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Comparative capillary electrophoretic and nuclear magnetic resonance studies of the chiral recognition of racemic metomidate with cyclodextrin hosts

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Abstract

Detailed capillary electrophoretic (CE) and ^{1}H NMR studies of the chiral recognition of racemic metomidate with various cyclodextrin hosts show that ^{1}H NMR spectrometry is a useful technique for the investigation of the chiral recognition mechanism in CE. NMR spectrometry provides data about the inherent chiral recognition abilities of a given chiral selector and allows to choose the optimal conditions for enantioseparations by CE.

The effects of the type of the cyclodextrin host, the pH and the ionic strength of the separation medium on the chiral recognition were studied with both techniques. Enantioselectivities observed in CE can be explained on the basis of self-mobilities of the chiral selectors and apparent binding constants which were calculated using ^{1}H NMR experiments.

Keywords: Nuclear magnetic resonance spectrometry; Enantiomer separation; Metomidate; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) has developed explosively over the past few years as a powerful technique for enantiomeric analysis [1]. Cyclodextrins (CDs) are most commonly used chiral selectors among others such as chiral micelles, linear oligosaccharides, peptides etc. The number of papers about CE enantioseparations is increasing rapidly, but mechanistic studies involving parallel to CE other experimental techniques are scarcely published [2,3]. A number of theoretical models of the enantioseparation are proposed in order to optimize the separation efficiency [4–8]. All these models are based on binding constants of enantiomers with chiral selectors and mobilities of the solute and selector. Nevertheless, the measurements of the apparent binding constants [5,6,9–11] and mobilities of the chiral selectors [12,13] are reported only in a few studies.

Among the nonseparation techniques used for the studies of stereoselective selector–solute interactions NMR spectrometry seems to be the technique of choice: (i) This technique supports considerable information about the environment of individual atoms and intermolecular interactions. Therefore, it is the most useful method to the analysis of the structure and molecular dynamics of complexes. (ii) NMR allows a clear differentiation between the inclusion and any other possible external interaction processes. This is an important advantage of this technique over all others, which are more global and

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do not provide any convincing proof for inclusion. (iii) Enantiotopic signals can be resolved principally in chiral medium in an NMR spectrum. This allows the racemic sample to be used for the study of enantioselective binding parameters (stoichiometry of the selector–solute complexes, apparent binding constants, free energy, enthalpy and entropy of binding etc.).

Detailed NMR studies of the chiral recognition of the racemic imidazole derivative metomidate using various native and derivatized cyclodextrins are reported in this work together with CE enantioseparations.

2. Experimental

2.1. CE equipment

Two CE systems – (a) a Grom capillary electrophoresis system 100 (Herrenberg, Germany), equipped with a Linear Instruments (Reno, NV, USA) UVIS 200 detector and a HP 3396 A integrator (Hewlett-Packard, Avondale, PA, USA) and (b) a P/ACE 2050 (Beckmann Instruments, Fullerton, CA, USA) – were used with an untreated fused-silica capillary (Grom) of (a) 61 cm and (b) 47 cm total length×50 μm I.D. The samples were introduced (a) hydrostatically (10 cm) during 5 s and (b) with low pressure for 2 s at the anodic end of the capillary. The detections of the solutes were carried out at (a) 210 nm and (b) 214 nm. The electric field was +400 V/cm, the temperature was 21±1°C.

The selectivity of the enantioseparation was characterized with α_{rel} which is the ratio of the effective mobilities of enantiomers. The resolution of enantiomers was calculated following Eq. 1:

$$R_s = \frac{2(t_2 - t_1)}{(w_1 + w_2)} \quad (1)$$

where t_2 and t_1 are the migration times and w_1 and w_2 baseline peak width of the first and second eluted enantiomers respectively.

2.2. NMR equipment

^{1}H and ^{13}C NMR, homonuclear correlated spectrometry (HOMCOR), heteronuclear chemical shift correlation (HETCOR), attached proton test (APT) and distortionless enhancement by polarization transfer (DEPT) spectral analysis were carried out with a Varian Gemini 200 NMR spectrometer at 200 MHz (^{1}H) and 50 MHz (^{13}C). ^2H_2O was used as a solvent and a solution of tetramethylsilane (TMS) in tetrachloromethane served as external standard. The peak assignment of (±)-metomidate [(±)-MET] in ^{1}H and ^{13}C NMR spectra was performed using HOMCOR, HETCOR, APT and DEPT spectra. CD signals in ^{1}H and ^{13}C NMR spectra were assigned using literature data for β-CD [14,15]. The stoichiometry of the selector–solute complexes was determined by the continuous variation method [16]. The total concentration of the interacting species in the solution was kept constant at 20 m*M* and the molar fraction of the guest was varied in the range of 0.2–0.8. Apparent binding constants of the enantiomers of (±)-MET with CD derivatives were calculated on the basis of Scott's modification of the Benesi–Hildebrand equation [17–19]. In ^{1}H-NMR studies concerning the effects of the ionic strength and the pH of the buffer solution, $K^2H_2PO_4$, NaO^2H and ^2HCl were used for the adjustment of the ionic strength and pH. The relevant correction between pH and p^{2}H was also made.

