

Chiral Separation of Some β -blockers Using Electrophoresis with Dual Cyclodextrin Systems

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Abstract

This paper shows the versatility of a dual cyclodextrin (CD) system in the direct chiral resolution of some β -blocks. The dual CD system consists of β -CD and β -CD-GLU. The enantiomeric separation of several β -blocks such as metoprolol, atenolol, isoproterenol, salbutamol and clenbuterol were investigated. The chiral resolution was strongly influenced by the concentration of the reaction mixture, pH and the organic modifier. The compounds studied can be enantiomerically resolved in less than 10 minutes with no capillary treatment. A possible separation mechanism and several factors influencing the chiral separation were discussed. The linearity, quantitative limit for the crude drug atenolol and the precision of the measurements were determined.

Keywords: Chiral separation, drug, capillary electrophoresis

Résumé

Nous montrons dans cet article la versatilité d'un système double cyclodextrine (CD) pour la résolution chirale directe de certains blocs- β , le double système CD étant formé de β -CD et β -CD-GLU. Nous avons étudié la séparation énantiomérique de plusieurs blocs- β tels que métoprolol, aténolol, isoprotérenal, salbutanol et clenbutérol. La résolution chirale a fortement été influencée par la concentration du mélange réactif, le pH et le modificateur organique. Les composés étudiés ont pu être résolus énantiomériquement en moins de 10 minutes sans traitement capillaire. Nous discutons d'un mécanisme possible de séparation et de plusieurs facteurs qui influencent la séparation chirale. Nous avons déterminé la linéarité, la limite quantitative pour le composé brut aténolol et la précision des mesures.

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Introduction

Since capillary electrophoresis (CE) and its related techniques represent high separation efficiencies, rapid analysis times, and low reagent consumption, they have been widely studied as promising analytical methodologies (1-12). Native cyclodextrin (CD), mostly β -CD, were the first most frequently used CD. Currently, its use is in decline. Native β -CD has limited solubility in water-based buffers and displays moderate selectivity spectra (13).

Applications of CDs were expanded with the introduction of alkylated derivatives of CDs. These derivatives show better solubility in various buffers and good solubilizing features and have broader chiral recognition spectra than native CDs (14). There is an approach known as the dual CDs system that can produce better CE chiral separations (15,16).

In a previous paper (3), we have proposed a very simple method to obtain an effective chiral selector (a reaction mixture-a dual CDs system- β -CD and β -CD-GLU), and we described its overall synthetic process including how the two chiral drugs (lobeline and benzhexol) were separated. In this work, we extended its application using more chiral drugs and separated the atenolol enantiomers from the crude drug.

Experimental

Apparatus

The experiments were carried out with a homemade CE apparatus, equipped with a multi-wavelength UV detector. The UV signals were recorded at 214 nm. A fused-silica capillary of 48 cm \times 75 μ m (i.d.) with an effective length of 40 cm (Hebei Yongnian Optical Fiber Factory, China) was used as a separation capillary.

Reagents

Phosphoric acid, Tris(Hydroxymethyl)aminomethane and sodium hydrate were of analytical reagent grade.

Metoprolol, atenolol, isoprottereliol, salbutamol and clenbuterol were purchased from Sigma. Their structures are shown in Figure 1. The β -CD was purchased from Yunan Cyclodextrin Factory, China. Double distilled water was used for the preparation of all solutions. The dual CDs system (β -CD and β -CD-GLU) was synthesized in our laboratory according to the reference (3) (use b-CDr as the dual CD system of β -CD and β -CD-GLU in the following. The structure of β -CD-GLU is shown in Figure 2).

Preparation of crude drug

Solutions of the crude drug form (tablet) of atenolol were preprocessed as follows. First, the sugar coating of a tablet (0.1 g sample) was removed. Each tablet was weighed out accurately. It was then placed into a 100 mL volumetric flask, and 50 ml of methanol was added. After shaking the mixture for 20 min, methanol to added to the mark. Then, it was filtered, and a portion of the filtrate solution was diluted using the running buffer to 300 μ g/ml before being analyzed by CE.

