Comparative capillary electrophoresis and NMR studies of enantioseparation of dimethindene with cyclodextrins

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Abstract

Enantioseparation of the antihistaminic drug dimethindene (DIM) (as maleate and tartrate salts) was studied using various cyclodextrins (CD) as chiral selectors in capillary electrophoresis (CE). NMR spectroscopy was used in order to elucidate the reason for the opposite migration order of the enantiomers of dimethindene (DIM) when native β-CD or commercially available carboxymethyl-β-CD (CM-β-CD) were used. This study demonstrated that the complexation-induced chemical shift of enantiomers does not always definitely show the chiral recognition pattern. The binding constants between the analyte and chiral selector need to be determined. NMR spectroscopy provided clear evidence on the multimodal (at least bimodal) complexation between DIM and CM-β-CD. These complexes seem to have a different stoichiometry. Moreover, the chiral recognition of DIM seems to be opposite in these complexes. The effect of additives such as urea on the interaction between DIM and β-CD was studied with both techniques. © 1998 Elsevier Science B.V.

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1. Introduction

Capillary electrophoresis (CE) is becoming one of the major techniques of analytical-scale enantioseparations [1–3]. Several approaches have been proposed in order to optimize enantioseparations in CE. In the mathematical model proposed by Wren and Rowe [4] it is intended to optimize the concentration of a chiral selector and the organic modifier of the background electrolyte (BGE). The extended model by Rawjee et al. [5] includes the pH of BGE, the electroosmotic flow (EOF), and other factors besides the concentration of chiral selector. Later, Williams and Vigh [6–8] also considered electrodispersion

phenomena and proposed a solution for this problem by extending a mobility matching principle [9,10] for chiral separations.

Another approach for the optimization of chiral separations is the statistical experimental design which allows a significant reduction in the number of experiments required for the optimization of a separation and, additionally, demonstrates the interdependence of separation parameters [11–13]. Molecular mechanics and molecular dynamics calculations may also provide useful information on the stereoselective analyte–selector interactions [14,15]. CE itself can also be used in order to obtain some information about analyte–selector interactions [4,5,16].

Most of these techniques do not provide experimentally proven structural information, which is a disadvantage. The data about the structure of solute–selector complexes and the thermodynamic

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quantities of these interactions need to be obtained for the explanation of some special effects in chiral CE. NMR spectroscopy is one of the powerful experimental techniques for the investigation of intermolecular interactions. This technique provided the very first evidence of the inclusion complex formation by CDs in the liquid phase [17]. NMR spectroscopy can also be used for the elucidation of chiral separation mechanisms using CD hosts [3,18–22]. The most important advantages of NMR spectroscopy in these studies are the following: (i) NMR is a liquid phase technique similar to CE and when required NMR experiments can be performed in exactly in the same medium as CE separations; (ii) the NMR spectrum provides considerable information about the environment of individual atoms and thus allows multimodal interactions between the analyte and the chiral selector to be followed in certain cases; (iii) NMR spectroscopy allows a clear differentiation between inclusion and other interactions. The last two are advantages of NMR spectroscopy over almost all other instrumental techniques, which are more global and do not provide any convincing proof for multimodal interactions or the inclusion; (iv) enantiotopic signals should be distinguishable in a chiral medium in NMR spectra. This allows racemic samples to be used for the study of enantioselective binding parameters; (v) NMR also provides information on the structure and dynamics of intermolecular complexes.

NMR spectroscopy was used in the present study for the elucidation of the reason of the opposite migration order of dimethindene (DIM) enantiomers using carboxymethyl-β-CD (CM-β-CD) as a chiral selector compared with the migration order obtained by using native β-CD.

