

Separation of Uniconazole by Enantiomers Capillary Electrophoresis with Dual Cyclodextrin Systems

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Résumé

Nous avons développé une méthode à électrophorèse capillaire micellaire (MEKC) modifiée par la cyclodextrine en se servant de mélanges de β -cyclodextrines (β -CD) et de mono-3-O-phénylcarbamoyle- β -CD comme additifs chiraux, pour la séparation chirale de uniconazole avec les deux systèmes CD. Les énantiomères ont pu être résolus en utilisant un tampon de 50 mmol/L de borate à pH 9.5, contenant 10 mmol/L de β -CD et 10 mmol/L de mono-3-O-phénylcarbamoyle- β -CD, 50 mmol/L de laurylsulfate de sodium et 5% de 1-propanol. Nous avons effectué une étude de l'influence respective de la concentration de β -CD et du mono-3-O-phénylcarbamoyle- β -CD afin de déterminer les conditions optimales par rapport à la résolution.

Abstract

A cyclodextrin-modified micellar capillary electrophoretic method (MEKC) was developed using mixtures of β -cyclodextrins (β -CD) and mono-3-O-phenylcarbamoyle- β -CD as chiral additives for the chiral separation of uniconazole with the dual CD systems. The enantiomers were resolved using a running buffer of 50 mmol/L borate pH 9.5 containing 10 mmol/L β -CD and 10 mmol/L mono-3-O-phenylcarbamoyle- β -CD containing 50 mmol/L sodium dodecyl sulfate and 5% 1-propanol. A study of the respective influence of the β -CD and the mono-

3-O-phenylcarbamoyle- β -CD concentration was performed to determine the optimal conditions with respect to the resolution.

Keywords: chiral separation, drug, uniconazole, capillary electrophoresis

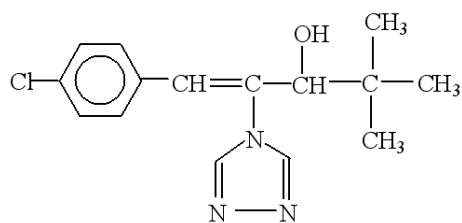
Introduction

Micellar electrokinetic chromatography (MEKC), which was introduced by Terabe *et al.* (1), is a variation of capillary electrophoresis (CE). The separation is a function of the distribution of the solutes between a micellar phase, working as a pseudo-stationary phase, and an aqueous mobile phase; the two phases are moving at different velocities within a fused-silica capillary.

Although most separation studies on MEKC have been performed with sodium dodecyl sulfate (SDS) (2-4), some other surfactants and bile salts (5) have also been successfully employed. A prerequisite for an accurate study of the stereoselective effects of the action of chiral drugs is the development of a versatile and accurate method for the resolution of enantiomers. The application of new types of chiral selectors with higher efficiency is presently of primary importance in this area.

Uniconazole (Figure 1), which is vinyl triazole, has fungicidal and plant growth-regulating activities. It has an asymmetric carbon, and its enantiomers are known to differ significantly in their biological properties. The *R*-enantiomer demonstrates stronger fungicidal activity than the *S*-enantiomer, whereas the *S*-enantiomer is more active than the *R*-enantiomer with regard to plant growth-regulating activity. Reliable and efficient methods for separating the enantiomers are therefore necessary. The

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Uniconazole

Figure 1. Structure of uniconazole

aim of our work was to develop a MEKC method for the separation of uniconazole and to investigate the synergistic effect of dual CDs for enantioseparation. The effects of the concentration of the chiral selectors, the buffer concentration and its pH on the enantioseparation were studied.

Experimental

Apparatus:

The experiments were carried out on a laboratory-assembled CE apparatus equipped with a multiwavelength UV detector. The UV signals were recorded at 214 nm. A fused silica capillary of 60 cm length (effective length of 50 cm) and of 75 μm i.d. (Hebei Yongnian Optical Fiber Factory, China) was used as a separation tube. A high-voltage power supply that could provide a voltage from 0 to 30 kV was used to generate electroosmotic flow (EOF).

