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Transient state of chiral recognition in a binary mixture of cyclodextrins in capillary electrophoresis

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Abstract

The transient state (as the defined point where no enantioseparation is obtained in a dual chiral selector system) of chiral recognition of aminoglutethimide in a binary mixture of neutral cyclodextrins (CDs) was studied by capillary electrophoresis (CE). The following three dual selector systems were used: α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD); α-CD and heptakis(di-*O*-methyl-β-cyclodextrin) (DM-β-CD); α-CD and heptakis(tri-*O*-methyl-β-cyclodextrin) (TM-β-CD). The *S*-(−) enantiomer of the analyte was more strongly retained in the presence of either α-CD or TM-β-CD at pH 2.5, 100 m*M* phosphate buffer, while the *R*-(+) enantiomer was more strongly retained in the presence of either β-CD or DM-β-CD. In the more simple case, the elution order is invariably kept if the enantiomers have the same elution order in either one of the two hosts of the binary mixture. In contrast, the elution order may be switched by varying the concentration ratio of two hosts that produce opposite elution order for this particular analyte. In such a dual selector system, the enantioselectivity will disappear at the transient state at a certain ratio of $host_1$:$host_2$. Moreover, the migration times of the two enantiomers with $host_1$ alone (diluted in buffer) is approximately equal to the migration times at the corresponding concentration of $host_2$ alone (diluted in buffer), where the ratio of concentrations of $host_1$:$host_2$ is the same as in the binary mixture at the transient state. As found by nuclear magnetic resonance experiments, the analyte is forming a 1:1 complex with either one of the CDs applied. From this finding, a theoretical model based on the mobility difference of the two enantiomers was derived that was used to simulate the transient state.

Keywords: Enantiomer separation; Chiral selectors; Transient state; Buffer composition; Cyclodextrins; Aminoglutethimide

1. Introduction

The separation of enantiomers is one of the most attractive and intriguing issues in separation science. Capillary electrophoresis (CE) has become a convenient and fruitful methodology for the direct analysis of enantiomers, bearing the advantages of high separation efficiency, short analysis time and low operating cost [1]. At present, the most versatile chiral additives are cyclodextrins (CDs), followed by macrocyclic antibiotics, protein and polysaccharides [2]. Various CDs are employed, i.e., native and derivatized CDs, neutral and charged CDs, in order to meet the requirements of different solutes [3–5]. Although in most cases only one CD was added to

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the running buffer to resolve the enantiomers of interest, a dual CD system became increasingly popular for this purpose; thus, neutral CDs were employed in combination with either other neutral CDs [6,7] or with charged CDs [8–15], in order to adjust the enantioselectivity. However, Mechref and El Rassi reported that no separation was achieved for the chiral compound silvex in the presence of both heptakis(tri-*O*-methyl-β-cyclodextrin) (TM-β-CD) and β-CD [6], while the enantiomers could be separated with a 5 m*M* solution of either one of the two selectors. Likewise, a mixture of 5 m*M* heptakis(di-*O*-methyl-β-cyclodextrin) (DM-β-CD) and 1 m*M* anionic β-CD-sulfobutyl ether (β-CD-SBE) failed to adequately resolve cathinone enantiomers, as compared to DM-β-CD or β-CD-SBE alone [8]. Notably, these interesting findings have not yet been thoroughly investigated, though theoretical models concerning enantioseparation in CE have been proposed [16–18].

In this paper, three dual neutral CD systems, i.e., α-CD and β-CD; α-CD and DM-β-CD; α-CD and TM-β-CD, were examined for the separation of aminoglutethimide enantiomers. A transient state is defined where no separation of enantiomers occurs, and a theoretical model is presented that serves to explain the annihilation of chiral recognition at this point. As found by nuclear magnetic resonance (NMR) experiments, the analyte is forming a 1:1 complex with either one of the CDs applied, thus supporting the straightforward approach proposed in this paper.

2. Experimental

2.1. Instrumentation

All CE experiments were carried out on an automatic BioFocus 3000 capillary electrophoresis system (Bio-Rad Labs., Hercules, CA, USA) equipped with an on-column high-speed scanning UV–Vis detector. Data acquisition was performed with CE3000 software. A laboratory-made fused-silica polyacrylamide-coated capillary [32 cm (27.5 cm to the detection window)×50 μm I.D.×375 μm O.D.] was used. The capillary temperature was thermostated at 25°C with water as a coolant, and the sample vials were maintained at 20°C. The run voltage for all separations was 15 kV, and samples were injected by electromigration, 15 kV for 3 s. Prior to use, the capillary was rinsed with deionized and distilled water for 5 min, and subsequently for 5 min with plain buffer. Between sample runs, the capillary was rinsed with deionized and distilled water for an additional 45 s, followed by the chiral selector solution for 60 s. ^{1}H-NMR spectra were recorded on a Bruker AMX-400 MHz spectrometer (Bruker, Germany) referenced to $H^{2}HO$ peak (^{1}H-NMR δ=4.81 ppm), and the spectra were processed with the software WinNMR (Bruker, Germany).