2. Experimental

2.1. Chemicals

Racemic DIM as maleate salt was a gift from Zyma (Munich, Germany). $R(-)$ and $S(+)$ enantiomers of DIM were obtained in our laboratory using diastereomeric crystallization with optically pure tartaric acid in ethanol as described [23]. β-CD, CM-β-CD with an averaged substitution degree

(D.S.) 3.5, succinyl-β-CD (SUC-β-CD) with D.S.= 2.8 and sulfoethyl-β-CD (SEE-β-CD) with D.S.=2.8 were a gift from Wacker Chemie (Munich, Germany). Heptakis-2,6-dimethyl-β-CD (DM-β-CD) and heptakis-2,3,6 trimethyl β-CD (TM-β-CD) were from Fluka (Buchs, Switzerland). Sulfobutyl-β-CD (SBE-β-CD) with D.S.=4.0 was a gift from Professor J. Stobaugh (University of Kansas, Lawrence, KS, USA). 6-Deoxy-6-monocarboxy-β-CD was prepared in our laboratory according to the literature procedure [24]. β-CD-6-phosphate was a gift from Dr. E. Tarelli (Kings College School of Medicine and Dentistry, London, UK). The structures of the synthesized CDs were confirmed using elemental analysis, ^{1}H- and ^{13}C-NMR spectroscopy and matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. Schematic representation of all CDs used in this study is given in Fig. 1. Analytical grade KH_2PO_4, Na_2HPO_4, H_3PO_4, triethanolamine and NaOH were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

2.2.1. CE

CE separations were performed using a Grom capillary electrophoresis modular system 100 (Grom, Herrenberg, Germany) equipped with a Linear Instrument UVIS 200 detector (Reno, NV, USA) and
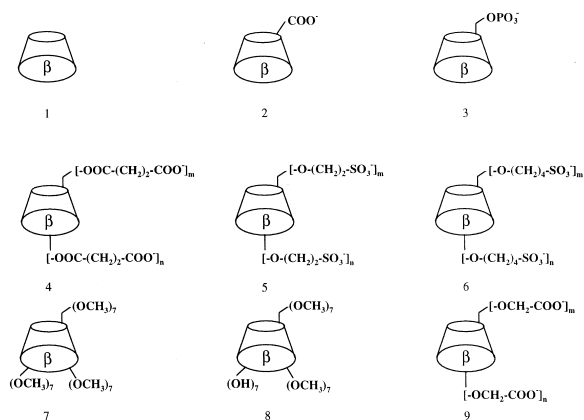

Fig. 1. Schematic representation of CDs used in this study. (1) β-CD; (2) 6-deoxy-6-monocarboxy-β-CD; (3) β-CD-6-monophosphate; (4) succinyl-β-CD; (5) sulfoethyl-β-CD; (6) sulfobutyl-β-CD; (7) heptakis-(2,3,6 trimethyl)-β-CD; (8) heptakis-(2,6-dimethyl)-β-CD; (9) carboxymethyl-β-CD.

an HP 3396 A integrator (Hewlett–Packard, Wald-bronn, Germany). The samples were introduced hydrostatically (10 cm). A fused-silica capillary (Grom, Germany) with 50 μm I.D., 60-cm total length and 43-cm effective length was used. Other experimental conditions are shown in the legends to the figures.

2.2.2. NMR-spectroscopy

^{1}H and ^{13}C NMR, homonuclear correlated spectroscopy (HOMCOR), heteronuclear chemical shift correlation (HETCOR) and destortionless enhancement polarization transfer (DEPT) spectral analyses were performed using a Varian Gemini 200 NMR-spectrometer at 200 MHz (^{1}H) and 50 MHz (^{13}C). [2]H_2O was used as a solvent, and a solution of tetramethylsilane (TMS) in tetrachloromethane served as an external standard. The stoichiometry of the DIM–CD complexes was determined by the continuous variation method [18–21,25] based on the chemical shift of the H(3) proton of (±)-DIM.

The apparent binding constants of the enantiomers of DIM with β-CD were calculated using Scott's technique [20,21,26–28].

2.2.3. Mass spectrometry

MALDI-TOF mass spectra were taken using a Finigan MAT Vision 2000 equipment with a laser Speser 600 and an excitation wavelength of 355 nm. The analytes were dissolved in water (1 μg/ml). 5–10 μl of the solutions were mixed with an aliquot of 30 mM NaCl solution and the matrix [0.2 mol 2,5-dihydroxybenzoic acid and 0.6 mol 1-hydroxy-isoquinoline in 1:1 (v/v) aqueous acetonitrile].