2.3. Chemicals and reagents

The racemic (±)-MET was a gift from Dr. J. Dingenen (Janssen, Beerse, Belgium). Sulfobutyl ether of β-CD (SBE-β-CD) [substitution degree (s.d.) approx. 4.0] was a gift from Professor J.F. Stobaugh and Professor V.J. Stella (Center for Drug Delivery Research, The University of Kansas, Lawrence, Kansas, USA). α-, β-, γ-CD, permethyl-β-CD (Me-β-CD) (s.d. approx. 12.6), hydroxypropyl-β-CD (HP-β-CD) (s.d. approx. 4.2), carboxymethyl-β-CD (CM-β-CD) (s.d. approx. 2.1) and sulfoethyl ether of β-CD (SEE-β-CD) (s.d. approx. 2.8) were from Wacker Chemie (Munich, Germany).

Analytical grade KH_2PO_4, Na_2HPO_4, H_3PO_4 and NaOH were purchased from Merck (Darmstadt, Germany).

2.4. Buffer and sample preparation

Stock solutions of 50 m*M* KH_2PO_4 and 50 m*M* Na_2HPO_4 were prepared in double distilled, deion-

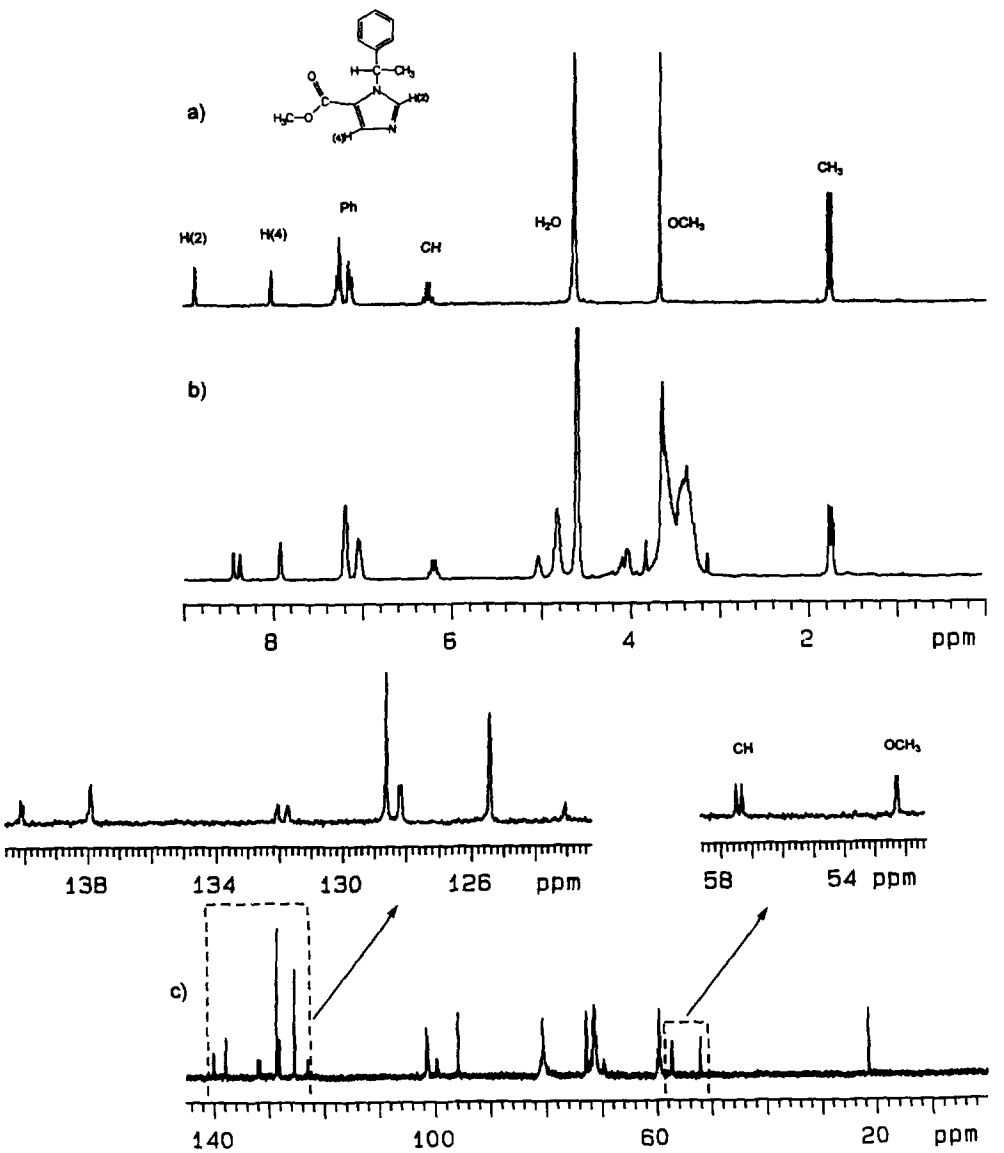

Fig. 1. ^{1}H NMR spectrum of 10 m*M* solution of (±)-MET in ^2H_2O (a); ^{1}H NMR (b) and ^{13}C NMR (c) spectra of equimolar solution (10 m*M* each) of (±)-MET and CM-β-CD.

ized water. The pH was adjusted with 0.17 *M* H_3PO_4 or 0.5 *M* NaOH. The run buffers were prepared accordingly after the addition of appropriate amounts of the chiral selectors. All solutions were filtered and degassed by sonication before use.

A stock solution of 1 mg/ml of the racemic (±)-MET was prepared, stored at 4°C and diluted to 60 μg/ml before use.

3. Results and discussion

3.1. NMR studies of selector–solute interactions

In order to evaluate the potential of NMR spectrometry for the study of the chiral recognition in (±)-MET–CD complexes ^{1}H and ^{13}C NMR spectra of the equimolar mixture (10 m*M* of each) of (±)-

MET with CM-β-CD were taken in ^2H_2O using tetramethylsilane dissolved in tetrachloromethane as an external standard.