2.2. Chemicals and reagents

α-CD, β-CD and DM-β-CD (degree of substitution, DS=1.8) were obtained from Wacker (Munich, Germany), TM-β-CD (purity>99%) was purchased from Cyclolab (Budapest, Hungary). The aminoglutethimide enantiomers were kindly donated by Novartis (Basel, Switzerland), chemical structure as shown in Fig. 1. For peak assignment, the concentration of the *R*-(+) enantiomer is about two times higher than the *S*-(−) form. All other chemicals were analytical grade. The plain buffer contained 100 m*M* sodium dihydrogenphosphate, adjusted to pH 2.5 with phosphoric acid. The CD was added to the plain buffer to give the concentration required. The analyte was dissolved in the plain buffer to yield a sample concentration of approximately 0.1 mg/ml. The p^{2}H 2.5, 100 m*M* phosphate buffer for NMR experiments was prepared by dissolving sodium dihydrogenphosphate and phosphoric acid in $^{2}H_{2}O$. Each solution was filtered with 0.45-μm diameter nylon syringe filters.

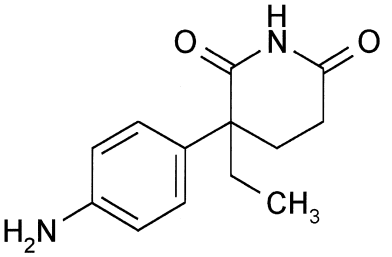

Fig. 1. Molecular structure of aminoglutethimide.

3. Results and discussion

Aminoglutethimide is an anticonvulsant, and its enantiomers have successfully been resolved by CE [15,19] and also by high-performance liquid chromatography (HPLC) [20,21]. In this paper, the enantiomers of aminoglutethimide were separated by CE upon addition of either two out of four neutral CDs, i.e., α-CD, β-CD, DM-β-CD and TM-β-CD, to the running buffer. The *R*-(+) enantiomer eluted before its *S*-(−) antipode in the presence of either α-CD or TM-β-CD alone, while the elution order was reversed with either β-CD or DM-β-CD alone, as illustrated in Figs. 2, 3 and 4, respectively. In the following, three dual CD systems were chosen, i.e., (α-CD and β-CD), (α-CD and DM-β-CD) and (α-CD and TM-β-CD). The total concentration of the two CDs in the binary mixture was kept constant at 15 m*M*, while the relative concentrations were varied step by step (nine data points, see Table 1). Electropherograms obtained with the dual selector system (α-CD and β-CD) are shown in Fig. 2. Upon changing the ratio of the two selectors, the elution order of the enantiomers was reversed at a certain point; thus, the separation vanished for a mixture of 90% α-CD and 10% β-CD. As demonstrated in Fig. 3, the separation of enantiomers in the dual selector system (α-CD and DM-β-CD) showed a similar pattern: the *R*-(+) enantiomer was eluted first with 100% α-CD, and no separation was achieved when DM-β-CD reached a concentration of approximately 3 m*M* (20% relative concentration); after that, the separation increased gradually as the DM-β-CD concentration was increased further, however, with a reversed elution order of the enantiomers. Apparently, the two cases have in common that a dual selector system is composed of two selectors with opposite elution order of the analyte enantiomers, thus leading to a complete loss of resolution at a certain concentration ratio of the two selectors.

The third dual selector system (α-CD and TM-β-CD) proved also applicable to the enantiomer separation of aminoglutethimide. Here, the elution order of enantiomers was maintained during variation of the concentration ratio of the two selectors, and the resolution of the two peaks was fairly constant (see Fig. 4), due to the fact that the enantiomers have the same elution order with both α-CD and TM-β-CD alone.

In Fig. 5, the concentration dependence of the migration times $t_{m(R)}$ and $t_{m(S)}$, respectively, of the two enantiomers with either one of the two selectors alone is compared with the concentration dependence of the migration separation factor $t_{m(R)}/t_{m(S)}$ in the

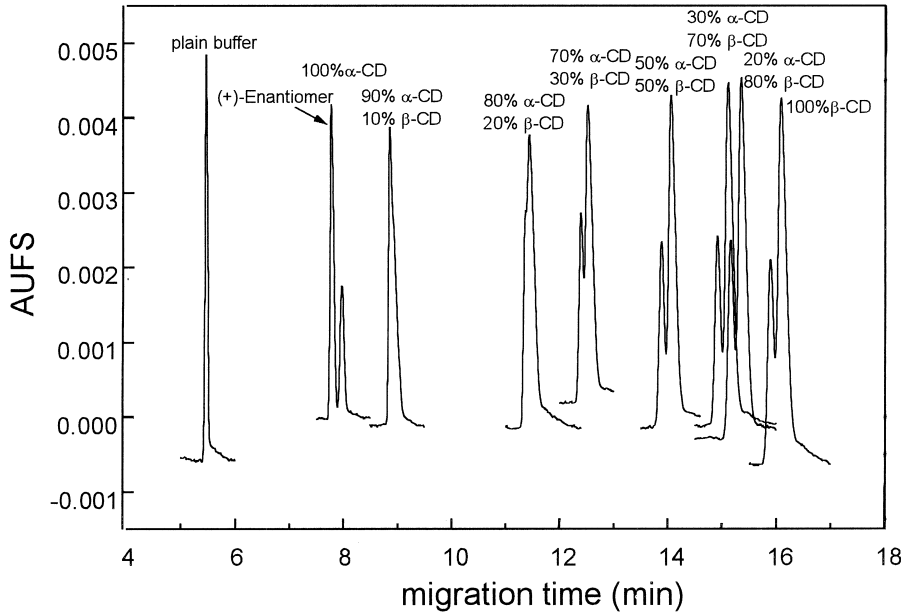

Fig. 2. Separation of enantiomers of aminoglutethimide in the dual selector system (α-CD and β-CD) at different concentration ratios.

