ELSEVIER

Journal of Chromatography A, 717 (1995) 245–253

JOURNAL OF CHROMATOGRAPHY A

Enantioseparation of mianserine analogues using capillary electrophoresis with neutral and charged cyclodextrin buffer modifiers
^{13}C NMR study of the chiral recognition mechanism☆

Bezhan Chankvetadze[1], Gabriele Endresz, Dieter Bergenthal, Gottfried Blaschke*

Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58–62, 48149 Münster, Germany

Abstract

The enantiomers of the antidepressant drug (±)-1,2,3,4,10,14b-hexahydro-2-methyldibenzo[*c*,*f*]pyrazino[1,2-*a*]azepine (mianserine, Tolvin) [(±)-MN] and its 11 structural analogues were resolved with capillary electrophoresis (CE) using native β-cyclodextrin (β-CD) and three charged CD-derivatives as chiral buffer modifiers.

The effect of the nature and position of substituents of the chiral solute on the resolution is discussed. Sulfonated β-CD derivatives such as sulfobutyl (SBE-β-CD) and sulfoethyl (SEE-β-CD) ethers of β-CD permit adequate enantioseparations at rather lower concentrations as chiral selectors in comparison with carboxymethyl-β-CD (CM-β-CD) and especially with native β-CD. In a previous paper, we ascribed the high chiral resolving power of SBE-β-CD to the counter-current mobility of this chiral selector. Another important advantage of SBE-β-CD, its more stereoselective binding to the chiral selectand, is proved here on the basis of ^{13}C NMR studies. This last technique seems to be useful for estimation of the stoichiometry of the host–guest complexes as well as for determination of the apparent binding constants. A number of well-resolved ^{13}C NMR signals which belong to the CD complexes with the (+)- or (−)-enantiomer of the chiral solute enable racemic samples to be used for the study of the enantioselective binding parameters. Additionally, these CD derivatives can be recommended as useful water-soluble chiral shift reagents for the enantiomeric excess determination by ^{13}C NMR technique.

1. Introduction

Enantioseparation via capillary electrophoresis (CE) is a relatively new and very rapidly developing technique [1,2]. The most popular non-charged (native α-, β-, and γ-cyclodextrin, CD, hydroxypropyl-, and methylated-β-CD) derivatives in the early stage of the development of this technique [3–6] are being gradually substituted with increasing frequency by more versatile, charged CD-derivatives [6–18]. In 1989 Terabe reported the application of the positively charged ethylene diamine derivative of β-CD for the resolution of dansylated amino acid enantiomers [7]. At the present time, anionic CD-derivatives are more commonly used as chiral selectors in CE [6,9–18] than cationic ones [7,8]. Among anionic derivatives, besides carboxylated

* Corresponding author.

☆ Dedicated to Professor Dr. H. Möhrle on the occasion of his 65th birthday.

[1] Permanent address: Department of Chemistry, Tbilisi State University, Chavchavadze ave 1, 380028, Tbilisi, Republic of Georgia.

0021-9673/95/$09.50
SSDI 0021-9673(95)00489-0

cyclodextrins [6,10,11], sulfonated derivatives show very interesting chiral recognition properties [12–18].

The very first application of the sulfobutyl ether (SBE-β-CD), one of the members of this series, did not demonstrate any remarkable advantages. In contrast, undesirable effects, such as large variation in the run-to-run migration times and no elution, were observed [9]. The same derivative in our studies showed a very high enantiomer resolving ability and permitted baseline separations of a number of racemates in the concentration range 20–100 μM in the run buffer [12,17]. The direct measurement of the electrophoretic mobility of the chiral selector [19] allowed us to ascribe the high effectiveness of SBE-β-CD to the counter-current mobility of this chiral selector, which was comparable to the migration of the chiral analytes [12]. Other authors also observed chiral separations using SBE-β-CD or its sulfopropyl analogue in relatively low concentration (1 mM), and ascribed that effect to the self-mobility of the chiral selector [13–16].

Basically, enantioseparations in CE are achieved as a result of (i) different binding constants of the enantiomers to the chiral selector and (ii) differences in the mobilities of the bound and free analytes. An oppositely charged selector and selectand pair is advantageous for achieving the greatest difference between the mobilities of the bound and free analytes and is consequently superior for enantiomer resolution, as already emphasized in our previous study [12]. The former of these two effects, the stereoselectivity of binding of a racemic solute to the chiral selector, is the subject of the present study. The role of the nature and position of substituents on the chiral solutes in their chiral resolution and correlations between the data of ^{13}C NMR spectrometry and CE are also reported.