Fig. 1a shows the ^1H NMR spectrum of the (±)-MET without CD additive. A clear splitting of the H(2)-singlet at approx. 8.85 ppm is observed in the ^1H NMR spectrum of an equimolar mixture of (±)-MET and CM-β-CD (Fig. 1b). The effect of splitting of lines is obvious in the ^{13}C NMR spectrum of the same sample (Fig. 1c). ^{13}C NMR signals at about 52 ppm (OCH_3), 57 ppm (CH) and further in the range of 128–141 ppm are duplicated as a result of the complexation of (±)-MET with CM-β-CD.

As our previous study showed [3], the interchange between the complexed and the free solute is fast on the NMR time scale and averaged signals between free and complexed solutes can be observed in NMR spectra. It means that the above-mentioned signal splittings in NMR spectra are a result of the enantiomeric composition of the sample.

The Job plots of (±)-MET–CM-β-CD solutions (Fig. 2) show the predominance of complexes of 1:1 stoichiometry of both enantiomers with the chiral selector. Further, for the complexes of known 1:1 stoichiometry the Scott's modification [17] of the Benesi–Hildebrand method [18] was used to calculate the apparent binding constants (K_a). In Scott's Eq. 2:

$$\frac{[\text{selector}]_t}{\Delta\delta_{obs}} = \frac{[\text{selector}]_t}{\Delta\delta_c} + \frac{1}{K_a\Delta\delta_c} \quad (2)$$

$[\text{selector}]_t$ is the molar concentration of the chiral selector, $\Delta\delta_{obs}$ is the observed chemical shift difference for a given $[\text{selector}]_t$ concentration, $\Delta\delta_c$ is the chemical shift difference between a pure sample of complex and the free component at the saturation.

The slope of the plot of $[\text{selector}]/\Delta\delta_{obs}$ against [selector] is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K_a\Delta\delta_c$, allowing the estimation of K_a.

It should be mentioned that most of CDs used in this study are heterogeneous mixtures of components with different degrees of substitution and maybe with different regio- and position-isomerism. Therefore, calculated parameters ($\Delta\delta_c$, K_a) can not be considered as absolutely correct thermodynamic values. Nevertheless, these averaged apparent parameters are

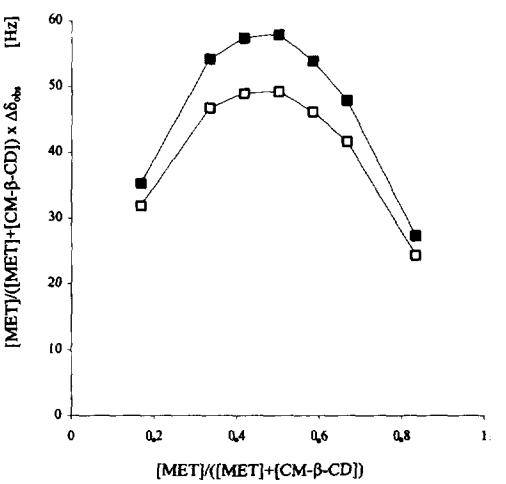

Fig. 2. Job plots for the (±)-MET–CM-β-CD complex.