3. Results and discussion

3.1. CE

Most of studies related to chiral CE separations discuss the effects of various experimental variables on the separation which is expressed by separation factor (α) or peak resolution (R_s). At present, less attention is paid to the migration order of enantiomers. The migration order of enantiomers can be reversed by changing the configuration of the chiral selector or the external separation parameters such as

the direction and magnitude of the EOF, the pH of the buffer, the concentration of the chiral selector, etc. [29,30]. Alternatively, the migration order of enantiomers may be opposite, even for the chiral selectors possessing the same configuration but relatively small structural differences. In addition to its practical value, the latter is of importance for the elucidation of chiral recognition mechanisms.

The CE separation of the enantiomers of the chiral antihistaminic drug DIM has been described in [29–39]. The migration order of the enantiomers is addressed in some of them [29–33]. The initial goal of this study was to elucidate a mechanism for the opposite migration order of DIM enantiomers using native β-CD and its randomly substituted derivative CM-β-CD as chiral selectors in CE. However, other CD derivatives were involved in the study also.

The electropherograms of a nonracemic mixture of DIM-tartrate in the presence of 15 mg/ml β-CD and 1 mg/ml CM-β-CD are shown in Fig. 2a,b, respectively. The following two points seem worthy of mention: (a) a significantly lower concentration of CM-β-CD was required for an adequate separation compared to β-CD and (b) the opposite migration order of the DIM enantiomers with CM-β-CD and β-CD. Interestingly, some other anionic CD deriva-
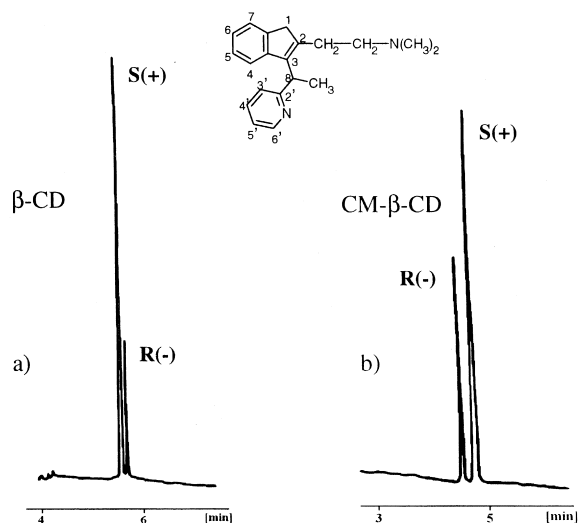

Fig. 2. Electropherograms of the mixture of DIM enantiomers [$R(-)/S(+)=1/3$] in the presence of 15 mg/ml β-CD (a) and 1 mg/ml CM-β-CD (b). Buffer: 50 mM potassium phosphate at pH 3.0. Other conditions as described in Section 2.

tives such as SEE-β-CD and SBE-β-CD also result in a baseline enantioseparation at low concentrations but do not cause a reversal of the enantiomer migration order (Table 1).

The predominant location of ionic substituents on the primary narrower CD rim has been suggested for sulfoalkyl CDs with a relatively low degree of substitution [40]. In order to examine the location of the carboxymethyl group in CM-β-CD, a DEPT-NMR spectrum of this CD derivative was recorded (Fig. 3). Based on this spectrum it can be concluded that in CM-β-CD the substituent is located predominantly on the secondary rim as confirmed by the downfield shift of the resonance signal of the C(2) atom where the carboxymethyl substituent is located (approx. 10 ppm) as well as the upfield shift of the resonance signal of the neighboring C(1) atom (approx. 2 ppm) [41]. However, the substitution on the primary CD rim also occurs. The indication for this is two negative peaks for CH_2 groups located in the range between 73.10–73.30 ppm. One of these signals belongs to the methylene carbon in the carboxymethyl group and the another one to the substituted C(6) which is shifted downfield. Thus, a carboxymethyl group seems to enter both the primary and the secondary CD rims even for a low D.S. However, as the most recent study shows, the same applies also for SBE-β-CD even for a substitution degree as low as 1.0 [42].

