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Journal of Chromatography A, 971 (2002) 261–266

JOURNAL OF CHROMATOGRAPHY A

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Short communication

# Enantioseparation of phenothiazines in cyclodextrin-modified micellar electrokinetic chromatography

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Received 14 December 2001; received in revised form 8 July 2002; accepted 11 July 2002

**Abstract**

In this study, enantioseparations of five phenothiazines in cyclodextrin (CD)-modified micellar electrokinetic chromatography (MEKC) were investigated using a citrate buffer containing tetradecyltrimethylammonium bromide (TTAB) as a cationic surfactant at low pH. β-Cyclodextrin (β-CD) and hydroxylpropyl-β-CD (HP-β-CD) were selected as chiral selectors. The results indicate that the separation window is greatly enlarged by β-CD concentration and that the separability and selectivity of phenothiazines are remarkably influenced by the concentrations of both β-CD and TTAB, as well as buffer pH. The interaction of thioridazine with β-CDs is considerably reduced in the presence of TTAB micelles due to competitive complexation of thioridazine with TTAB micelles, which is pH-dependent. As a result, effective enantioseparation of thioridazine is simultaneously achievable with that of trimeprazine and promethazine or ethopropazine in MEKC with addition of either β-CD or HP-β-CD, respectively, to a micellar citrate buffer containing TTAB at pH 3.5. Better enantioresolution of thioridazine in MEKC than in capillary zone electrophoresis can be obtained.


*Keywords:* Enantiomer separation; Pharmaceutical analysis; Phenothiazines; Cyclodextrins

## 1. Introduction

Phenothiazines, used as antipsychotics, neuroleptics and antihistamines, are a group of basic drugs possessing a phenothiazine ring with different substituents attached at the 2-position and 10-position [1]. Among them, promethazine, ethopropazine, trimeprazine, methotrimeprazine, and thioridazine possess a chiral center. The structures of these phenothiazines are depicted in Fig. 1.

Phenothiazines have previously been separated by high-performance liquid chromatography (HPLC) [2–9] and capillary electrophoresis (CE) [10–19]. The enantiomers of promethazine [13,14], trimeprazine [15], or thioridazine [16] in capillary zone electrophoresis (CZE) were separated using either proteins [13,14] or cyclodextrins (CDs) [15,16,18,19] as chiral selectors. Chiral separations of the enantiomers of thioridazine, trimeprazine and ethopropazine in nonaqueous CD-modified capillary zone electrophoresis (CZE) were also studied [16]. Very recently, enantioseparations of seven chiral phenothiazines in CZE using various native CDs and some of neutral and charged CD derivatives as chiral selectors were investigated [18]. In our previous paper [19], enantioseparations of promethazine, ethopropazine, tri-

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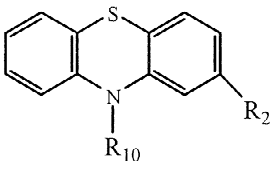
0021-9673/02/$ – see front matter 
PII: S0021-9673(02)01044-0



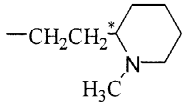
| | $R_2$ | $R_{10}$ |
|---|---|---|
| Promethazine (**1**) | H | $-CH_2\overset{*}{C}H(CH_3)N(CH_3)_2$ |
| Ethopropazine (**2**) | H | $-CH_2\overset{*}{C}H(CH_3)N(C_2H_5)_2$ |
| Trimeprazine (**3**) | H | $-CH_2\overset{*}{C}H(CH_3)CH_2N(CH_3)_2$ |
| Methotrimeprazine (**4**) | $OCH_3$ | $-CH_2\overset{*}{C}H(CH_3)CH_2N(CH_3)_2$ |
| Thioridazine (**5**) | $SCH_3$ |  |

Fig. 1. Structures of the five phenothiazines studied.

meprazine and thioridazine in CD-modified CZE with the use of CDs at low concentrations in a phosphate buffer were reported. On the other hand, separation of phenothiazines by micellar electrokinetic chromatography (MEKC) was rarely reported [10]. Nine out of 14 phenothiazines were separated with β-CD as an electrolyte modifier using a Tris–acetate buffer containing a fluorinated cationic surfactant and cetyltrimethylammonium bromide (CTAB) at pH 5.0 [10]. In fact, enantioseparation of phenothiazines in MEKC is completely blank and the migration behavior of phenothiazines in MEKC still remains incompletely understood.