Standard solution: a stock solution of pure racemic atenolol was prepared in methanol (1.0 mg/ml). It was diluted with a running buffer to the appropriate concentrations before use.

Procedures

Tris- H_3PO_4 buffer (50 mmol/L) was prepared by dissolving 3.025 g of Tris in water, titrating it to pH (2.5, 3.0, 3.5, 4.0) with phosphoric acid, then diluting the solution to volume in a 500 mL-volumetric flask. The chiral selector was dissolved in the above buffer. The buffers were filtered through a 0.45 μ m membrane filters and were degassed by sonication prior to use. A new capillary was conditioned by flushing successively with 1.0 mol/L NaOH (60 min), 0.1 mol/L NaOH (30 min) and double distilled water (30 min) before use. Between each injection, the capillary was rinsed with 0.1 mol/L NaOH (2 min), double distilled water (2 min) and with the respective running buffer (2 min).

Samples were injected electrokinetically at 10 kV for 5 s. The applied voltage was constant at 15 kV. The UV signals were recorded at 214 nm.

Capillary electrophoresis procedure and resolution calculation

A new capillary was conditioned by flushing successively with 1.0 mol/L NaOH (60 min), 0.1 mol/L NaOH (30 min) and double distilled water (30min) before use.

Between each injection, the capillary was rinsed with 0.1 mol/L NaOH (2 min), double distilled water (2 min) and with the respective running buffer (2min).

Resolution (R_s) for a pair of enantiomers was calculated using the following equation:

$$R_s = \frac{2(t_2 - t_1)}{w_2 + w_1}$$

where t_1 and t_2 are the migration times of the enantiomers measured in minutes; w_1 and w_2 are the peaks widths at the baseline of each enantiomer designated as "1" and "2", and are also measured in minutes.

Results and Discussion

Effect of the pH on Resolution (R_s)

Buffer pH is an important parameter in the CE chiral separation because pH affects resolution and migration time. All the enantiomers studied are basic drugs; they exist as positively charged ions in acidic condition.

Basic drugs and their complexes will migrate towards the cathode. At low pH, the surface charge of the capillary and the adsorption of cationic analytes to the bare fused silica surface can be reduced (17). At the same time, the electroosmotic flow (EOF) will be much less than that at high pH. All these factors provide analytes with a longer time for interaction with CDs as they migrate through the capillary. Therefore, lower pH seems to be more favorable for the separation of the basic drugs.

The separation was investigated at pH 2.5, 3.0, 3.5 and 4.0. Electroosmotic flow decreased with decreasing pH and migration times increased. Figure 3 shows the effect of pH on resolution. It can be seen that the optimized resolution was obtained at pH 2-2.5. The optimum separation for all the β -blockers was found at pH 2.5.

Effect of the chiral selector concentration on R_s

The β -CD was investigated for the chiral recognition of the enantiomeric drugs. When β -CD was used, no baseline separation was observed for all the β -blocks. We evaluated the effect of the concentration of b-CDr on the resolution of the isomers, when the concentration of b-CDr was raised from 5-25 g/100 mL buffer. Usually, maximum resolution values are obtained at a given cyclodextrin concentration, which depends on the affinity of the analyte enantiomers for the selector. The resolution increases with increasing β -CD concentration, a maximum resolution is reached, and further increase in