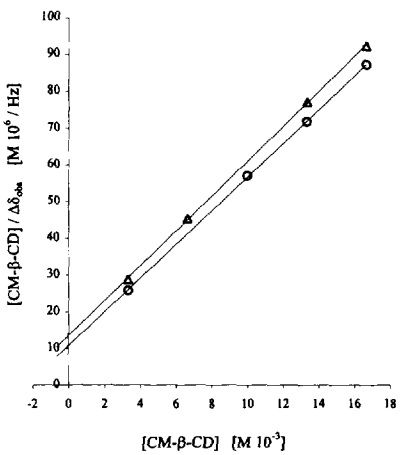

Fig. 3. Scott's plots for the (±)-MET–CM-β-CD complex.

Table 1
Complexation-induced chemical shift differences at saturation ($\Delta\delta c$) and apparent binding constants (K_a [M^{-1}]) of (±)-MET with various CDs

CD	$\Delta\delta c$ (Hz)		K_a (M^{-1})		α_i^a
	$\Delta\delta c_1$	$\Delta\delta c_2$	K_{a1}	K_{a2}	
β-CD	20.9	16.5	483	655	1.36
CM-β-CD	210.8	218.1	350	423	1.21
SBE-β-CD	77.8	87.3	397	564	1.42

All binding parameters in case of CM-β-CD and SBE-β-CD were calculated on the basis of H(2)-signal at 8.85 ppm and for β-CD on the basis of the quadruplet at 6.28 ppm.

[a] $\alpha_i = \frac{K_{a2}}{K_{a1}}$.

determined in the conditions used in CE separations and therefore they are very valuable for understanding the mechanism and for optimization of the CE enantioseparations.

For CM-β-CD, SBE-β-CD and β-CD the binding constants and the chemical shift differences at saturation were calculated for both enantiomers of (±)-MET. The H(2)-signal of the imidazole moiety at 8.85 ppm was used for the calculation of binding constants for CM-β-CD and SBE-β-CD and the CH quadruplet at approx. 6.28 ppm for β-CD. In latter case the H(2)-signal was substantially destroyed by increasing CD concentration and correct measurement of the signal position was impossible.

The plot of [solute]/$\Delta\delta_{obs}$ versus [solute] for the calculation of the binding parameters of (±)-MET with CM-β-CD as an example are given on Fig. 3. The chemical shift differences at the saturation ($\Delta\delta_c$) and the apparent binding constants (K_a) calculated on the basis of these plots and similar plots for β-CD and SBE-β-CD are given in Table 1.

As these results show the complexation-induced chemical shift differences at saturation for the diastereomeric complexes of different enantiomers with a given cyclodextrin are not the same. This means that the chemical shift due to the complexation can not be used directly in this case for characterising the binding strength differences between the enantiomers. More reliable parameters are the apparent binding constants. It seems noteworthy that β-CD binds the enantiomers of (±)-MET slightly stronger than SBE-β-CD and markedly stronger than CM-β-CD. Higher binding selectivity of SBE-β-CD in comparison with CM-β-CD is in good agreement with the results of CE enantioseparations given in Table 2 and Fig. 4a–c.

3.2. Effect of the cyclodextrin type on the chiral recognition

In order to study the effect of the CD type on the chiral recognition of (±)-MET, the eight most commonly used native and derivatized (neutral and charged) CDs were tested as chiral selectors in an equal molar concentration using the same conditions.

NMR spectra of equimolar mixtures of (±)-MET with these CDs were taken (Fig. 5) and show that the effect of α-CD on the ^{1}H NMR spectrum of (±)-MET is negligible (Fig. 5b). This CD does not show any measurable chiral recognition also in CE even at a concentration as high as 50 m*M* [20]. Like α-CD no substantial change of ^{1}H NMR spectrum was observed in the γ-CD–(±)-MET solution (Fig. 5d).