In order to examine the role of the ionic substituents on the primary CD rim on the chiral recognition ability towards the enantiomers of DIM, two single-isomer monosubstituted anionic CD de-
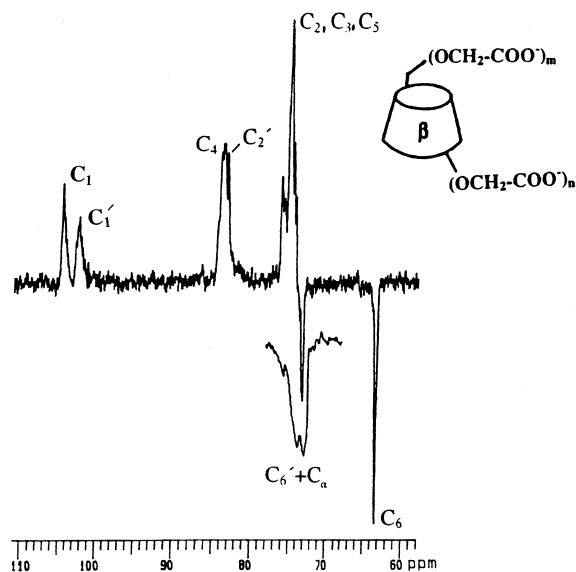

Fig. 3. DEPT-NMR spectrum (only part is depicted) of commercially available CM-β-CD.

rivatives, 6-deoxy-6-monocarboxy-β-CD and β-CD-6-monophosphate were studied as chiral selectors (Fig. 4). No reversal of the migration order of DIM enantiomers was observed with these CD derivatives compared to β-CD. Thus, the introduction of anionic substituents on the primary CD rim does not appear to cause the opposite chiral recognition towards the enantiomers of DIM compared with β-CD.

The reversal of the migration order of the DIM enantiomers was also observed using the neutral CD derivative TM-β-CD. However, the chiral resolving ability of this CD derivative was significantly lower.

Table 1
Enantioseparation of (±)-dimethindene with various cyclodextrins (experimental conditions as in Fig. 2)

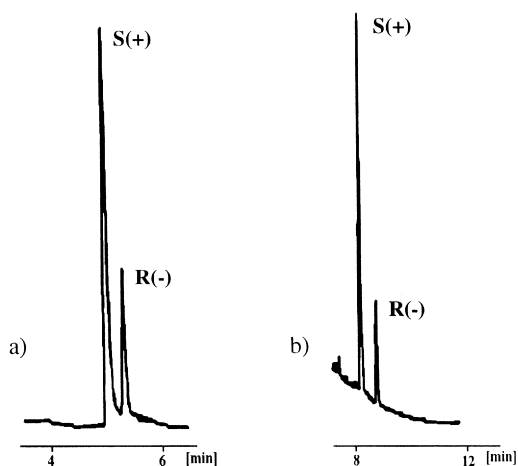| Cyclodextrin	Concentration (mg/ml)	Migration times (min)		Migration order
		t_1	t_2	
β-CD	15	4.5	4.7	*S* before *R*
6-Deoxy-6-monocarboxy-β-CD	15	5.5	5.7	*S* before *R*
β-CD-6-monophosphate	5	9.4	9.7	*S* before *R*
SBE-β-CD	1	5.0	5.5	*S* before *R*
SEE-β-CD	2	6.5	6.8	*S* before *R*
Succinyl-β-CD	15	7.0	7.1	*S* before *R*
CM-β-CD	1	4.4	4.7	*R* before *S*
DM-β-CD	25	14.0	14.1	*R* before *S*
TM-β-CD	60	7.3	7.5	*R* before *S*

Fig. 4. Electropherograms of the mixture of DIM enantiomers $[R(-)/S(+)=1/3]$ in the presence of 15 mg/ml 6-deoxy-6-mono-carboxy-β-CD (a) and 5 mg/ml β-CD-6-monophosphate (b). Other conditions as in Fig. 2.

Fig. 5. ^{1}H-NMR spectra of 6.9 mg mixture of DIM enantiomers $[R(-)/S(+)=2/1]$ in the absence (a) and in the presence of 13.2 mg β-CD (b) or 16.5 mg CM-β-CD (c). Only the aromatic parts of the spectra are depicted. Other conditions as described in Section 2.