Chemicals and reagents:

Racemic uniconazole was purchased from Sigma (St. Louis, MO, USA). Mono-3-*O*-phenylcarbamoyl- β -CD (6), which had been used to separate many drugs by us before, was synthesized in our laboratory. α -, β -, γ -CD, 2,6-di-*O*-methyl- β -CD (DM- β -CD), 2,3,6-tri-*O*-methyl- β -CD (TM- β -CD) and Hydroxypropyl- β -CD (HP- β -CD) were obtained from Sigma (St. Louis, MO, USA). All organic solvents and other chemicals were of analytical grade; sodium dodecyl sulfate (SDS), boric acid (H_3BO_3), sodium hydroxide (NaOH), 1-propanol and disodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) were obtained from Beijing Chemical Factory. Double-distilled water was used to prepare of all solutions; 0.25 μm pore size filters was used to filter all of the solutions.

Capillary electrophoresis:

A new capillary was conditioned by flushing

Table 1. Influence of the concentration of mono-3-*O*-phenylcarbamoyl- β -CD on the chiral resolution of uniconazole in a single CD system

Concentration (mmol/L)	5	10	15	20
Resolution (R_s)	0.25	0.91	0.65	0.57

Electrophoretic conditions: 50 mmol/L borate (pH 9.5) containing 50 mmol/L SDS and 5% 1-propanol; applied voltage: 10 kV; temperature: 25°C; detection wavelength: 214 nm; injection: 10 kV per 5 s; analyte concentration: 300 $\mu\text{g}/\text{ml}$.

successively with 1.0 mol/L NaOH (overnight) and 0.1 mol/L NaOH (30 minutes) and then equilibrated with double-distilled water and running buffer each for 30 minutes before use. Between each injection, the capillary was rinsed with 0.1 mol/L NaOH (2 minutes), double distilled water (2 minutes) and with the respective running buffer (5 minutes). Samples were injected by an electrokinetic method at the anode. MEKC operations were run under a constant voltage at ambient temperature.

Results and Discussion

Single CD systems as a chiral selector

In CD-MEKC, the solutes are distributed among three phases: aqueous, micellar and CD. Solute form inclusion complexes with CDs based on their size, geometry and physicochemical properties, while interactions with micellars are based on the solute hydrophobicity. The solute is partitioned between the micellar and CD cavity. Therefore, the type CD is the most important factor for separation. We investigated the type of CD (α -, β -CD, DM- β -CD, HP- β -CD, TM- β -CD, mono-3-*O*-phenylcarbamoyl- β -CD) on the resolution of uniconazole using a 50 mmol/L borate buffer, pH = 9.5, containing 50 mmol/L SDS and 5% 1-propanol.

The enantiomers of uniconazole could not be separated by α -CD, β -CD, HP- β -CD, DM- β -CD and TM- β -CD. They were only partly separated when mono-3-*O*-phenylcarbamoyl- β -CD was used. The effect of the mono-3-*O*-phenylcarbamoyl- β -CD concentration on the resolution was examined by varying the concentration in steps from 5 to 20 mmol/L. As can be seen in table 1, the maximum resolution was reached at a certain concentration of mono-3-*O*-phenylcarbamoyl- β -CD (10 mmol/L). The experimental results are consistent with a simple model proposed by Wren *et al.* (7).

Table 2. Enantioseparation of uniconazole using dual CD systems containing β -CD and mono-3-*O*-phenylcarbamoyl- β -CD in different concentration ratios

[β -CD/ mono-3- <i>O</i> -phenylcarbamoyl- β -CD] (mmol/L)	5/10	10/10	15/10
R_s	1.42	3.05	2.66

Electrophoretic conditions were the same as Table 1.

In the inclusion-complexation mechanism, the compound fits the CD cavity with the whole molecule or with the hydrophobic part, and the CD cavity size has a very important role in the separation process. The hydrophobic interaction with the cavity alone is not sufficient to enable the separation of chiral drugs; weak bonds between substituent groups on the asymmetric center of analytes and secondary and/or primary groups of the CD ring are responsible for chiral recognition. This result underlines the fact that mono-3-*O*-phenylcarbamoyl- β -CD is more suitable for the inclusion of uniconazole than the other CDs.