Table 2
Results of the enantioseparation of (±)-MET with various CDs (5 m*M*) at pH 3.5 and 6.0

CD type	pH 3.5				pH 6.0			
	t_1 (min)	t_2 (min)	α_{rel}	R_s	t_1 (min)	t_2 (min)	α_{rel}	R_s
α-CD	8.27	–	1.00	–	3.09	–	1.00	–
	8.24	–	1.00	–	3.08	–	1.00	–
β-CD	10.27	10.80	1.05	1.07	3.07	–	1.00	–
	10.32	10.86	1.05	1.09	3.06	–	1.00	–
γ-CD	8.91	9.02	1.01	0.45	3.04	–	1.00	–
	8.94	9.05	1.01	0.42	3.04	–	1.00	–
HP-β-CD	9.41	9.72	1.03	0.79	3.03	–	1.00	–
	9.56	9.88	1.03	0.82	3.03	–	1.00	–
Me-β-CD	9.75	10.18	1.04	1.03	3.04	–	1.00	–
	9.71	10.14	1.04	1.04	3.04	–	1.00	–
SBE-β-CD	14.49	15.41	1.06	1.86	4.56	4.73	1.04	0.71
	14.44	15.33	1.06	1.67	4.56	4.73	1.04	0.75
SEE-β-CD	12.38	13.07	1.06	1.36	4.57	4.75	1.04	0.69
	12.34	13.00	1.05	1.30	4.56	4.74	1.04	0.69
CM-β-CD	12.35	12.68	1.03	0.66	3.54	3.62	1.02	0.42
	12.36	12.70	1.03	0.69	3.54	3.61	1.02	0.45

Conditions: P/ACE 2050, 400 V/cm, 214 nm, 50 m*M* phosphate buffer, 25±1°C.