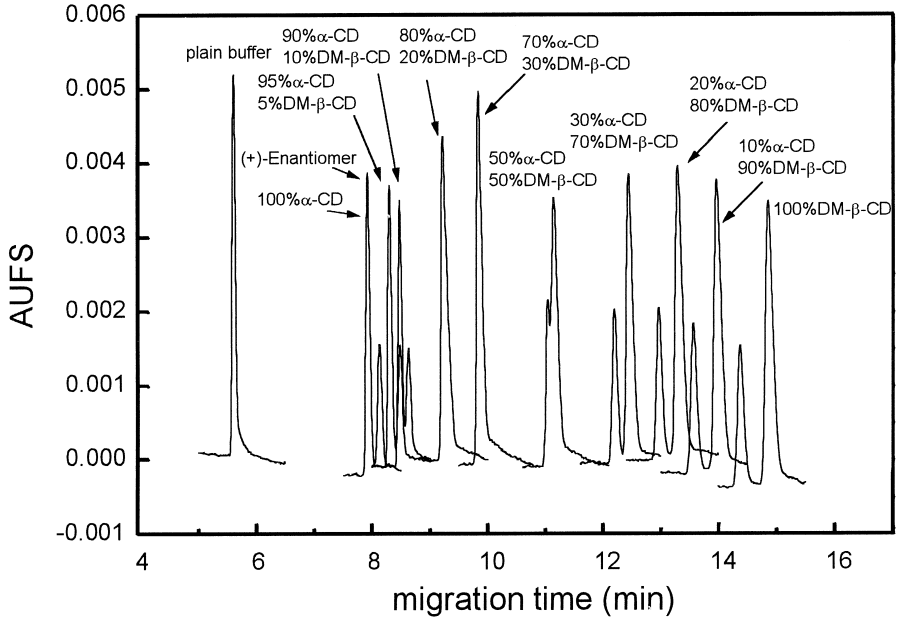

Fig. 3. Separation of enantiomers of aminoglutethimide in the dual selector system (α-CD and DM-β-CD) at different concentration ratios.

binary mixture of the two selectors. It is interesting to note that the migration times of the two enantiomers in 1.5 m*M* β-CD solution (10%, v/v, of a 15 m*M* β-CD solution was mixed with 90% of plain buffer) were approximately equal to the migration times in a 13.5 m*M* α-CD solution (90%, v/v, of a 15 m*M* α-CD solution was mixed with 10% of plain buffer). Likewise, no separation (migration sepa-

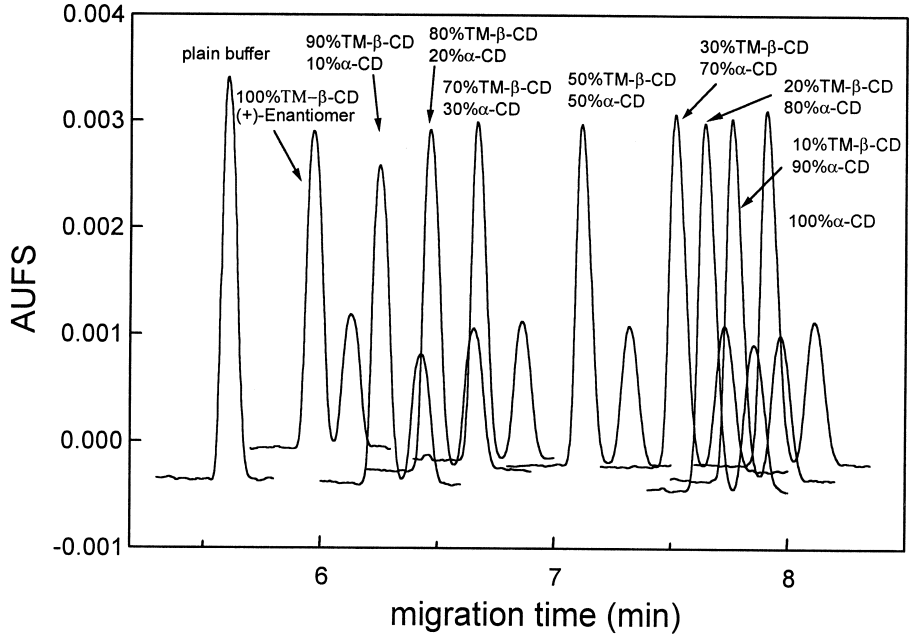

Fig. 4. Separation of enantiomers of aminoglutethimide in the dual selector system (α-CD and TM-β-CD) at different concentration ratios.