A 60-fold higher concentration of TM-β-CD was required for obtaining an enantioseparation comparable to that obtained using 1 mg/ml CM-β-CD. Another methylated β-CD derivative, DM-β-CD, does not exhibit a measurable chiral recognition ability at lower (<20 mg/ml) and higher (>25 mg/ml) concentrations. Only a poor enantiosepara-tion was observed at 25 mg/ml DM-β-CD. The migration order of the DIM enantiomers with this CD was the same as in the presence of TM-β-CD. Thus, a negative charge is apparently not a determin-ing factor for the recognition pattern of β-CDs toward the enantiomers of DIM, but it seems to be very important for the resolution ability of these CDs. Both the mobility of CM-β-CD as well as its binding pattern (stoichiometry, enantioselectivity of formation of transient diastereomeric complexes and their stability) may contribute to this effect.

3.2. NMR spectroscopy

In order to obtain more detailed information on selector–selectant interactions of the complexes of the DIM enantiomers with β-CD and CM-β-CD, NMR studies were performed.

^{1}H- and ^{13}C-NMR spectra of a 1:2 mixture of $S(+)$ and $R(-)$-DIM are depicted in Figs. 5 and 6, respectively. The splitting of some resonance lines

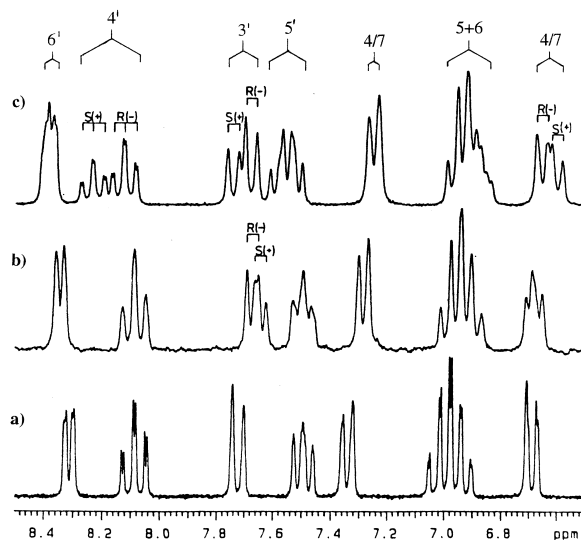due to the nonequivalent complexation-induced chemical shifts for the enantiomers was observed in ^{1}H-NMR spectra in the presence of both β-CD and CM-β-CD (Fig. 5). The stereoselective effect was highest on the H(3́) (at 7.7 ppm) proton of DIM in the presence of β-CD, whereas the H(4́) (8.1–8.2 ppm) proton was affected most in the presence of CM-β-CD. These data indicate some differences in the interaction of these two CDs with the enantio-mers of DIM.

In the presence of CM-β-CD, the resonance signal corresponding to H(4́) is more strongly shifted downfield for $S(+)$-DIM than for $R(-)$-DIM. If the complexation-induced chemical shifts are a correct measure of the complex stability, then $S(+)$-DIM would be preferentially complexed with CM-β-CD and, consequently, it must migrate as the second peak in CE. This is actually the case (Fig. 2b). However, the above-mentioned chemical shift pat-tern does not hold for a wide range of the DIM/CM-β-CD ratio. This is discussed in more detail below.

Surprisingly the H(3́) doublet of the DIM mole-cule tends to shift in the opposite direction for the enantiomers in the presence of CM-β-CD. The downfield shift for $S(+)$-DIM is less pronounced