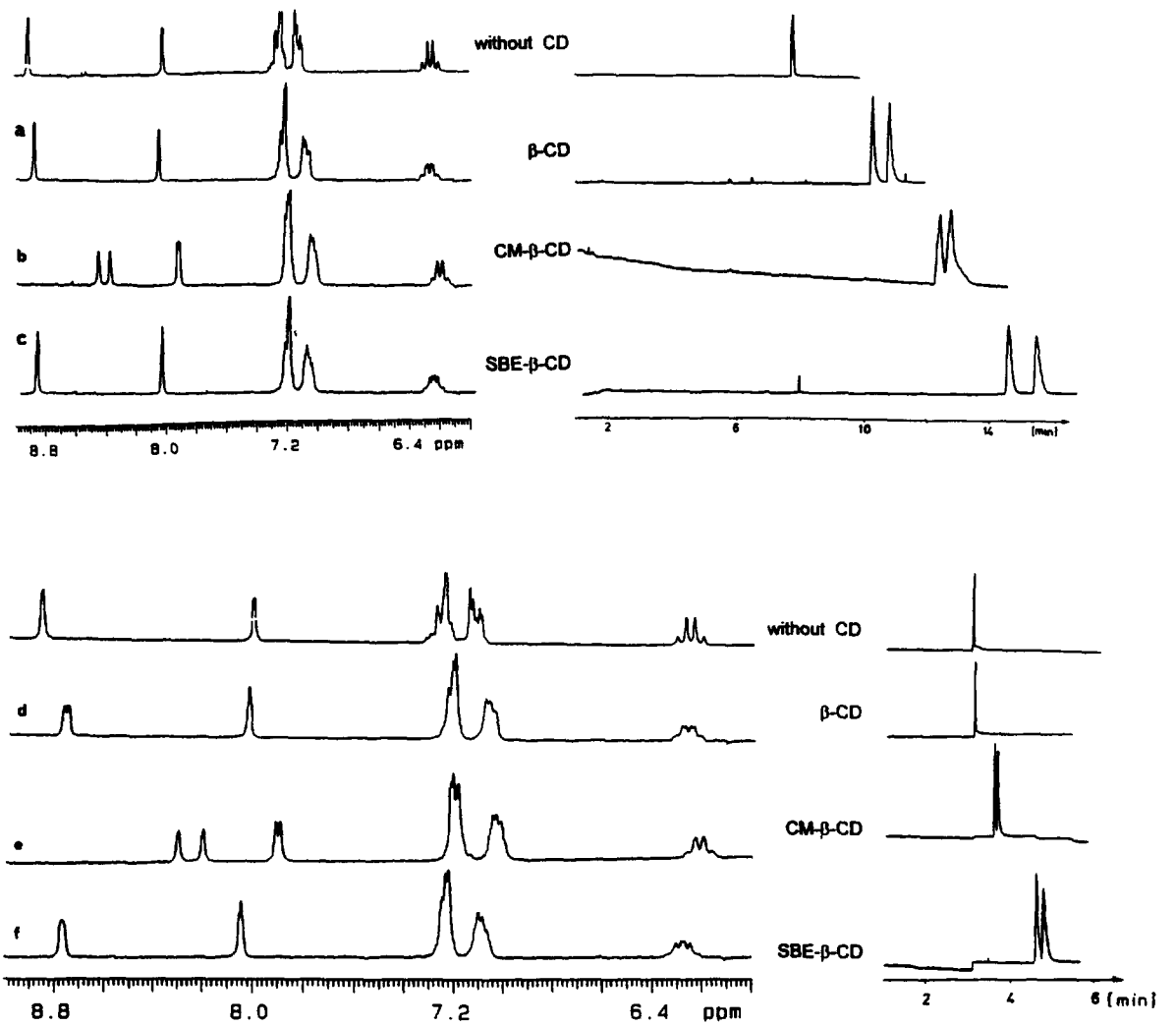

Fig. 4. ^{1}H NMR spectra of equimolar mixtures (10 m*M* each) of (±)-MET with β-CD (a, d), CM-β-CD (b, e) and SBE-β-CD (c, f) at pH 3.5 (a, b, c) and pH 6.0 (d, e, f) together with CE enantioseparations with the same chiral selectors at the same pH.

This CD exhibits only a weak chiral recognition of (±)-MET in CE [20].

Unlike to α- and γ-CD, β-CD shows a substantial effect on the ^{1}H NMR spectrum of (±)-MET (Fig. 5c). The downfield shift and a clear splitting of the CH-quadruplet centered approx. at 6.28 ppm and the upfield shift accompanied with a line splitting of the signals of H(2)-protons of the imidazole moiety at 8.85 ppm are most remarkable in this case. As for derivatized CDs, neutral derivatives show some measurable changes in ^{1}H NMR spectra of (±)-MET and even small but measurable splitting of the signals of the H(2)-proton was observed in the Me-β-CD–(±)-MET complex. The effect is much more substantial in the (±)-MET complexes with all charged CD derivatives. It seems worth mentioning that in the complexes of (±)-MET with CM-β-CD the signal of the H(2)-proton in the imidazole ring is exclusively affected whereas in the complexes of both sulfated CD derivatives the CH quadruplet at