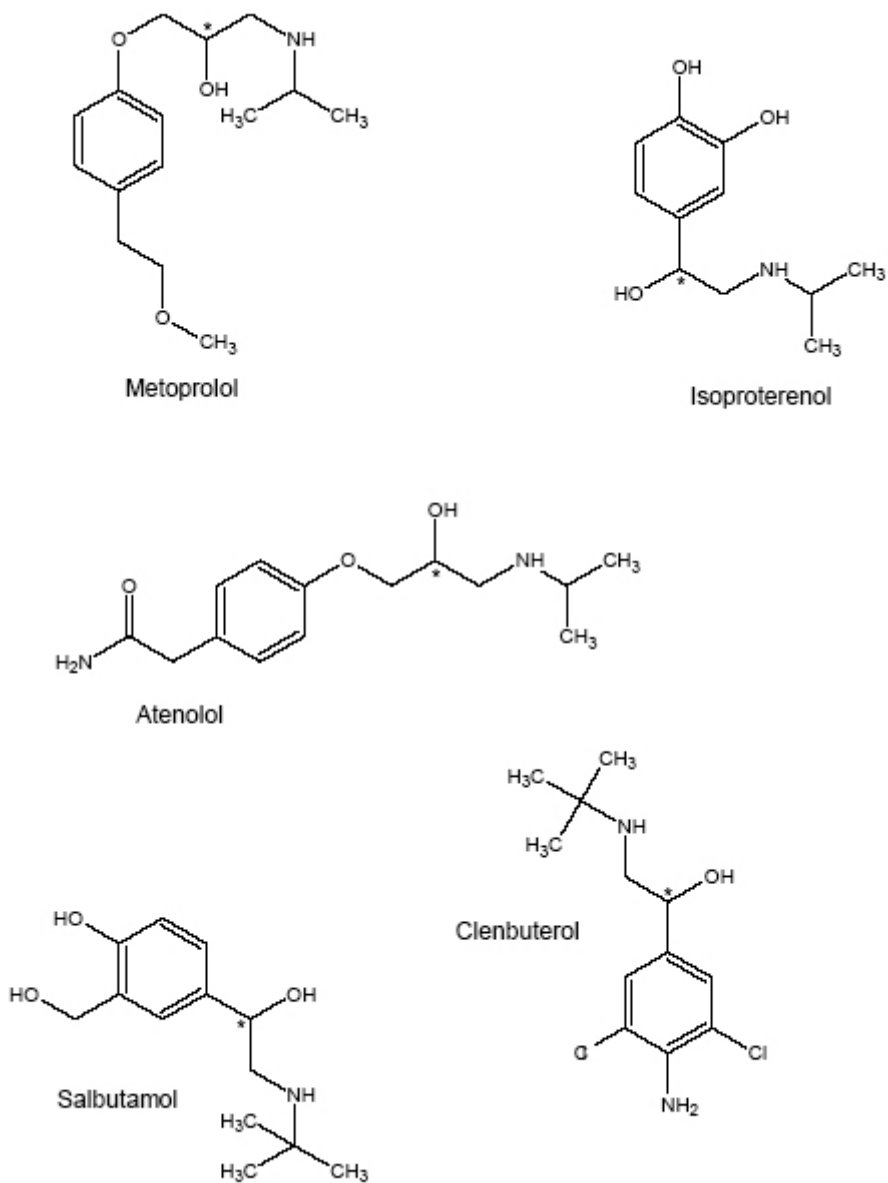


Figure 1. The structures of chiral drugs.

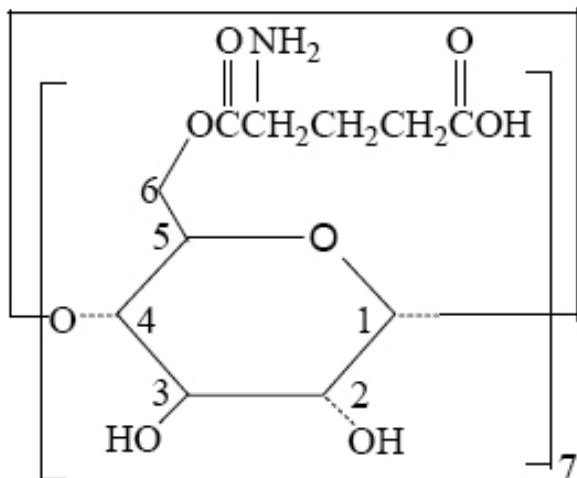


Figure 2. The structure of β -CD GLU.

the β -CD concentration can result in a slow decrease in resolution. The experimental results are consistent with a simple model proposed by Wren *et al.* (18). As can be seen in table 1, the optimum separating concentration of b-CDr (g/100 ml) for metoprolol, atenolol, isoproterenol, salbutamol and clenbuterol was 20, 20, 15, 25 and 15. Figure 4 showed the best resolutions of the drugs. It has been shown that they were all baseline separated by the

reaction mixture. We have already discussed the chiral separation mechanism in reference (3).