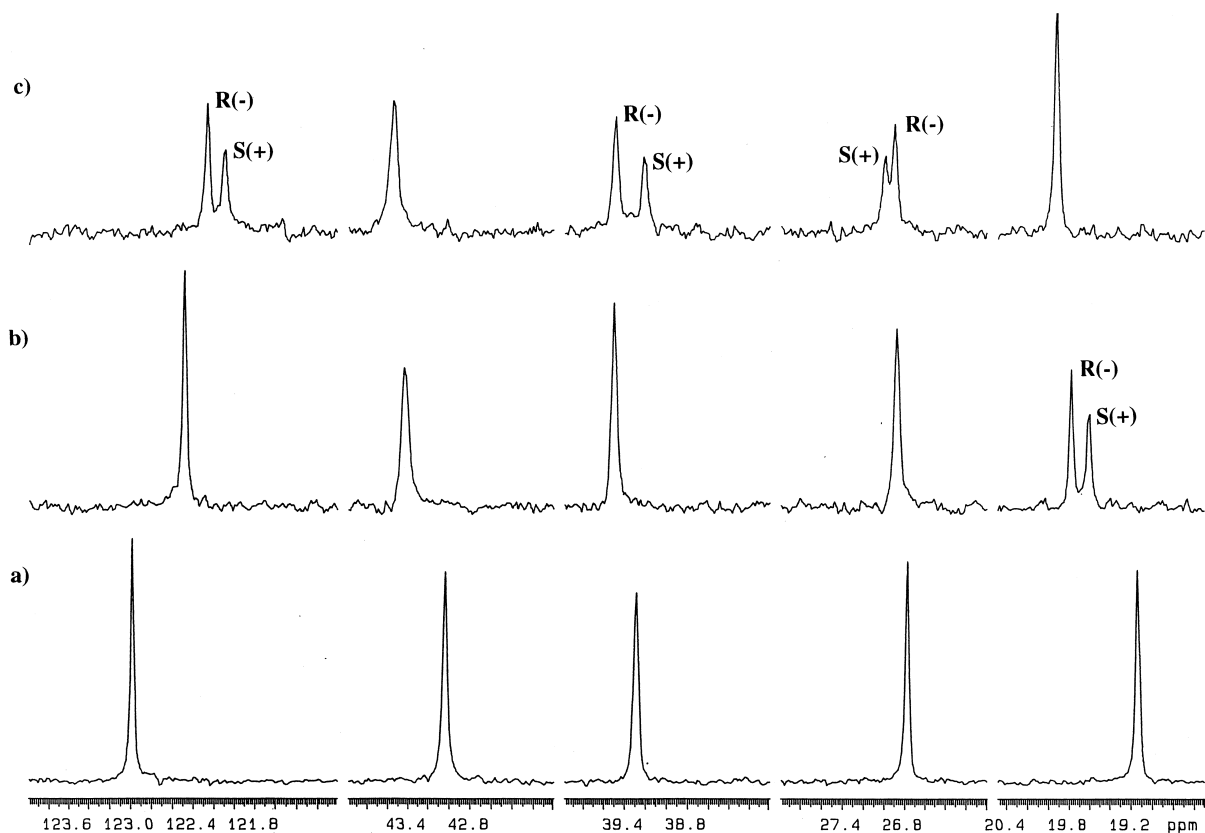Fig. 6. ^{13}C-NMR spectra of 6.9 mg mixture of DIM enantiomers $[R(-)/S(+)=2/1]$ in the absence (a) and in the presence of 13.2 mg β-CD (b) or 16.5 mg CM-β-CD (c). Only the aromatic parts of the spectra are depicted. Other conditions as described in Section 2.

than the upfield shift for $R(-)$-DIM. The data do not correlate with the enantiomer migration order observed in CE. A similar trend was observed in the ^{13}C-NMR spectrum of DIM in the presence of CM-β-CD. In this case, from nine definitely splitted resonance lines at higher CD/DIM ratios only four correlated with the enantiomer migration order observed in CE (these data are not shown in figures). This indicates a possibility of multiple complexation between DIM and CM-β-CD. However, as is shown in Fig. 6, the shifting pattern observed at low and intermediate of CM-β-CD/DIM ratios correlates well with the enantiomer migration order observed in CE. In particular, the complexation-induced chemical shifts are always stronger for $S(+)$-DIM irrespective of the shifts upfield or downfield.

The upfield shift of the H(3́) proton in the presence of β-CD was higher for $S(+)$-DIM than for

$R(-)$-DIM (Fig. 5). This means that in the first approximation $S(+)$-DIM should preferentially be complexed with β-CD, i.e. it must migrate as the second peak in CE. This is in contradiction to the enantiomer migration order actually observed in CE (Fig. 2a). However, it must be noted that a complexation-induced chemical shift correctly reflects the stability of intermolecular complexes only in those cases where those complexes possess the same NMR characteristics. This is not always true for diastereomeric complexes. Therefore, the calculation of the binding constants is required in order to obtain more reliable data [21]. Contrary to the case of the ^{1}H-NMR spectrum, the correlations between the complexation-induced chemical shift pattern in the ^{13}C-NMR spectrum and the enantiomer migration order in CE using β-CD is fairly good (Fig. 6b).