2. Experimental

2.1. Equipment

Two CE systems: (a) a Grom 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 detection of the solutes was carried out at (a) 210 nm and (b) 214 nm. The electric field was 400 V/cm, the temperature 21 ± 1°C. The anode and cathode buffers had the same pH and molarity as the run buffer and contained no chiral selectors.

The selectivity of the enantioseparation was characterized with α_{rel}, which is the ratio of the effective mobilities of enantiomers and is an average value of two measurements. The resolution of enantiomers was calculated according to the following equation:

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

where t_2 and t_1 are the migration times and w_1 and w_2 the baseline peak width of the first and second eluted enantiomers, respectively. Baseline resolutions of enantiomers were achieved in almost all cases where $\alpha_{rel} \geq 1.02$.

2.2. Chemicals and reagents

The racemic (±)-MN and its analogues (Fig. 1) were gifts from Dr. F.A. van der Vlugt (Organon, Netherlands). The optically pure *R*-(−)- and *S*-(+)-MN were prepared in our laboratory by enantioselective preparative liquid chromatography using cellulose triacetate as chiral packing material and a methanol–ethanol mix-

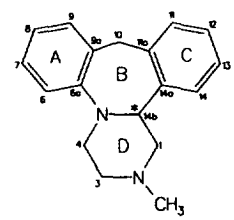

Fig. 1. Structures of (±)-MN and its analogues.

ture (1:1, v/v) as mobile phase. SBE-β-CD (substitution degree ca. 3.14, $M_r = 1684$) was a gift from Prof. J.F. Stobaugh and Prof. V.J. Stella (Center for Drug Delivery Research, The University of Kansas, Lawrence, KS, USA). β-CD, CM-β-CD (substitution degree ca. 2.1, indicated by the manufacturer), and the sulfoethyl ether, SEE-β-CD (substitution degree ca. 2.8, indicated by the manufacturer) 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).

2.3. 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, deionized water. The pH was adjusted with 1.5 *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.

Stock solutions of 1 mg/ml of the racemic compounds were prepared, stored at 4°C, and diluted to 60 μg/ml before use.

2.4. NMR

^1H and ^{13}C NMR, homonuclear correlated spectroscopy (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 (^1H) 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 (±)-MN in ^1H NMR and ^{13}C NMR spectra was performed using HOMCOR, HETCOR, APT, and DEPT spectra of (±)-MN and of some analogues. CD signals in ^1H NMR and ^{13}C NMR spectra were assigned using literature data [20,21] for β-CD. Stoichiometry of the selectand–selector complexes was determined by the continuous variation method [22,23] using the chemical shift of C(6) carbon atom of the (±)-MN molecule. The total concentration of the interacting species was kept constant at 17 m*M*, and the molar fraction of the guest was varied in the range 0.2–0.8.

3. Results and discussion

3.1. Effect of the nature and position of the substituents on (±)-MN molecule on chiral recognition

The results of the enantioseparations of (±)-MN and its analogues are summarized in Table 1. The quite different effectiveness of each chiral selector given in Table 1 did not allow us to use them in the same or similar concentrations.

Aromatic A and C moieties of the (±)-MN molecule are most probably involved in a host–guest complex, and therefore, substituents in both aromatic moieties A and C should play a critical part in chiral recognition. Geometric considerations on the basis of the crystal structure study of (±)-MN [24] and cavity size of β-CD suggest a multimodal complex formation between (±)-MN and its analogues on the one hand and CDs on the other. For example, the (±)-MN molecule (or analogues) could be complexed with the chiral selector via aromatic rings A or C. Alternatively, both A and C can simultaneously partially enter the cavity of CD. All above-mentioned complexes are characterized with a 1:1 stoichiometry. A sandwich-like (±)-MN–CD complex with 1:2 stoichiometry is also expected when the (±)-MN molecule is complexed via rings A and C simultaneously with two different CD molecules. A number of effects were found depending on the nature and position of the substituents.

Position 7

A methyl substituent in position 7 has a minor influence on the separations, whereas a chloro substituent changes the chiral recognition significantly. The enantioseparation of (±)-7-Cl-MN was markedly better than that of (±)-MN when the sulfonated SBE-β-CD and SEE-β-CD