Dual CD systems for the enantioseparation of uniconazole

Since various CDs exhibited different chiral selectivity, it is obvious that electrolyte systems composed of mixed CDs should yield a unique chiral selectivity that cannot be achieved by either of the CDs alone. In order to be able to enhance the resolution in the CE enantioseparation of uniconazole, dual CD systems were employed. The β -CD was added at different concentrations (0 - 15 mmol/L) to a pH 9.5 borate buffer containing SDS and 1-propanol with 10 mmol/L mono-3-*O*-phenylcarbamoyl- β -CD. As can be seen in table 2, the resolution was significantly increased. The addition of β -CD resulted in complete enantioseparation. Mixtures of these two cyclodextrins were employed due to the possible increase in selectivity that could be obtained (8).

Effect of the buffer pH

The pH of the buffer is a very important parameter to be studied. The change in the pH influences the charge of both the analytes and the chiral selector and thus their electrophoretic mobility. Furthermore, the electrostatic interactions between the analyte and the CDs as well as the solubility of CDs are also influenced. It is obvious that EOF increases with an increase in the pH. At a low pH, the migration time is long; this easily causes such

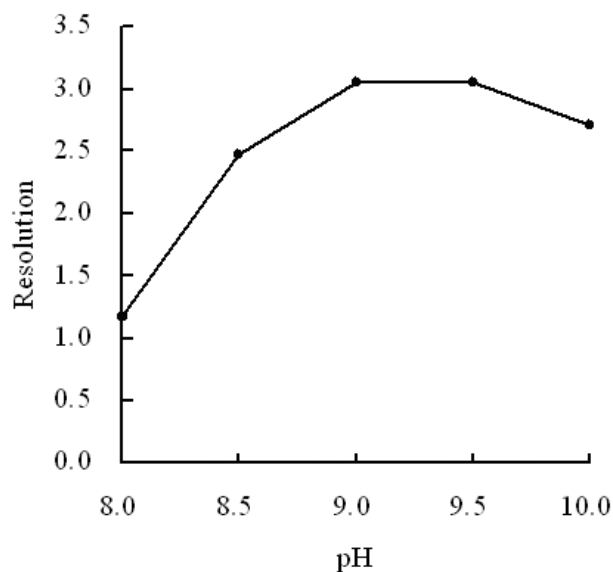


Figure 2. Effect of the pH on the resolution. Electrophoretic conditions: β -CD/ mono-3-*O*-phenylcarbamoyl- β -CD (mmol/L): 10:10; 50 mmol/L borate containing 50 mmol/L SDS and 5% 1-propanol at different pH : (8.0, 8.5, 9.0, 9.5, 10.0); applied voltage, 10 kV; temperature, 25°C; detection wavelength, 214 nm; injection, 10 kV per 5 s.

results as severe diffusion and lower efficiencies. At the same time, borate buffer can maintain a good buffer capacity in the pH range of 8-10. Thus the pH dependence of the chiral resolution was investigated in the pH range from 8 to 10. Figure 2 shows the effect of the pH of the buffer on the resolution of uniconazole. The best resolutions were obtained for uniconazole at pH 9.0- 9.5.

Effect of the buffer concentration

The effect of the borate concentration on resolution was also investigated over the range of 0 to 50 mmol/L at pH 9.5. When the concentration of borate was increased from 0 to 50 mmol/L, we observed an improvement in the enantiomeric resolution and a lengthening of the migration time. This is consistent with the publication that emphasized the use of a high ionic concentration of buffer solutions to improve the resolution and the peak shape (9). A high buffer concentration gives significantly higher efficiencies and enhances the resolution. It also prevents analyte-analyte or analyte-wall adsorption in the capillary and thus leads to better quantification and reproducibility (10). On the other hand, high ionic concentration buffers increase the current generation and may lead to Joule heating. Figure 3 shows the effect of the borate concentration on resolution. A 50