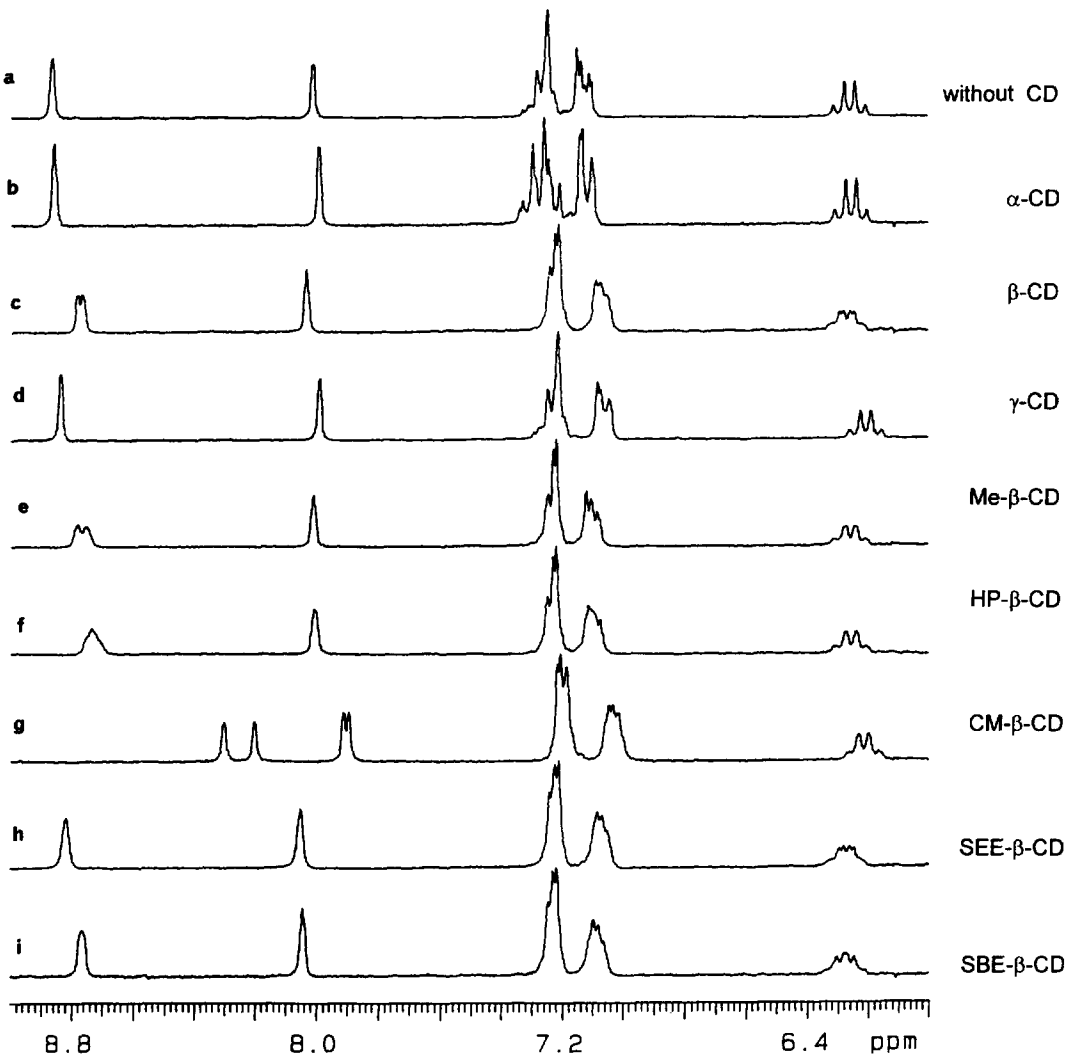

Fig. 5. ^{1}H NMR spectra of equimolar mixtures (10 m*M* each) of (±)-MET with various CD in $^{2}H_{2}O$ at pH 6.0.

6.28 ppm is preferentially split. For the latter derivatives at higher selector–solute ratios the upfield chemical shift and line splitting of the imidazole H(2)-signal is also easily observable.

The enantioseparation of (±)-MET in CE at pH 3.5 is achieved with all CDs which exhibit a signal splitting in NMR (Fig. 5, Table 2). In contrast, at pH 6.0 an enantioseparation was observed only with anionic CDs (Table 2, Fig. 4e,f). The reason of this discrepancies is that the apparent binding constants K_{a1} and K_{a2} of the enantiomers (1) and (2) with a given chiral selector as well as the mobilities of the free solute (μ_s) and of the selector–solute complex (μ_c) determine the selectivity (α) observed in CE. If we assume that diastereomeric transition complexes of both enantiomers of (±)-MET with CDs have the same mobility than the precondition for a successful CE enantioseparation is $K_{a1} \neq K_{a2}$ and $\mu_s \neq \mu_c$ at the same time. Furthermore, the magnitudes of K_{a1} and K_{a2} and the difference between them affect α.

As Fig. 4 and Table 2 show, for noncharged CDs the above-mentioned precondition fulfills only at pH 3.5, whereas at pH approx. 6.0 (±)-MET becomes neutral (pK 5–6) and it migrates in the electroosmotic flow (EOF) with the same effective mobility as noncharged CDs, i.e. $\mu_s = \mu_c$, and no enantioseparation is observed.

3.3. Effect of the ionic strength of the run buffer on the chiral recognition

The ionic strength of the buffer solution is one of the important parameters in CE enantioseparations. The effect of this variable can be dual with the nature. At first, as it was found by MacNicol and Rycroft [21], the "salt out" effect may force substance to complex tighter with the selector by increasing the ionic strength of the buffer. The second is that in uncoated capillaries the ionic strength of the buffer solution may substantially affect the double electric layer formed on the capillary wall and consequently the EOF. ^{1}H NMR spectrometry is a suitable technique to differentiate between these two effects. The electropherograms of the CE enantioseparation of (±)-MET using 5 mM CM-β-CD in the buffer solutions of various ionic strengths are depicted in Fig. 6. As this figure shows the migration time increases and the enantioseparation improves substantially by increasing the ionic strength of the buffer, whereas no definitive effects were observed in ^{1}H NMR spectra of the equimolar solutions of the same selector–solute pairs in the same buffer solution. These results indicate that the inherent chiral recognition ability of CM-β-CD should not be modified substantially and the improvement of the peak resolution is mainly caused by the change of the effective mobility of (±)-MET. Another reason of the improved peak resolution can be a better suppression of the electrophoretic dispersion with increasing ionic strength of the buffer.