Table 1
Concentrations of the two selectors applied in this study; in the binary mixture, the total concentration was kept constant at 15 m*M*

Cyclodextrin A								
0	10	20	30	50	70	80	90	100%
0	1.5	3.0	4.5	7.5	10.5	12.0	13.5	15.0 m*M*
Cyclodextrin B								
100	90	80	70	50	30	20	10	0%
15.0	13.5	12.0	10.5	7.5	4.5	3.0	1.5	0 m*M*

ration factor $t_{m(R)}/t_{m(S)} \approx 1$, see Fig. 5b) was obtained for a 15 m*M* binary mixture composed of 10% (v/v) β-CD and 90% α-CD. The percentage of α-CD where the cross-over occurred is indicated as a vertical line; this percentage appeared to be slightly higher in Fig. 5b than in Fig. 5a, for reasons discussed below. Due to the reversal of the elution order of the enantiomers, the $t_{m(R)}/t_{m(S)}$ value drops below 1 at higher α-CD concentration.

In a similar fashion (see Fig. 6a), the migration times of the two enantiomers of aminoglutethimide in 3.0 m*M* DM-β-CD solution (20% of a 15 m*M* DM-β-CD solution was mixed with 80% of plain buffer) were the same as in a 12 m*M* α-CD solution (80%, v/v, of a 15 m*M* α-CD solution was mixed with 20% of plain buffer). There was no separation in a 15 m*M* binary mixture consisting of 20% DM-β-CD and 80% α-CD ($t_{m(R)}/t_{m(S)}=1$, see Fig. 6b).

Typically, the separation will vanish in these experiments if the migration separation factor is less than 1.002; thus, a ±0.002 deviation must be assumed if the migration separation factor appears to be 1.0 [22]. Nevertheless, there is also a 1 to 5% deviation in the determination of the migration time. Within these experimental errors, it is difficult to judge whether the slight shift in the ratio of the two selectors from Fig. 5a to b, as well as from Fig. 6a to b, is a meaningful aberration from the theory outlined below, or whether it is just an experimental artifact. In a first order approach, the concentration ratio of the two hosts in the dual selector system at the cross-over point of the enantiomers is fairly close to the concentration ratio of the single selectors in plain buffer, at the point where the average analyte migration times are the same for the two hosts.

Thus, if the enantiomers have opposite elution order with two different hosts, there is a certain ratio of the two hosts where no enantioseparation can be obtained, and this point is defined as the transient state in the dual selector system. In such a system, the elution order will be switched upon the variation of the concentration ratio of the two selectors if only the concentration ratio range covers both sides of the transient state. Adversely, given that the enantiomers have the same elution order for two different hosts, even varying the concentration ratio of these two hosts over the full range will not affect the elution order. In the following, a theoretical model will be established to support this hypothesis.

Under the operating conditions applied, the aminoglutethimide enantiomers are positively charged and the two hosts are neutral in either case. Moreover, it is assumed that the host–guest interaction leads to a 1:1 binding ratio. Thus, the apparent mobility of the (+) and (−) enantiomer in the dual host system can be expressed as given in Eqs. (1) and (2):

$$\mu_{(+)} = \frac{\mu_0 + K_{1(+)}[L_1]\mu_1 + K_{2(+)}[L_2]\mu_2}{1 + K_{1(+)}[L_1] + K_{2(+)}[L_2]} \quad (1)$$

$$\mu_{(-)} = \frac{\mu_0 + K_{1(-)}[L_1]\mu_1 + K_{2(-)}[L_2]\mu_2}{1 + K_{1(-)}[L_1] + K_{2(-)}[L_2]} \quad (2)$$

Here, $\mu_{(+)}$ and $\mu_{(-)}$ are the apparent mobilities of the (+) and (−) enantiomers, respectively, in the dual host system. μ_0 is the mobility of the analyte (similar for both enantiomers) in the absence of any host. μ_1 and μ_2 are the mobilities of the complexes formed with $host_1$ and $host_2$, respectively; as the two enantiomers have the same molecular mass, they should still have approximately the same mobility. $K_{1(+)}$ and $K_{2(+)}$ are the binding constants of the (+) enantiomer of the analyte with $host_1$ and $host_2$, respectively; accordingly, $K_{1(-)}$ and $K_{2(-)}$ are the binding constants of the (−) enantiomer. $[L_1]$ and $[L_2]$ are the free concentrations of $host_1$ and $host_2$, respectively, in the dual host system. The mobility difference $\Delta\mu$ between the (+) and (−) enantiomers of the analyte in the binary mixture of the two hosts is outlined in Eq. (3):

