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Short Communication

Separation of ferrocenylethanol enantiomers by microcolumn liquid chromatography with β-cyclodextrin as mobile phase additive

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ABSTRACT

Ferrocenylethanol enantiomers can be separated by microcolumn liquid chromatography with β-cyclodextrin (β-CD) as a mobile phase additive. The effects of the mobile phase composition on the retention behaviour of the analytes were examined. The separation factor achieved under various mobile phase conditions was 1.1–1.2, and association constants of the analytes with β-CD were determined from the experimental results. Addition of α- and γ-CD to the mobile phase did not result in separation of the enantiomers.

INTRODUCTION

Cyclodextrin (CD)-bonded stationary phases have been successfully applied to the separation of enantiomers in liquid chromatography (LC) [1–4]. When there is a good match between the size of the analyte and the cavity of the CD, enantiomeric resolution can be achieved as a result of a difference in the stability of the inclusion complex. Three kinds of CD stationary phases are commercially available, which differ in the number of glucose units. β-CD and its derivatives are the most widely used as chiral stationary phases in LC. The use of CDs as mobile phase additives in LC is another approach to establishing chiral resolution [5,6].

Inclusion complexation of ferrocenes (FERs) into CDs has been investigated [7,8]. It was found that FERs form 1:1 complexes with β- and γ-CD in a crystalline state, while α-CD forms 2:1 (α-CD–FER) complexes with FER. This phenomenon was applied to the separation of ferrocenylalcohol enantiomers by LC with a polyamide stationary phase and α-CD as a mobile phase additive [9]. Enantiomers of various FER derivatives were also separated by a β-CD-bonded stationary phase [10].

In this paper the separation of 1-ferrocenylethanol (FET) enantiomers by microcolumn LC with different CDs as mobile phase additives is examined. Microcolumn LC facilitated the examination of wide range of mobile phase conditions in detail.

EXPERIMENTAL

Apparatus

The microcolumn liquid chromatograph was assembled from a microfeeder (Azumadenki

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Kogyo, Tokyo, Japan) equipped with an MS GAN-050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan) as a pump, an ML-522 microvalve injector with an injection volume of 20 nl (Jasco, Tokyo, Japan), a 150 × 0.35 mm I.D. micropacked separation column, a Uvidec-100V UV absorbance detector (Jasco) with a laboratory-made flow cell and a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan). The detector was operated at 220 nm. The separation column was made from fused-silica tubing with 0.35 mm I.D. (GL Science, Tokyo, Japan) or 0.5 mm I.D. PTFE tubing (GL Science). The packing materials employed were Develosil ODS-5 (5 μm; Nomura Chemical, Seto, Japan) and Cyclobond I (37–53 μm β-CD-bonded silica; Astec, Whippany, NJ, USA). The columns were prepared by the slurry-packing technique reported previously [11] and dipped into the water bath in operation to minimize the variation in the column temperature. The separation was carried out at room temperature, *ca.* 20°C.

The enantiomers were isolated by using a conventional LC system, comprising an LC-6AD pump (Shimadzu), a 250 × 4.6 mm I.D. Develosil ODS-5 column, a loop injector (21 μl) and a Uvidec-100V UV detector (280 nm).

Reagents

The reagents employed were of guaranteed reagent grade and obtained from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. The reagents were employed without any further treatment. Distilled water was of HPLC grade. Racemic FET was of extra-pure grade from Tokyo Chemical Industry (Tokyo, Japan). α- and β-CD were from Tokyo Chemical Industry, while γ-CD was from Wako (Osaka, Japan).