Effect of organic modifier on resolution

The organic modifier can affect both migration time and chiral resolution when the inclusion-complex mechanism is involved in the separation process. Some researchers (19) reported that the addition of organic solvents to the buffer improved the chiral separation. Some researchers (20) reported the contrary results. The reason is that the organic modifier can have two roles (21): (a) improving the solubility of chiral substances, which may reduce the adsorption of analyte to the capillary wall and leads to improved resolution. (b) Decreasing the interaction of the analyte with the hydrophobic cavity of chiral selectors, which may result in poor separation.

The addition of methanol for the separation of metoprolol enantiomers was studied here. The effects of methanol on the resolution and migration time of metoprolol are shown in Table 2. It shows that the addition of methanol decreased the chiral resolution of metoprolol and increased the migration time. Therefore, the addition of organic solvents to the buffer decreased the interaction of the analytes with the hydrophobic cavity of chiral selectors, which resulted in poor separation.

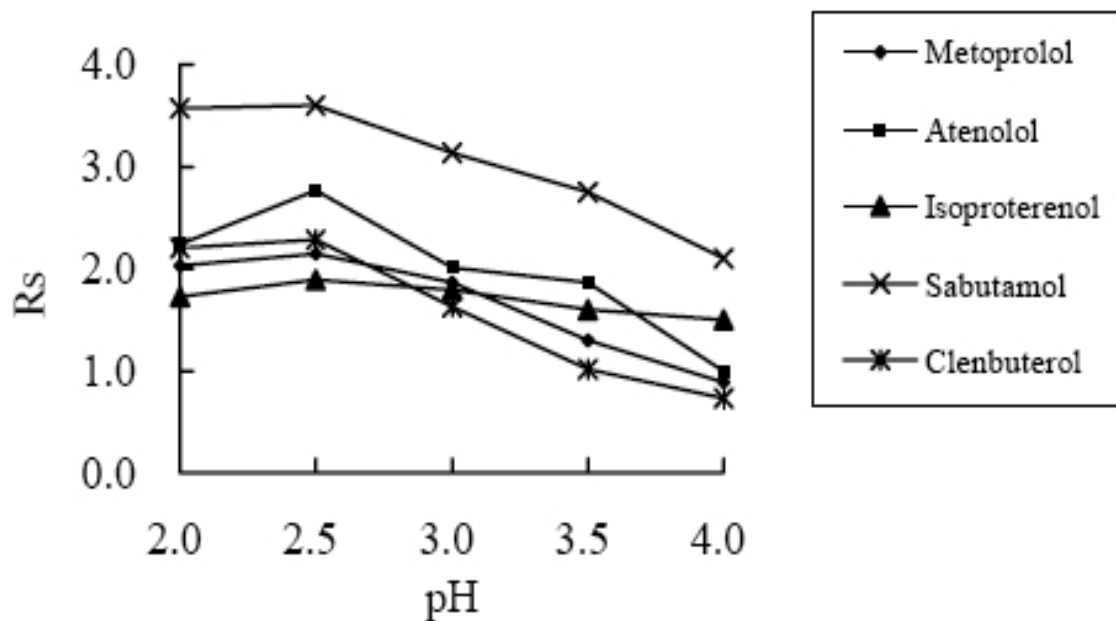


Figure 3. Effect of buffer pH on resolution (R_s). Separating conditions: Buffer: 50 mmol/L Tris- H_3PO_4 ; b-CDr, 20 g/100ml buffer; applied voltage, 15 kV; injection, 10 kV/5 s; detection wavelength, 214 nm.

Table 1. Influence of cyclodextrin concentration on chiral resolution (R_s).