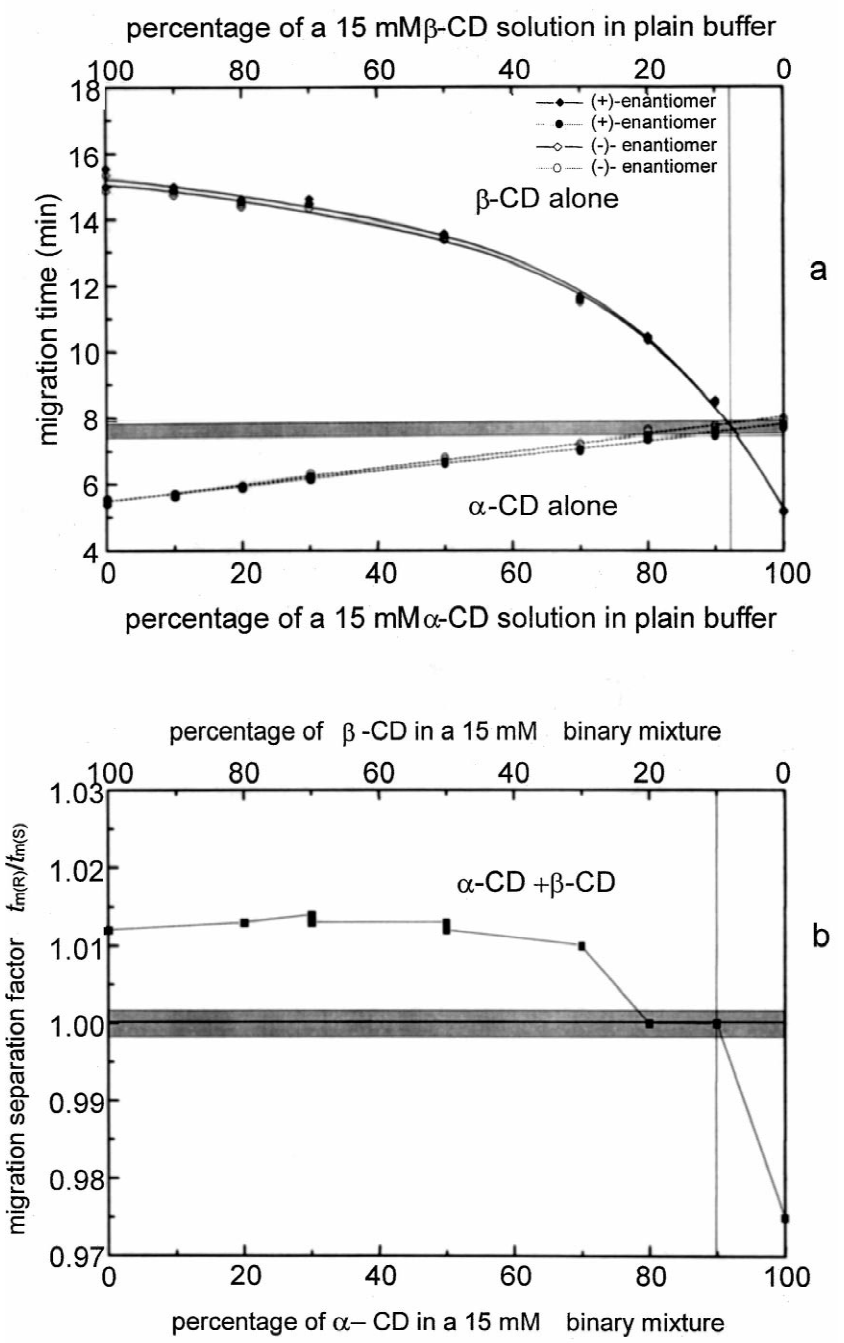

Fig. 5. Migration times of (+)- and (−)-aminoglutethimide in α-CD alone and β-CD alone at various concentrations in buffer (a). Migration separation factors $t_{m(R)}/t_{m(S)}$ of enantiomers [$t_{m(R)}$ and $t_{m(S)}$ the migration times of the (*R*) and (*S*) enantiomers in the dual selector system, respectively] in a binary mixture of α-CD and β-CD at various ratios and a constant total concentration of 15 m*M* (b). Hatches indicate the experimental error.