Table 1
Enantioseparations of the (±)-MN and its analogues

Mianserine and analogues with various substituents		20 m*M* β-CD		5 m*M* CM-β-CD		0.1 m*M* SBE-β-CD		0.1 m*M* SEE-β-CD	
		α_{rel}	R_S	α_{rel}	R_S	α_{rel}	R_S	α_{rel}	R_S
(±)-MN		1.08	2.04	1.24	2.98	1.06	0.89	1.04	0.91
7	$-CH_3$	1.06	1.70	1.27	4.27	1.06	1.18	1.08	1.53
	−Cl	1.04	1.20	1.15	4.08	1.16	1.84	1.23	2.78
8	$-CH_3$	1.05	1.45	1.24	3.93	1.19	2.68	1.12	1.67
	$-OCH_3$	1.04	1.40	1.25	6.36	1.16	2.25	1.13	2.25
	−Cl	1.03	0.81	1.15	4.72	1.21	2.04	1.23	1.36
	−F	1.07	1.88	1.24	4.59	1.09	1.30	1.05	0.90
9	$-CH_3$	1.06	1.60	1.22	3.63	1.18	2.00	no separation	
								1.02[a]	1.13[a]
10	*cis*-OH	1.02	0.94	1.05	1.45	no separation		no separation	
						1.02[a]	2.81[a]	1.02[a]	3.33[a]
	trans-OH	1.10	2.57	1.17	3.97	1.02	0.60	1.02	0.74
12	−Cl	1.01	0.20	1.10	2.10	1.08	0.79	1.01	0.15
13	$-CH_3$	1.08	2.07	1.23	4.02	1.08	1.20	1.04	0.99

[a] 0.5 m*M* chiral selector.
Conditions: Beckmann P/ACE; 50 m*M* phosphate buffer, pH 3.0.

were used as chiral selectors. In contrast, the enantioselectivity is substantially worse for (±)-7-Cl-MN in comparison with (±)-MN in the case of β-CD and CM-β-CD as chiral selectors.

Position 8

The effect of the nature of substituents on chiral recognition was studied in more detail for the position 8.

The native β-CD and CM-β-CD behave similarly. The enantioselectivity of the separation for the (±)-MN derivatives decreases in the order $H \geq F > CH_3 \geq OCH_3 > Cl$ for native β-CD and $H \approx F \approx CH_3 \approx OCH_3 > Cl$ for CM-β-CD. Both sulfonated derivatives are almost similar again in this case. The dependence of enantioselectivity on a substituent can be given as follows: $Cl \geq CH_3 > OCH_3 > F > H$ for SBE-β-CD and $Cl \gg OCH_3 \geq CH_3 > F \approx H$ for SEE-β-CD. An absence of correlation between the electron withdrawing ability of substituents on the (±)-MN molecule and their chiral recognition indicates that electron interactions do not play an important role in this case. Rather, the geometric dimension of the substituents should be the more important factor for chiral recognition. Nearly the reverse order of the selectivity dependence on substituents was observed for pairs of the native β-CD and CM-β-CD on the one hand and for SBE-β-CD and SEE-β-CD on the other. This indicates that the structure of the diastereomeric complexes formed in the first two cases is different from that in the latter two selectors.

Position 10

The CH_2 group which bridges both phenyl rings in position 10 seems to be the key position for stereoselective complex binding. Thus, the chiral recognition ability of all CD derivatives under study is markedly lower for (±)-*cis*-10-hydroxy-MN than for (±)-MN. The same substituent in the *trans*-C(10) position also decreases the chiral recognition ability of the sulfonated CDs, but does not alter markedly that of the native and carboxymethyl derivate of β-CD.

Methyl substituents in various positions

The selectivity of the enantioseparation does not strongly depend on the position of the methyl substituent for β-CD and CM-β-CD,

whereas this dependence is very substantial for SBE-β-CD. In particular, the introduction of a methyl substituent in position 7 in ring A or its equivalent position 13 in ring C does not affect the selectivity of the enantioseparation, whereas the selectivity increases drastically by the introduction of the same substituent in position 8 or 9.

As already mentioned above, the dependence of selectivity on the nature and position of substituents is almost similar for the two sulfonated derivatives of β-CD. A substantial difference between these two derivatives was established when the methyl group is substituted in position 8 and, moreover, an even more drastic difference when the same group is substituted in position 9. These two chiral selectors possess the same ionic end group, but they have different lengths of the alkyl spacer and different degrees of substitution. Which one of these two factors is responsible for the considerable variation in chiral recognition abilities in this particular case is the subject of further studies.

Rings A and C

In order to estimate the role of rings A and C of (±)-MN in the complex with CDs, it was interesting to study the effect of the introduction of bulky substituents like chloro groups in the most likely complex positions such as 7, 8, 9, 12, or 13. Both sulfonated CDs, SBE-β-CD and SEE-β-CD, separate the enantiomers of 7- or 8-chloro-substituted (±)-MN derivatives better than the native (±)-MN. The reverse effect is observed again for β-CD and CM-β-CD, which separate the (±)-MN enantiomers with a substantially higher selectivity than any of its monochloro-substituted analogues. As for the Cl-substituent in equivalent positions of rings A and C, it seems worth mentioning that all CD derivatives in this study separate 8-Cl-substituted (±)-MN with a substantially higher enantioselectivity than 12-Cl-substituted (±)-MN.