3.4. Effect of the pH of the run buffer on the chiral recognition

The role of the pH of the separation medium is crucial for the enantioseparation in CE. The electrophoretic mobility of the solute as well as the EOF

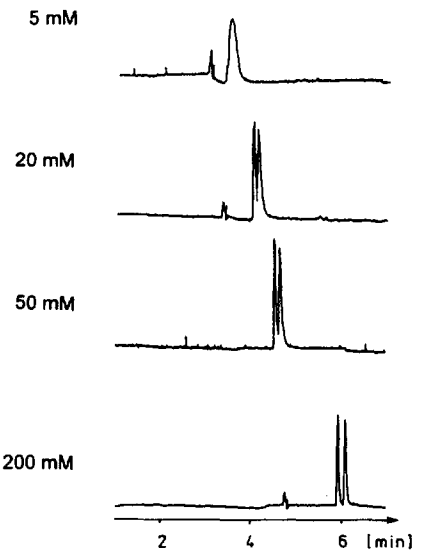

Fig. 6. CE enantioseparation of (±)-MET at pH 6.0 using 5 mM CM-β-CD in phosphate buffer with various ionic strengths.

and sometimes the self-mobility of the chiral selector are substantially pH dependent. Beside these parameters pH can markedly affect the inherent complexation ability of the chiral selector and a structure of the selector–solute complex. NMR techniques support in addition an important information on the pH dependence of the selector–solute interactions. The ^{1}H NMR spectra of equimolar solutions of (±)-MET–CM-β-CD at various pH values are shown in Fig. 7 parallel to the electropherograms of the enantioseparation obtained at the same pH in CE. A good correlation between CE and ^{1}H NMR spectrometry can be observed in this instance. Particularly, no complexation-induced splitting of the spectral lines was observed at pH 2.4 in ^{1}H NMR spectra of the equimolar (±)-MET–CM-β-CD solution in ^2H_2O and very poor enantioseparation was observed in CE. The spectral signal of the H(2)-proton of the imidazole moiety shifted markedly upfield and split ($\Delta\sigma = 0.05$ ppm) in the same solution at pH 3.5, which was accompanied by substantial improvement of the peak resolution in CE. The upfield chemical shift of the signal of the H(2)-proton of the imidazole moiety and the complexation-induced chemical shift nonequivalence between (±)-MET enantiomers increased by further increase of the pH up until 6.0.

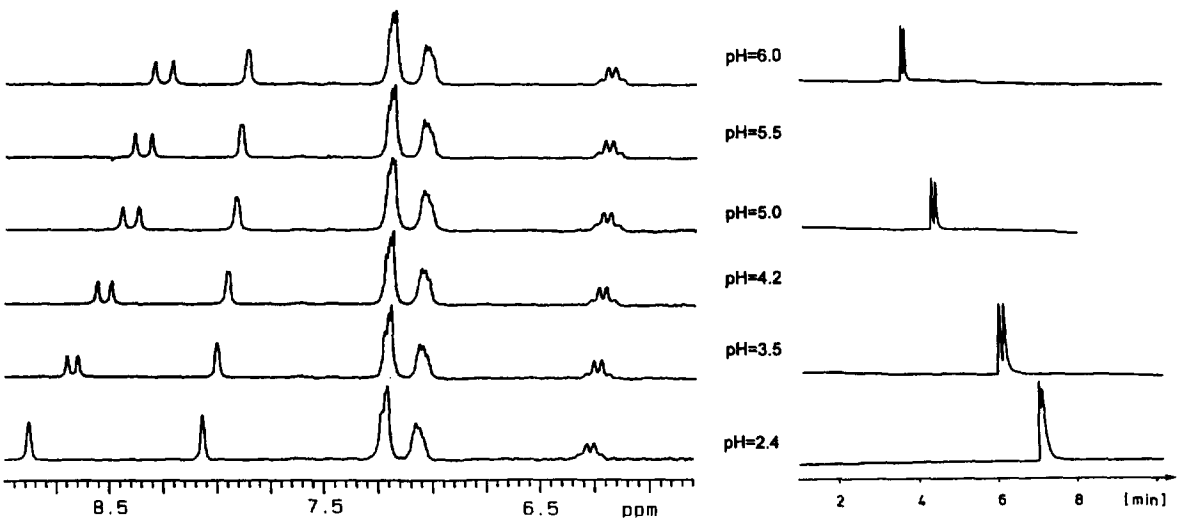

Fig. 7. CE enantioseparation of (±)-MET using 5 m*M* CM-β-CD in 50 m*M* phosphate buffer at various pH. ^{1}H NMR spectra of equimolar solutions of (±)-MET and CM-β-CD (10 m*M* each) at various pH.

Thus, in this study the different complexation-induced chemical shifts of the enantiomers of (±)-MET with CDs in ^{1}H NMR were used for calculations of the stoichiometry and stereoselective apparent binding constants of the inclusion complexes of (±)-MET with CM-β-CD, SBE-β-CD and β-CD. Definitive qualitative correlations between CE and ^{1}H NMR spectrometry were established in studies relevant to the role of CD type, the ionic strength and the pH of the separation buffer in the chiral recognition of (±)-MET.

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