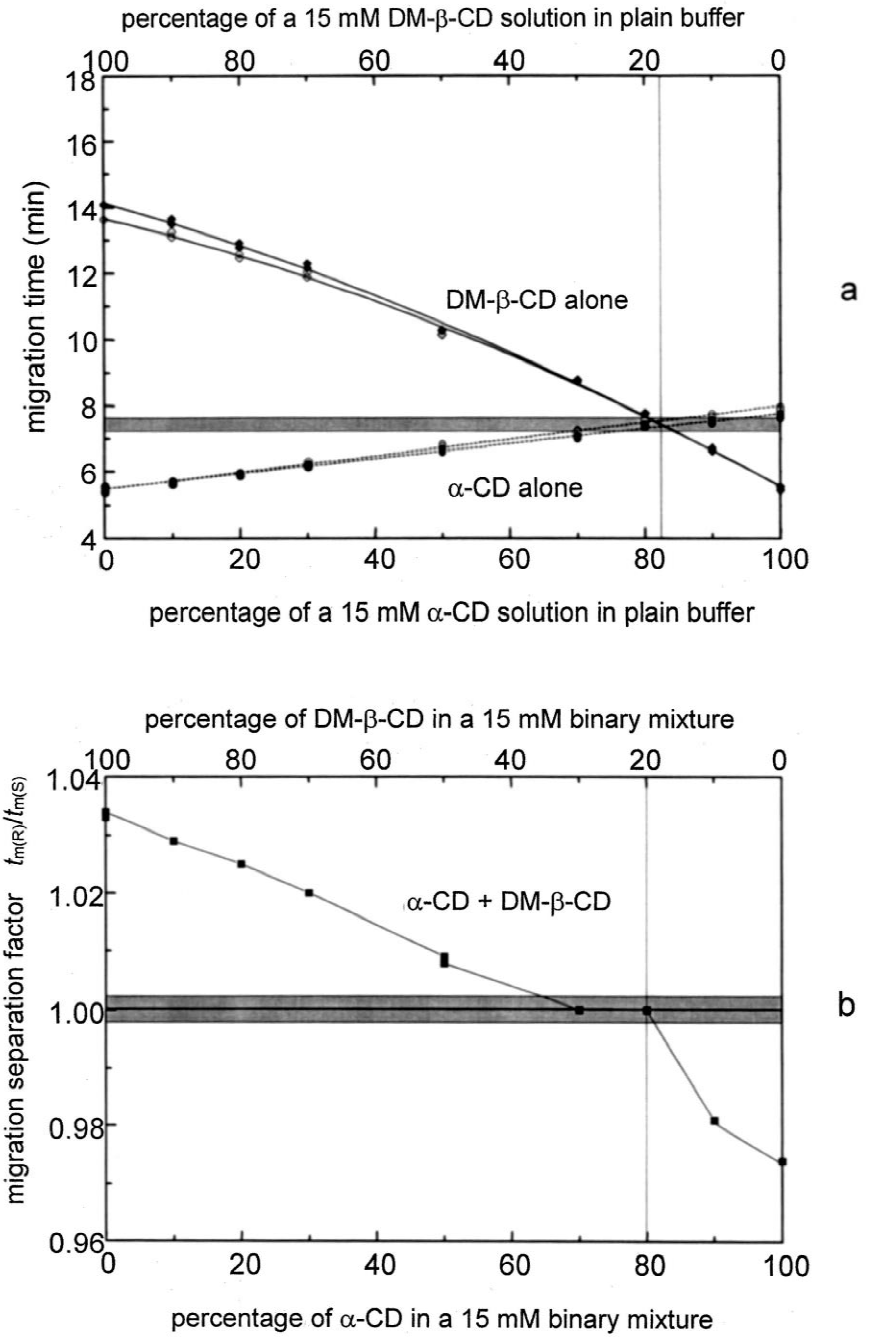

Fig. 6. Migration times of (+)- and (−)-aminoglutethimide in α-CD alone and DM-β-CD alone at various concentrations in buffer (a). Migration separation factors $t_{m(R)}/t_{m(S)}$ of enantiomers in a binary mixture of α-CD and DM-β-CD at various ratios and a constant total concentration of 15 m*M* (b). Hatches indicate the experimental error.

$$
\begin{aligned}
\Delta\mu &= \mu_{(+)} - \mu_{(-)} \\
&= \frac{\mu_0 + K_{1(+)}[L_1]\mu_1 + K_{2(+)}[L_2]\mu_2}{1 + K_{1(+)}[L_1] + K_{2(+)}[L_2]} - \frac{\mu_0 + K_{1(-)}[L_1]\mu_1 + K_{2(-)}[L_2]\mu_2}{1 + K_{1(-)}[L_1] + K_{2(-)}[L_2]} \\
&= \frac{\mu_0 + K_{1(-)}[L_1]\mu_0 + K_{2(-)}[L_2]\mu_0 + K_{1(+)}[L_1]\mu_1 + K_{1(+)}K_{1(-)}[L_1]^2\mu_1 + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_1 + K_{2(+)}[L_2]\mu_2 + K_{2(+)}K_{1(-)}[L_1][L_2]\mu_2 + K_{2(+)}K_{2(-)}[L_2]^2\mu_2}{(1 + K_{1(+)}[L_1] + K_{2(+)}[L_2])(1 + K_{1(-)}[L_1] + K_{2(-)}[L_2])} \\
&\quad - \frac{\mu_0 + K_{1(+)}[L_1]\mu_0 + K_{2(+)}[L_2]\mu_0 + K_{1(-)}[L_1]\mu_1 + K_{1(+)}K_{1(-)}[L_1]^2\mu_1 + K_{2(+)}K_{1(-)}[L_1][L_2]\mu_1 + K_{2(-)}[L_2]\mu_2 + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_2 + K_{2(+)}K_{2(-)}[L_2]^2\mu_2}{(1 + K_{1(+)}[L_1] + K_{2(+)}[L_2])(1 + K_{1(-)}[L_1] + K_{2(-)}[L_2])} \\
&= \frac{(K_{2(-)}[L_2]\mu_0 + K_{1(+)}[L_1]\mu_1 + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_1) + (K_{1(-)}[L_1]\mu_0 + K_{2(+)}[L_2]\mu_2 + K_{1(-)}K_{2(+)}[L_1][L_2]\mu_2)}{(1 + K_{1(+)}[L_1] + K_{2(+)}[L_2])(1 + K_{1(-)}[L_1] + K_{2(-)}[L_2])} \\
&\quad - \frac{(K_{2(+)}[L_2]\mu_0 + K_{1(-)}[L_1]\mu_1 + K_{1(-)}K_{2(+)}[L_1][L_2]\mu_1) + (K_{1(+)}[L_1]\mu_0 + K_{2(-)}[L_2]\mu_2 + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_2)}{(1 + K_{1(+)}[L_1] + K_{2(+)}[L_2])(1 + K_{1(-)}[L_1] + K_{2(-)}[L_2])}
\end{aligned} \tag{3}
$$