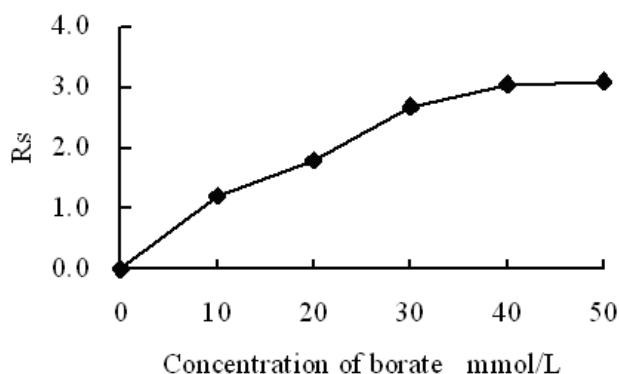


Figure 3. Effect of the borate concentration on R_s . The electrophoretic conditions were the same as in Figure 2 (pH = 9.5)

Table 3. Effect of the SDS concentration on the resolution of uniconazole

SDS concentration (mmol/L)	Migration time (min)		R_s
10	9.41	9.41	0.00
30	10.31	10.73	1.50
50	15.02	15.71	3.05
70	22.54	23.29	3.09

Electrophoretic conditions: 50 mmol/L borate (pH 9.5) containing different concentration of SDS and 5% 1-propanol; Other electrophoretic conditions were the same as Table 1.

mmol/L borate buffer was used in our experiments.

Effect of SDS concentration

The concentration of SDS is also one of the most important parameters for the resolution. SDS monomers can have their hydrophobic tails end in the CD cavity along with the solute. This could change the nature of the solute and the CD interaction. At the same time, the increases in the fraction of the solute partitioning into the CDs micellar phase at higher SDS concentrations delays the migration time of the solute. We therefore tested the effect of the SDS concentration on the resolution. As shown in Table 3, when the concentration of SDS was less than 10 mmol/L, uniconazole could not be separated. When the concentration of SDS was 70 mmol/L, though the resolution was slightly improved, the migration time was too long, and thus 50 mmol/L SDS was chosen in our experiment.

Table 4. Influence of organic modifiers on the enantiomeric separation of uniconazole

	t min		
	t_1	t_2	
None	12.31	12.73	2.25
5	14.74	15.35	2.76
10	17.27	17.77	1.91
5	15.02	15.71	3.05
10	18.34	18.85	2.13

The electrophoretic conditions were the same as in Figure 4.

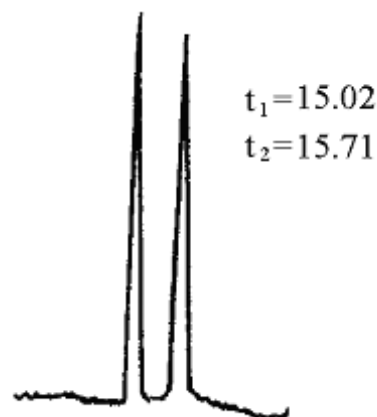


Figure 4. Electropherograms of uniconazole. Electrophoretic conditions: β -CD/mono-3-*O*-phenylcarbamoyl- β -CD (mmol/L): 10:10; 50 mmol/L borate (pH 9.5) containing 50 mmol/L SDS and 5% 1-propanol; applied voltage, 10 kV; temperature, 25°C; detection wavelength, 214 nm; injection, 10 kV per 5 s.

Effect of an organic modifier

The resolution in MEKC can be improved by modifying the buffer by adding some short-chain alcohols, which decrease the electroosmotic flow (EOF) and affinity of the hydrophobic solute for the micellar phase (9,11).

In our study, methanol and 1-propanol were used as organic modifiers. The concentration of each organic modifier was 5% and 10%. Table 4 lists the effect of the content of each organic modifier on the resolution and migration times. It can be seen that the effect with 1-propanol (5%) seems to be most pronounced.

Reproducibility of this method

Once the method had been optimized, we tested its repeatability (9 injections per day). For the migration times and peak area, the RSD values we obtained were

0.45 and 5.2%, respectively.

Conclusion

We studied the optimal conditions for enantiomeric separation of uniconazole using CD-MEKC as a routine analysis method. Dual CD systems of β -CD and mono-3-*O*-phenylcarbamoyl- β -CD have good synergistic effects for the enantiomeric separation of uniconazole.

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