RESULTS AND DISCUSSION

The enantiomeric resolution of racemic FET using 20% aqueous acetonitrile solution including 8 m*M* β-CD as the mobile phase is demonstrated in Fig. 1. Baseline separation of the enantiomers was achieved. In principle, the elution order of the FET enantiomers observed under the conditions in Fig. 1 should be opposite to that obtained when a β-CD-bonded stationary phase is employed. In order to confirm this, the enantiomers were isolated by using the conventional LC system, in which a 250 × 4.6 mm I.D. column packed with Develosil ODS-5 and 10 mM β-CD dissolved in acetonitrile–water (15:85) were employed as the separation column and the mobile phase. The isolated components were then injected into a 380 × 0.5 mm I.D. column packed with Cyclobond I (β-CD-bonded silica). In fact, the elution order observed when using the β-CD-bonded column was opposite to that observed in Fig. 1. Since the optical rotation of each enantiomer at the sodium D line was not measured, (+) and (−) in this paper refer to the definition given by Armstrong *et al.* [10]. These data show that the stability of the inclusion complex for the (+)-isomer is larger than that for the (−)-isomer. In addition, in the work with α-CD as the mobile phase additive, it was also found that (*S*)-(+)-FET eluted before (*R*)-(−)-FET [9].

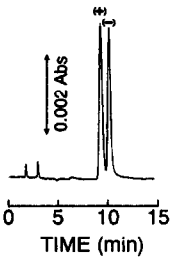

Fig. 1. Separation of FET enantiomers with β-CD as mobile phase additive. Column: Develosil ODS-5, 150 × 0.35 mm I.D. Mobile phase: acetonitrile–water (20:80) containing 8 m*M* β-CD. Flow-rate: 2.8 μl/min. Wavelength of UV detection: 220 nm. Analytes: (+) = (+)-FET and (−) = (−)-FET (0.05% total concentration). Injection volume: 20 nl.

The effect of mobile phase composition on the resolution was examined. Fig. 2 illustrates the relationship between the reciprocal of the capacity factor and the concentration of β-CD in the mobile phase for FET. The acetonitrile concentration in the mobile phase was kept constant at 15, 20 or 30% (v/v). The capacity factor was calculated by assuming that nitrate was not retained on the stationary phase. A linear relationship between the two parameters is observed in the figure.

Since the distribution of β-CD in the stationary phase can be disregarded under the present

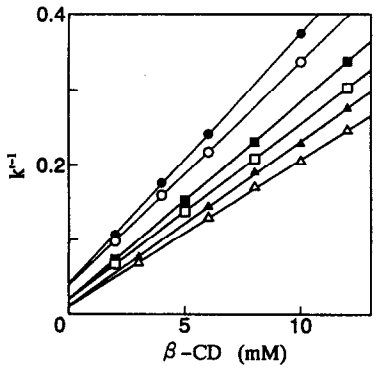

Fig. 2. Relationship between the reciprocal of the capacity factor (k^{-1}) and the β-CD concentration in the mobile phase. Operating conditions as in Fig. 1 except for the mobile phase. Mobile phase: acetonitrile–water mixture containing β-CD; ●, ○ = 25% acetonitrile; ■, □ = 20% acetonitrile; ▲, △ = 15% acetonitrile. Closed symbols = (+)-FET; open symbols = (−)-FET.

system, there exist three phases in the present separation system. The three-phase model developed in micellar LC [12] can be applied to the present separation system, *i.e.*, CD instead of the micelle in micellar LC. The following equilibrium expressions are derived:

$$A_m + L_s \rightleftarrows AL_s \quad (1)$$

$$A_m + C_D \rightleftarrows AC_D \quad (2)$$

$$AC_D + L_s \rightleftarrows AL_s + C_D \quad (3)$$

where A_m represents the analyte existing in the mobile phase solvent, L_s represents the stationary phase, AL_s represents the analyte distributed in the stationary phase, C_D represents CD in the mobile phase and AC_D represents the analyte associated with CDs in the mobile phase. The following equation derived by Arunyanart and Cline Love [12] is therefore valid in the present system:

$$k'^{-1} = K_2[C_D]/K_1[L_s]\phi + 1/K_1[L_s]\phi \quad (4)$$

where K_1 and K_2 are the equilibrium constants of eqns. 1 and 2, ϕ is the ratio of the volume of the stationary phase to that of the solvent in the mobile phase, $[L_s]$ is the concentration of the stationary phase and $[C_D]$ is the concentration of CD. Eqn. 4 is valid when the solute concentration is within the linear range of the isotherm for the equilibrium shown in eqn. 2. Since the stationary phase is achiral in the present system, K_1 for (+)- and (−)-FET should be identical. Therefore, a difference in K_2 for (+)- and (−)-FET is the key to the separation of the enantiomers.