The apparent mobilities of the (+) and (−) enantiomers with host$_1$ alone are expressed as given in Eqs. (4) and (5), respectively:

$$\mu_{1(+)} = \frac{\mu_0 + K_{1(+)}[L_1]\mu_1}{1 + K_{1(+)}[L_1]} \tag{4}$$

$$\mu_{1(-)} = \frac{\mu_0 + K_{1(-)}[L_1]\mu_1}{1 + K_{1(-)}[L_1]} \tag{5}$$

Likewise, the apparent mobilities of the (+) and (−) enantiomers with host$_2$ alone are outlined in Eqs. (6) and (7), respectively:

$$\mu_{2(+)} = \frac{\mu_0 + K_{2(+)}[L_2]\mu_2}{1 + K_{2(+)}[L_2]} \tag{6}$$

$$\mu_{2(-)} = \frac{\mu_0 + K_{2(-)}[L_2]\mu_2}{1 + K_{2(-)}[L_2]} \tag{7}$$

In the case of peak-reversal of the enantiomers with two different chiral selectors, for the mobilities of the enantiomers it holds true that $\mu_{1(+)} = \mu_{2(-)}$ and $\mu_{1(-)} = \mu_{2(+)}$ at the point where the migration times of the two enantiomers with host$_1$ are equal to the one with host$_2$ (see Figs. 5a and 6a, respectively). From this equality, Eqs. (8) and (9) are derived:

$$
\begin{aligned}
&K_{2(-)}[L_2]\mu_0 + K_{1(+)}[L_1]\mu_1 + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_1 \\
&= K_{1(+)}[L_1]\mu_0 + K_{2(-)}[L_2]\mu_2 \\
&\quad + K_{1(+)}K_{2(-)}[L_1][L_2]\mu_2
\end{aligned} \tag{8}
$$

$$
\begin{aligned}
&K_{2(+)}[L_2]\mu_0 + K_{1(-)}[L_1]\mu_1 + K_{1(-)}K_{2(+)}[L_1][L_2]\mu_1 \\
&= K_{1(-)}[L_1]\mu_0 + K_{2(+)}[L_2]\mu_2 \\
&\quad + K_{1(-)}K_{2(+)}[L_1][L_2]\mu_2
\end{aligned} \tag{9}
$$

From Eqs. (8) and (9) follows for the point of transient state that the mobility difference of the two enantiomers, as outlined in Eq. (3), gives $\Delta\mu = 0$ ($m^2\ s^{-1}\ V^{-1}$). Hence, there is no separation of the two enantiomers of the analyte under these particular conditions, in good agreement with the experimental findings.

The relationship between mobility difference and selector concentration, as expressed in Eq. (3), was simulated assuming the following test parameters: $\mu_0 = 10$ (10^{-9} $m^2\ s^{-1}\ V^{-1}$), $\mu_1 = 8$ (10^{-9} $m^2\ s^{-1}\ V^{-1}$), $\mu_2 = 6$ (10^{-9} $m^2\ s^{-1}\ V^{-1}$), $K_{1(+)} = 50$ (M^{-1}), $K_{1(-)} = 100$ (M^{-1}), $K_{2(+)} = 250$ (M^{-1}), $K_{2(-)} = 200$ (M^{-1}). The concentration of host$_1$ was varied from 0 to 15 m*M*, complemented by host$_2$ to a constant total concentration of 15 m*M*. The simulation curves are displayed in Fig. 7.

While the upper curve of Fig. 7 shows only a slight variation in $\Delta\mu$ [$K_{2(+)} = 200$ (M^{-1}), $K_{2(-)} = 250$ (M^{-1})], the lower curve, for opposite elution order of the analyte enantiomers with two different hosts, deserves further comment. Here, the mobility difference $\Delta\mu$ is negative at the left side of the transient state and positive at the other side, and the enantiomers have opposite elution order at different sides. At the transient state, there is a cross-over at $\Delta\mu = 0$ ($m^2\ s^{-1}\ V^{-1}$).

The theoretical model is based on the assumption of a 1:1 binding ratio between analyte and either one of the two hosts. In the real case with neutral cyclodextrins as hosts, the binding ratio was investigated by NMR experiments using the continuous variation method, i.e., Job's plot [23]. From these experiments, it was confirmed that the two analyte