Analyte	Concentration (g/100 mL buffer)				
	5	10	15	20	25
Metoprolol	0.69	1.13	1.97	2.15	1.86
Atenolol	0.88	1.02	2.15	2.77	2.02
Isoproterenol	0.56	1.14	1.89	1.55	1.02
Salbutamol	0.85	1.81	2.15	2.74	3.60
Clenbuterol	1.01	1.97	2.28	2.10	1.49

The separating conditions: buffer, 50 mmol/L Tris- H_3PO_4 (pH = 2.5); applied voltage, 15 kV; injection, 10 kV/5 s; detection wavelength, 214 nm.

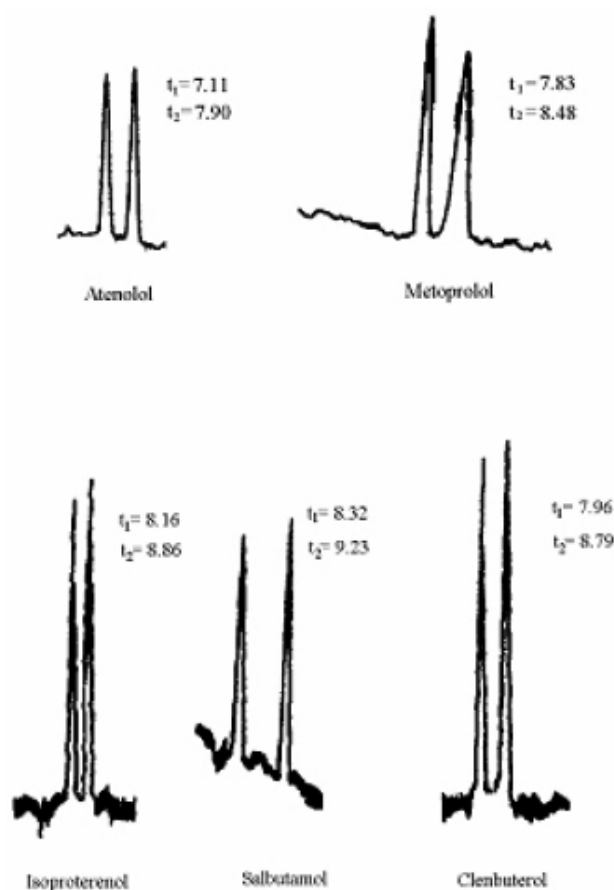


Figure 4. The electropherograms of chiral drugs. Separating conditions: buffer: 50 mmol/L Tris- H_3PO_4 (pH = 2.5); b-CDr, 20 g/100ml buffer; applied voltage, 15 kV; injection, 10 kV/5 s; detection wavelength, 214 nm.

Table 2 Influence of organic modifiers on the enantiomeric separation of metoprolol.

Methal Content (%)	t_1	t_2	R_s
0	7.83	8.48	2.15
10	10.14	10.55	1.76
20	11.07	11.32	1.00
30	12.61	12.87	0.87

Other separating conditions see Fig.4.

Electrophoretic analysis

Once the method had been optimized, its reproducibility (10 injections a per day) was tested. For the migration times and peak area, the RSD values that were obtained were 0.46 and 4.8%. The calibration curves for atenolol showed good linearity in the range 120-770 $\mu\text{g/ml}$ ($r \geq 0.99$). The limit of detection was 40 $\mu\text{g/ml}$. The recovery obtained from this method were 97.2, 98.0 and 102.2 %.

Conclusion

The results demonstrate that in CZE the mixture of β -CD and β -CD-GLU gave optimal separation of some β -blocks, which cannot be resolved by using only β -CD. The resolution is obtained in less than 10 minutes. The optimum separation conditions depend on the CD type, concentration and the pH of the buffer. Finally, it was demonstrated that this method has adequate sensitivity and reproducibility to separate the enantiomer of the crude drug.

The present method represents a simple and less expensive alternative to the synthesis of CD derivatives. The mixture of CDs was found to be an effective dual CDs system for the CE chiral separation.

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