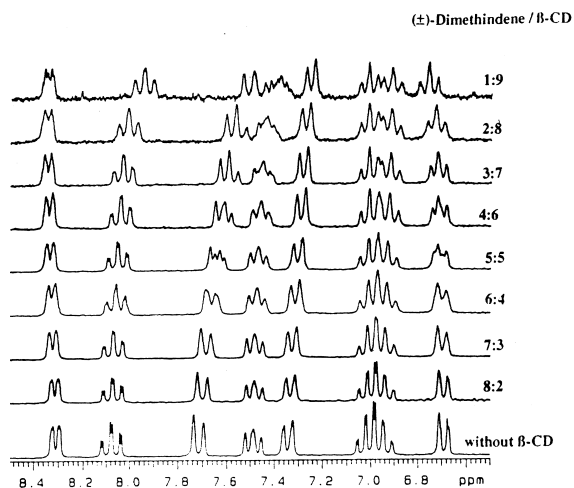The measurement of complexation-induced chemi-

Fig. 7. ^{1}H-spectra of the mixture of DIM enantiomers [$R(-)$/$S(+)$=2/1] at various DIM/β-CD ratios.

Fig. 9. Scott's plots for ($\pm$)-DIM/β-CD complex.

Fig. 8. Job's plots for ($\pm$)-DIM/β-CD complex.

cal shifts at different DIM/β-CD ratios (Fig. 7) showed that the intermolecular complex between these two species has a 1:1 stoichiometry (Fig. 8). The apparent binding constants (K_a) and complexation-induced chemical shifts at saturation ($\Delta\delta_s$) for both enantiomers of DIM with β-CD were calculated, based on the Scott's plots (Fig. 9). These data are summarized in Table 2. As it is clearly shown, the average binding constant is higher for $R(-)$-DIM than for $S(+)$-DIM. In contrast to the complexation-induced chemical shift pattern in the ^{1}H-NMR
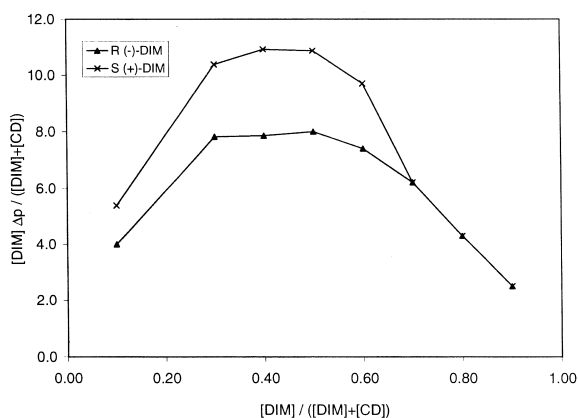
spectra, these data correlate well with the observed migration order of the DIM enantiomers in CE (Fig. 2a).

It seems worth mentioning that in one set of ^{1}H-NMR experiments the addition of urea was used in order to enhance the solubility of β-CD in the aqueous buffer. The calculation of the binding constants in this series demonstrated the adverse effect of urea (approx. 5-fold decrease of the binding constants) on the stability of complexes between the DIM enantiomers and β-CD. The enantioseparations performed in CE using β-CD in the presence and absence of urea clearly confirmed this detrimental effect (Fig. 10).

One of the main goals of this study was to elucidate the molecular mechanisms of the markedly higher separation ability of CM-β-CD toward the enantiomers of DIM compared with β-CD. The ^{1}H-NMR spectra of DIM at different DIM/CM-β-CD ratios are depicted in Fig. 11. Several interesting conclusions can be drawn from these data. First, in contrast to the DIM/β-CD case (Fig. 7) the complexation-induced chemical shift difference does not continuously increase with a decreasing DIM/CM-β-CD ratio. These data confirm the above-mentioned multiple complexation between DIM and CM-β-CD. Additionally, these complexes seem to possess a different stoichiometry as the extend of their formation changes with increasing DIM/CM-β-CD ratios. Moreover, the chiral recognition is apparently opposite to each other in these complexes. These conclusions are based on the fact that the directions of the

Table 2
Complexation induced chemical shifts for H(3′) proton at the saturation ($\Delta\delta_s$), averaged apparent binding constants [$K_{a(av)}$] and selectivity of binding (α) of $S(+)$ and $R(-)$-dimethindene to β-CD