As shown in Fig. 2, the capacity factors of the analytes decrease with increasing β-CD concentration. In other words, the increase in the β-CD concentration leads to a reduction in the analysis time. The data shown in Fig. 2 can be examined by linear regression analysis to determine the slope (m) and intercept (b). From eqn. 4, it is apparent that the slope ($m = K_2/K_1[L_s]\phi$) divided by the intercept ($b = 1/K_1[L_s]\phi$) will yield the equilibrium constant (K_2) for distribution of the enantiomer between β-CD and the mobile phase solvent. This equilibrium constant K_2 is tabulated for the (+)- and (−)-FET in the sixth column of Table I. It is seen that K_2 values calculated for (+)-FET are larger than those for (−)-FET. In the last column of Table I, the ratios of K_{2+} to K_{2-} are also shown.

An expression for the separation factor (α) can be derived directly from eqn. 4:

$$\alpha = k'_-/k'_+ = (m_+[C_D] + b_+)/(m_-[C_D] + b_-) \quad (5)$$

where the subscripts + and − represent the (+)- and (−)-isomers, respectively. Eqn. 5 indicates that b_+ and b_- will become negligible with increasing concentration of β-CD. The magnitudes of the product of m and $[C_D]$ as well as b can be estimated from the data in Table I. Thus, as the concentration of β-CD is increased, the separation factor should approach the value m_+/m_-, and become independent of the concentration of β-CD.

Fig. 3 shows the linear relationship between the logarithm of K_2 and the acetonitrile concentration. It is found that K_2 increases with decreasing acetonitrile concentration. K_2 for pure water can be determined from the data in Fig. 3 by extrapolation of the linear curves, *e.g.*, $6.6 \cdot 10^3$ M for both (+) and (−)-FET. Basically, K_2 for (+)-FET should be larger than (−)-FET. This may be because of an experimental error.

The separation factor (α) is plotted *versus* the

TABLE I

REGRESSION ANALYSIS

Operating conditions as in Fig. 2.

[CH_3CN] (%, v/v)	FET	m (M^{-1})	m_+/m_-	b	$K_2 = (m/b)$ (M^{-1})	K_{2+}/K_{2-}
15	+	$2.20 \cdot 10$	1.12	$1.18 \cdot 10^{-2}$	$1.86 \cdot 10^3$	1.04
15	−	$1.97 \cdot 10$		$1.10 \cdot 10^{-2}$	$1.79 \cdot 10^3$	
20	+	$2.64 \cdot 10$	1.12	$2.10 \cdot 10^{-2}$	$1.26 \cdot 10^3$	1.08
20	−	$2.36 \cdot 10$		$2.02 \cdot 10^{-2}$	$1.17 \cdot 10^3$	
25	+	$3.34 \cdot 10$	1.12	$4.14 \cdot 10^{-2}$	$8.07 \cdot 10^2$	1.07
25	−	$2.98 \cdot 10$		$3.95 \cdot 10^{-2}$	$7.54 \cdot 10^2$	

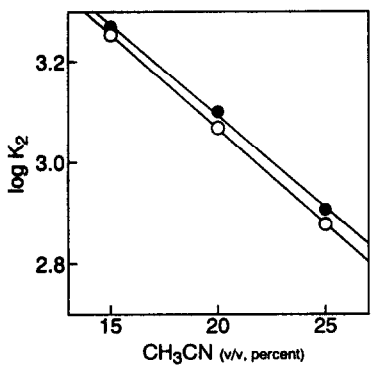

Fig. 3. Logarithm of the equilibrium constant (K_2) as a function of the acetonitrile concentration. Operating conditions as in Fig. 2. ● = (+)-FET; ○ = (−)-FET.