In this report, enantioseparation and migration behavior of phenothiazines in MEKC with β-CD and HP-β-CD (hydroxypropyl-β-CD) as chiral selectors using a citrate buffer containing a cationic surfactant at low pH were investigated. Tetradecyltrimethylammonium bromide 'TTAB' was selected as the cationic surfactant. The influences of the concentration of β-CDs on the enantioseparation of phenothiazines were examined. Also, the interactions of phenothiazines with β-CD in the presence of TTAB micelles were studied so that enantioseparation of phenothiazines in MEKC can be better understood. Here we present the results of our investigation.

## 2. Experimental

### 2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE System 5500 equipped with a UV detector for absorbance measurements. Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary are 57 cm×50 μm I.D. The effective length of the capillary is 50 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer and a laser printer. System Gold software from Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

### 2.2. Chemicals and reagents

Five phenothiazines were obtained from Sigma (St. Louis, MO, USA). β-CD was obtained from Merck (Darmstadt, Germany) and HP-β-CD from Sigma–Aldrich (St. Louis, MO, USA). TTAB was acquired from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). All other chemicals were of analytical grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of phenothiazines at a concentration of 10 μg/ml were prepared by dissolving analytes in an aqueous solution. The pH of a citrate buffer was adjusted to the desired pH value by mixing various proportions of a certain concentration of trisodiumcitrate solution with the same concentration of citric acid. All buffer solutions, freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

### 2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 30 min with 1.0 *M* NaOH solution, followed by 30 min with deionized water at 25 °C. Before each injection, the capillary was prewashed for 3 min with running buffer and postwashed for 3 min with deionized water, 3 min with 0.1 *M* NaOH, and 5 min with deionized water to maintain proper reproduci-

bility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 5 s under a pressure of 1.0 p.s.i. at 25 °C. The measurements were run at least in triplicate to ensure reproducibility. The relative standard deviation of migration time is less than 0.6% ($n=5$). An applied voltage of −20 kV was selected, and the total current measured was less than 50 μA. The detection wavelength was set at 254 nm. Peak identification was conducted by spiking with the analyte to be identified. Mesityl oxide was used as neutral marker.

### 2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times as described previously [19].

## 3. Results and discussion

For an effective separation of the five phenothiazines studied by MEKC, citrate buffer, rather than phosphate buffer, containing TTAB micelles at pH 3.5 was used. The concentrations of citrate buffer and TTAB micelles were optimally selected at 80 and 10 m*M*, respectively, throughout the study. β-CD and HP-β-CD were chosen as chiral selectors.

### 3.1. Enantioseparation with addition of β-CD

Fig. 2 shows the variation of the electrophoretic mobility of phenothiazines as a function of β-CD concentration in the range 0.2–7 m*M* using a micellar citrate buffer (80 m*M*) containing 10 m*M* TTAB at pH 3.5. As can be seen, the electrophoretic mobility of each individual phenothiazine decreases with increasing β-CD concentration to a different extent in the order thioridazine>methotrimeprazine>trimeprazine>ethopropazine>promethazine.

The interaction of an analyte with β-CD can be reflected from the extent of the variation of its electrophoretic mobility as a function of β-CD concentration. Thus the greater the extent of the variation of electrophoretic mobility, the stronger the interaction between an analyte and β-CD. Accordingly, the results shown in Fig. 2 reveal that phenothiazines interact strongly to moderately with β-CD

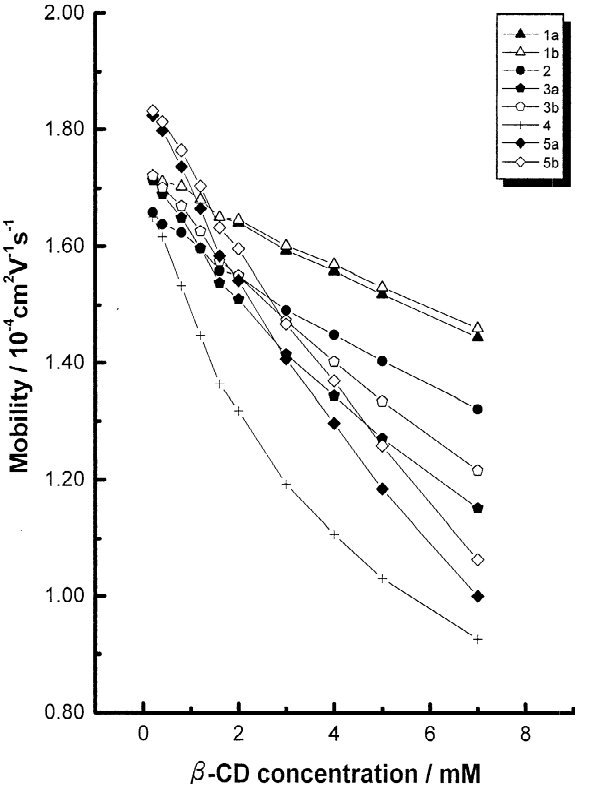



Fig. 2. Variations of the electrophoretic mobility of phenothiazines as a function of β-CD concentration in the range 0.2–7 m*M* using a citrate buffer (80 m*M*) containing 10 m*M* TTAB at pH 3.5. Capillary, 57 cm×50 μm, I.D; sample concentration, 10 μg/ml; detection wavelength, 254 nm; other operating conditions, −20 kV, 25 °C. Curve identification: (1) promethazine (▲, △); (2) ethopropazine (●); (3) trimeprazine (⬟, ⬠); (4) methotrimeprazine (+); (5) thioridazine (◆, ◇).