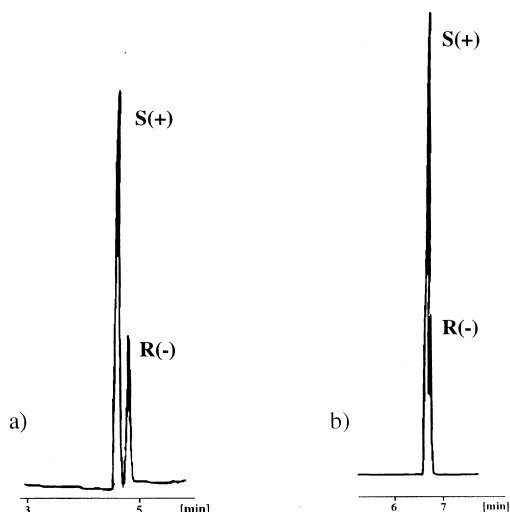| Enantiomers of dimethindene	Complexation induced chemical shifts at saturation, ($\Delta\delta_s$), H_z	Averaged apparent binding constants, K_a (M^{-1})	$\alpha = K_R / K_S$
$S(+)$-Dimethindene	40.6	457	1.10
$R(-)$-Dimethindene	28.0	504	

Fig. 10. Electropherograms of the mixture of DIM enantiomers [$R(-)/S(+)=1/3$] using 15 mg/ml β-CD in the absence (a) and in the presence (b) of 5 M urea. Other experimental conditions as in Fig. 2.

chemical shifts are opposite for almost all observed signals at lower and higher DIM/CM-β-CD ratios. Additionally, $R(-)$-DIM is shifted more at the lower ratios, whereas the shift is higher for the $S(+)$ enantiomer at higher DIM/CM-β-CD ratios. A stoichiometry-dependent reversal of the chiral recognition ability is an unique example for intermolecular inclusion complexes. However, one example was described recently where the chiral recognition which was absent in a 1:1 complex appeared in a 1:2 complex between a biphenyl-type chiral host and cyclohexyldiamine guest [43].

NMR-data alone are insufficient to determine the exact stoichiometry of DIM/CM-β-CD complexes. Further studies are in progress at the present time.

In conclusion, the formation of multiple complexes with a different stoichiometry, structure and chiral recognition pattern in the case of CM-β-CD may be responsible for the high chiral resolving ability of this CD towards the enantiomers of DIM compared
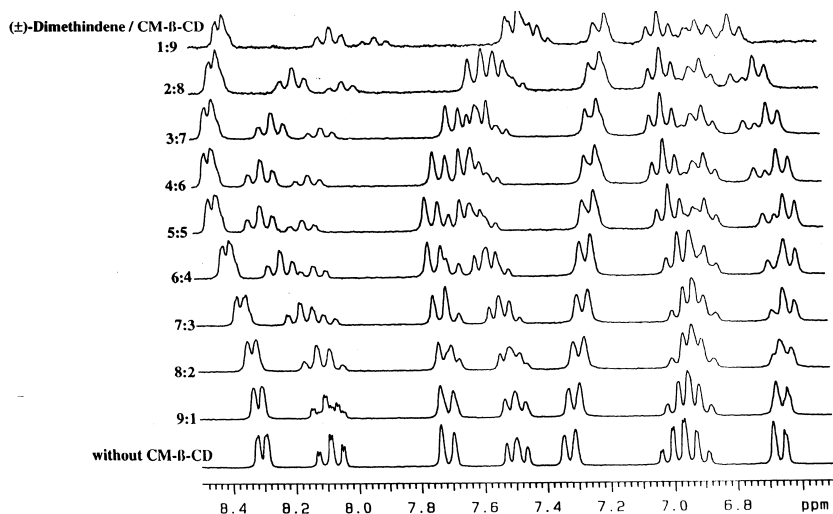

Fig. 11. ^{1}H-spectra of the mixture of DIM enantiomers [$R(-)/S(+)=1/2$] at various DIM/CM-β-CD ratios. Only the aromatic parts of the spectra are depicted.

with β-CD. The same may be true for the opposite migration order of the enantiomers of DIM using these two CDs. A chiral recognition pattern observed in CE mainly represents a statistically averaged sum of the interactions in multiple transient complexes which may possess different structure, stoichiometry and chiral recognition pattern.

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