β-CD concentration in Fig. 4, in which the acetonitrile concentration is kept constant at 20%. It is seen that the separation factor slightly increases with increasing β-CD concentration. The α-values observed were around 1.1 and they were nearly the same as the corresponding m_+/m_- values as shown in Table I.

By substituting the expression for slope given above, it is apparent that m_+/m_- is equal to $K_{2+}K_{1-}/K_{2-}K_{1+}$ and should be equal to K_{2+}/K_{2-} since the equilibrium constant K_1 for the distribution of each enantiomer between the mobile and the stationary phases should be identical. These values are also shown in Table I. The data in Table I show that m_+/m_- values are nearly equal to K_{2+}/K_{2-} values.

Fig. 5 shows the effect of acetonitrile concentration on the retention of the analytes. The logarithm of the capacity factor is plotted as a function of the acetonitrile concentration. Linear relationships are observed, as in common reversed-phase LC. The dependence of the separation factor on the acetonitrile concentration is

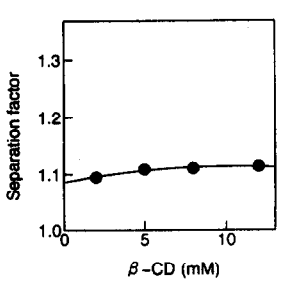

Fig. 4. Separation factor as a function of the β-CD concentration in the mobile phase. Mobile phase: acetonitrile–water (20:80) containing β-CD. Other operating conditions as in Fig. 2.

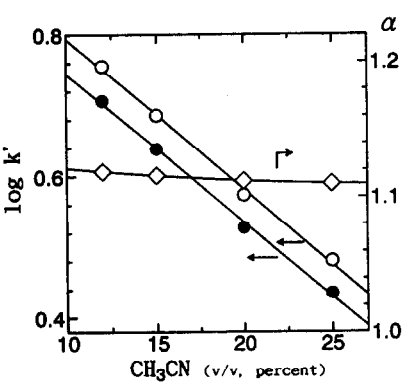

Fig. 5. Effects of the acetonitrile concentration on the retention behaviour of the FET enantiomers. Mobile phase: acetonitrile–water (mixture containing 10 mM β-CD). Other operating conditions as in Fig. 2.

also shown in Fig. 5. The separation factor slightly decreases with increasing acetonitrile concentration in the mobile phase.

The addition of α- or γ-CD to the mobile phase failed to separate the FET enantiomers. When 20% aqueous acetonitrile solution containing 40 m*M* γ-CD was used as the mobile phase, the retention time was 14.2 min. The retention time was much shorter than that achieved without γ-CD. This indicates that γ-CDs form inclusion complexes with FET molecules, resulting in a decrease in the retention time. Nevertheless, chiral recognition is not achieved for the inclusion complexation with γ-CD. According to the results obtained by nuclear magnetic resonance, infrared, UV and circular dichroism spectroscopy, Harada *et al.* [8] have proposed different orientations of a FER molecule incorporated into the cavity of different CDs in a crystalline state. A γ-CD cavity is large enough to accommodate a FER molecule equatorially, while a FER molecule could fit well into a β-CD cavity by axial inclusion. The difference in the orientation may be attributed to the difference in the stability of the inclusion complex formed. Such a difference in the orientation of the FET molecule in the cavity of β- and γ-CD may exist in solution and affect the chiral recognition.

On the other hand, when 30% aqueous acetonitrile solution containing 30 m*M* α-CD was used as the mobile phase, the retention time of the FET enantiomers was 41.7 min. The retention time was nearly the same as that achieved without α-CD. These FET enantiomers were separated on a polyamide column using a carbonate buffer (pH 11.4) containing 50 m*M* α-CD as the eluent [9]. An unsuccessful result in the present system may be attributed to the use of different stationary and mobile phases in comparison with those employed by Harada *et al.* [9].

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