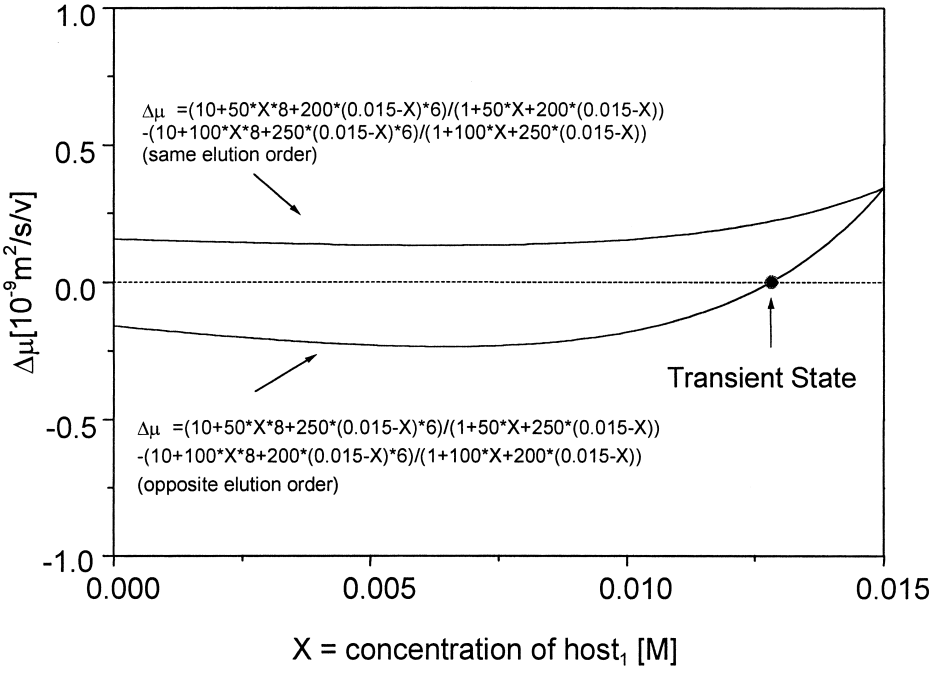

Fig. 7. Simulation results of the theoretical model with parameters quoted in the text; relationship between the mobility difference $\Delta\mu$ of two enantiomers and the concentration of host$_1$ in a 15 m*M* binary mixture.

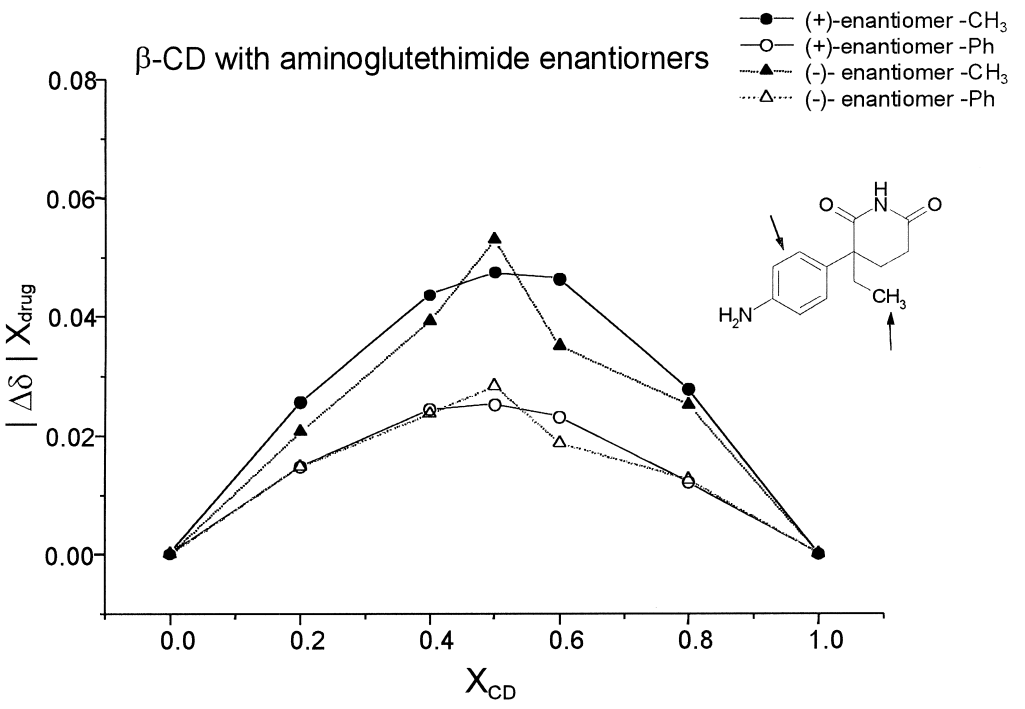

Fig. 8. Job's plot for the complexes of (+)- and (−)-aminoglutethimide, respectively, with β-CD, as investigated by ^{1}H-NMR spectroscopy.

enantiomers bind to any of the four CDs applied always in a 1:1 ratio, according to the chemical shifts of the methyl and aryl protons of aminoglutethimide. As can be seen in Fig. 8, the binding ratio for (+)- and (−)-aminoglutethimide, respectively, with β-CD is in excellent accordance with a 1:1 binding model, and the same was found, within the experimental error of this method, for α-CD, DM-β-CD and TM-β-CD.

4. Conclusion

In the enantioseparation by CE in a dual selector system, e.g., composed of two neutral cyclodextrins, there exists a transient state of chiral recognition if the two analyte enantiomers have opposite elution order with the two different hosts. Then, the enantioselectivity vanishes completely at the point of transient state. This case, although of theoretical interest, should be circumvented if one wants to improve the enantiomer separation using such a dual selector system.

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