in the order thioridazine>methotrimeprazine>trimeprazine>ethopropazine>promethazine. Compared with the results obtained previously [19], we can see that the interactions between phenothiazines and β-CD are significantly reduced in the presence of TTAB micelles. This is due to the competitive complexation of phenothiazines with TTAB micelles, in addition to the complexation between β-CD and TTAB.

The selectivity and separability of phenothiazines were found to be greatly affected when the concentration of β-CD varied from 0.2 to 7 m*M*. This phenomenon may be ascribed to the changes in the migration order resulting from the variation in the

electrophoretic mobility of phenothiazines as a function of β-CD concentration. In the absence of β-CD, thioridazine migrates toward the cathode much faster than trimeprazine which, in turn, migrates slightly faster than promethazine, but much faster than ethopropazine and methotrimeprazine, when using a micellar citrate buffer containing 10 m*M* TTAB at pH 3.5. In contrast, with addition of 7 m*M* β-CD to such a micellar citrate buffer, phenothiazines migrate with respect to the migration time in the order methotrimeprazine < thioridazine < trimeprazine < ethopropazine<promethazine.

The separation window was greatly enlarged and the enantioseparability of promethazine, trimeprazine, and thioridazine was remarkably enhanced as β-CD concentration increased from 0.2 to 7 m*M*. Effective enantioseparation of thioridazine and trimeprazine was achieved with addition of β-CD at a concentration as low as 0.4 m*M*, and the enantiomers of promethazine were baseline separated with addition of 7 m*M* β-CD. It should be pointed out that better enantioresolution of thioridazine in MEKC than in CZE is obtained. No enantioseparation of ethopropazine and methotrimeprazine could be achieved with the use of β-CD as a chiral selector as observed in the case of CZE separation [19]. Fig. 3 shows such electropherogram of phenothiazines obtained with addition of β-CD at 7.0 m*M* to the micellar citrate buffer at pH 3.5.

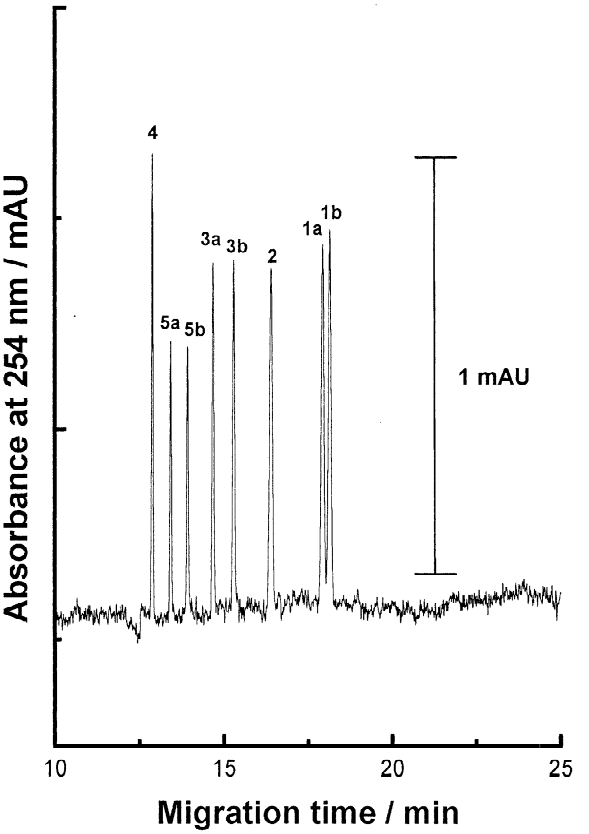



Fig. 3. An electropherogram of phenothiazines obtained with addition of β-CD at 7 m*M* using a citrate buffer (80 m*M*) containing 10 m*M* TTAB at pH 3.5.

