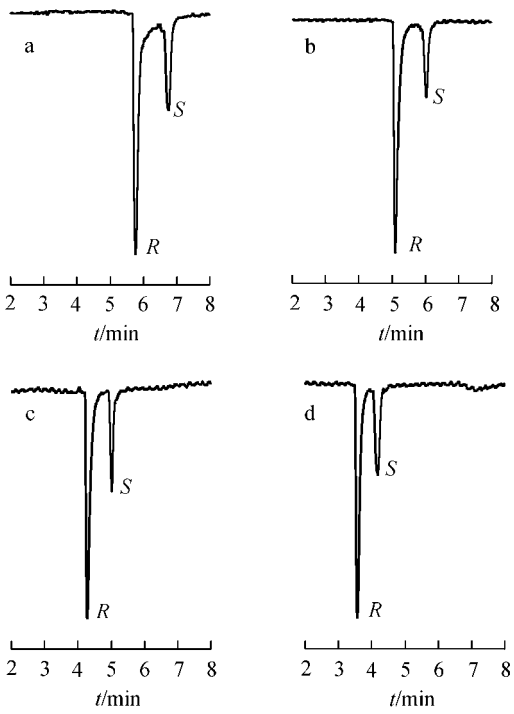


Figure 4 Effect of applied voltage on resolution of epinephrine. a, 6 kV; b, 8 kV; c, 10 kV; d, 12 kV.

Chiral recognition of CD

Native CDs are cyclic oligosaccharides which have a toroidal shape built up from six (α-), seven (β-) or eight (γ-) *D*(+)-glucopyranose units bonded through –(1,4) linkages. Cyclodextrins contain 18(α-CD), 21(β-CD) or 24(γ-CD) hydroxyl groups respectively. The interior of the CD cavities is relatively hydrophobic, thus allowing them to form inclusion complexes with a variety of molecules.

The basic property of CD that allows them to affect chiral separations is their ability to form enantioselective inclusion complexes with guest molecules. There are several requirements for chiral recognition by CDs. The size of the CD cavity with respect to the size of the enantiomer to be complexed is of critical importance, and tighter fitting molecules are preferable. Additionally, it is beneficial if the substituents attached to the chiral centre of the compound to be resolved interact with the secondary hydroxyl groups at the mouth of the CD cavity by hydrogen bongding. Under the optimal experimental condition, epinephrine and salbutamol are charged negatively. The migration direction of their β-CD inclusion complexes is opposite to that of EOF and towards the cathode. The stabilities of *R* and *S*-inclusion complexes are different, so enantiomer separation can be obtained. In this method, the stability of *S*-enantiomer is higher than that of *R*-enantiomer. And an increasing in the stability of inclusion complexes leads to an increase in the migration time, and *S*-enantiomer is retained longer than *R*-enantiomer. Under the optimized conditions, baseline separation of the enantiomers could be accomplished in 6 min and the capillary electrophorograms of the enantiomers of epinephrine and salbutamol are shown in Figure 5.

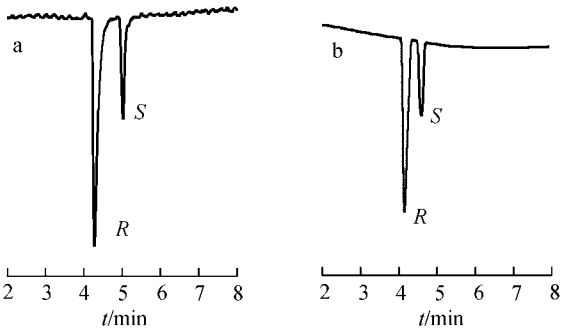


Figure 5 Electrophorograms for the enantiomeric separation of epinephrine (a) and salbutamol (b).

References

1 Furuta, R.; Doi, T. *J. Chromatogr., A* **1995**, *708*, 245.
2 Xia, L.-J.; Hu, J.-B.; Ding, Z.-D.; Jin, H.; Zhao, G. *Chin. J. Org. Chem.* **2002**, *22*, 60 (in Chinese).
3 Chen, X.-M.; Mao, X.-Q.; Zhang, Z.-Z.; Luo, Q.-Z.; Zou, H.-F. *Acta Chim. Sinica* **2002**, *60*, 93 (in Chinese).
4 Armstrong, D. W.; Faulkner, J. R.; Han, Jr. S. M. *J. Chromatogr., A* **1988**, *452*, 323.
5 Granville, C. P.; Gehrcke, B.; Konig, W. A.; Wainer, I. W. *J. Chromatogr.* **1993**, *622*, 21.
6 Runge, S. C.; Rizzi, A. *J. Chromatogr., A* **1997**, *759*, 157.
7 Lu, H.-J.; Yu, C.-T.; Guo, Y.-L. *Acta Chim. Sinica* **2002**, *60*, 882 (in Chinese).
8 Schmitt, T.; Engelhardt, H. *Chromatographia* **1993**, *37*, 475.
9 Zheng, Y.-P.; Mo, J.-Y.; Lai, R. *Acta Chim. Sinica* **2003**, *61*, 89 (in Chinese).
10 Wren, S. A. C.; Rowe, R. C. *J. Chromatogr., A* **1992**, *603*, 235.

(E0311249 ZHAO, X. J.)