### *3.2. Enantioseparation with addition of HP-β-CD*

The trends in the variation of the electrophoretic mobility of phenothiazines as a function of HP-β-CD concentration look similar to those obtained with the use of β-CD. Since the interactions of phenothiazines with HP-β-CD are considerably weaker than those with β-CD [19], addition of HP-β-CD at a concentrations greater than that of β-CD is necessary for achieving effective enantioseparations. For an effective enantioseparation of thioridazine and trimeprazine, addition of HP-β-CD greater than 2 m*M* to the micellar citrate buffer is needed. The enantiomers of ethopropazine could be baseline resolved with addition of HP-β-CD at a concentration greater than 4 m*M*, but complete separation of five phenothiazines, together with the enantiomers of thioridazine, trimeprazine and ethopropazine, could only be achieved with addition of 7–9 m*M* HP-β-CD. No enantioseparation of promethazine and methotrimeprazine could be achieved with the use of HP-β-CD, as observed in the case of CZE [19]. Fig. 4 shows such electropherogram of phenothiazines obtained with addition of HP-β-CD at 9 m*M* to the micellar citrate buffer at pH 3.5.

### *3.3. Enantioseparation with addition of mixed CDs*

Based on the facts that effective enantioseparation of thioridazine, trimeprazine and promethazine could simultaneously be achieved with addition of 6 m*M* β-CD in a micellar citrate buffer at pH 3.5 and that the enantiomers of thioridazine, trimeprazine and ethopropazine were effectively and simultaneously separated with addition of 7–9 m*M* HP-β-CD to the

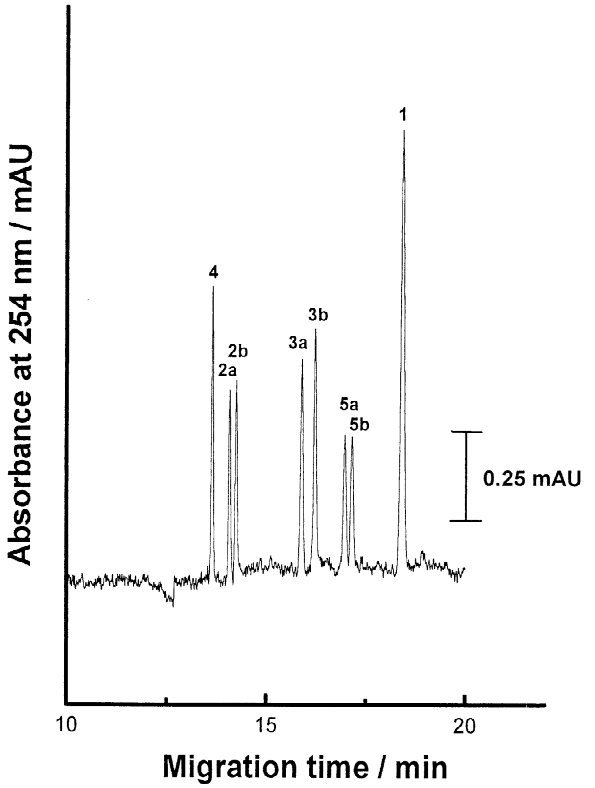



Fig. 4. An electropherogram of phenothiazines obtained with addition of HP-β-CD at 9 m*M* using a micellar citrate buffer (80 m*M*) containing 10 m*M* TTAB at pH 3.5.

same micellar citrate buffer, we are interested in finding out whether complete enantioseparation of thioridazine, trimeprazine, promethazine and ethopropazine can simultaneously be accomplished with addition of mixed CDs composed of β-CD and HP-β-CD. By fixing the β-CD concentration at 7 m*M* and varying the HP-β-CD concentration from 3 to 9 m*M*, the resolution of the two enantiomer peaks of ethopropazine was found to be very little improved, while the enantioresolution of promethazine was progressively reduced as the concentration of HP-β-CD increased. Apparently, the enantioseparation of phenothiazines is predominately controlled by the concentration of β-CD, because the interaction of a certain phenothiazine with β-CD is comparatively much stronger than that with HP-β-CD. Hence we may conclude that an effective and simultaneous enantioseparation of promethazine and ethopropazine in MEKC cannot be accomplished with mixed β-CDs using a micellar citrate buffer containing TTAB micelles.

## 4. Conclusion

As a result of substantially decreased interactions of phenothiazines with CDs in the presence of TTAB, effective enantioseparation of thioridazine, trimeprazine and promethazine or ethopropazine with the use of β-CD or HP-β-CD, respectively, can simultaneously be achieved by MEKC using a micellar citrate buffer containing TTAB at pH 3.5. This is not achievable with the use of β-CDs by CZE reported previously. The results of the present investigation may also suggest that effective separation of phenothiazine derivatives in MEKC is achievable using β-CDs as electrolyte modifiers.

## Acknowledgements

We thank the National Science Council of Taiwan for financial support.

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