3.2. pH dependence of the enantioseparation

One of the important advantages of the sulfonated CD derivatives in contrast to the native ones is the presence of a negative charge in the whole pH region acceptable in CE. As a result, SBE-β-CD as a chiral selector shows an optimum stereoselectivity for basic compounds in acidic buffer [12,17]. In contrast to Ref. [14], basic buffers are not necessary for separations. On the other hand, the stereoselectivity for basic compounds decreases drastically in basic medium (Fig. 2). The most likely explanation of this fact is that with increasing pH the electroosmotic flow (EOF) also increases, which is unfavourable for enantioseparation [12,15–17].

The compounds depicted in Fig. 2 are deprotonated at a pH above ca. 7.0–7.5 and behave as neutral compounds. Under these conditions, they can also be separated but with a higher concentration of chiral additives (ca. 0.5 m*M*).

The decrease in the electrostatic selectand–selector attraction could be another reason for the reduction of enantioselectivity in basic medium. This effect seems to be of much less importance because all anionic CD-derivatives studied are able to enantioselectively recognize not only positively charged and neutral, but also negatively charged chiral analytes [18].

3.3. ^{13}C NMR study of the chiral recognition mechanism

As already mentioned above, our aim was to test whether or not any remarkable differences exist in the binding pattern and stereoselectivity of the various CD derivatives towards the en-

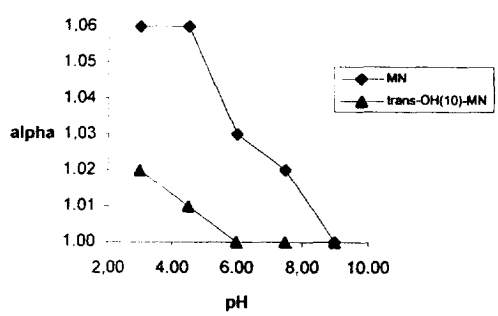

Fig. 2. pH dependence of the enantioselectivity of the separation. Conditions: Beckmann P/ACE, 50 m*M* phosphate buffer, 0.1 m*M* SBE-β-CD.

antiomers of (±)-MN. On the basis of the migration time measurements in CE, Stobaugh and co-workers [15] considered it unlikely that a large increase in binding constant for SBE-β-CD for (±)-ephedrine, as compared with those for other CDs, is responsible for the comparable separation of the (±)-ephedrine enantiomers using SBE-β-CD in 10–13 times smaller concentrations than native β-CD and (2,6-dimethyl)-β-CD. Here it seems worth mentioning that since the binding of the racemic solute with a chiral selector is a prerequisite for enantioseparation, no directly proportional dependence may necessarily exist between the binding strength and the stereoselectivity. A number of examples are known in high-performance liquid chromatography (HPLC) [25,26] and CE [27] in this respect. A factor of rather more importance in this case is certainly the difference between binding strengths of the enantiomers, although stronger binding does not always mean a higher binding-strength difference between the enantiomers.

The complexation-induced chemical shifts of selected CD protons (up-field) as well as of some protons of the (±)-MN molecule (down-field) were observed in our experiments using ^{1}H NMR spectroscopy. However, in 200 MHz spectra of (±)-MN–CD complexes, the quantitative assessment of the concentration dependence of the chemical shifts, either of CDs or (±)-MN protons, was impossible due to the overlapping of the selector and selectand signals. No ^{1}H NMR signal splitting was observed for any of the (±)-MN proton signals upon addition of any CD derivative in this study in the range of a host/guest ratio of 0.5–2.5. Therefore, the calculation of the stoichiometry of the selectand–selector complexes formed was based on ^{13}C NMR data. This technique is more and more used for the measurement of enantiomeric excess [28]. Although ^{13}C NMR experiments are more time-consuming, they have some advantages, such as less broadening of the spectral lines, broader spectral width and, as a result, better resolution and easier assignment of signals.

Since CDs are widely used as chiral shift reagents in ^{1}H NMR spectroscopy [29,30], only few data involving ^{13}C NMR have been published. The use of complexation-induced ^{13}C NMR chemical shifts for measurement of binding constants of the nonchiral [31] or optically pure but nonracemic [32] compounds has been also reported.

^{13}C NMR spectra of (±)-mianserine and SBE-β-CD

The ^{13}C NMR spectra of (±)-MN and equimolar mixtures (8.5 m*M* each) of SBE-β-CD/(±)-MN, SBE-β-CD/(−)-MN, and SBE-β-CD/(+)-MN are given in Fig. 3. As shown in these spectra, a number of ^{13}C NMR signals of (±)-MN are split as a result of nonequivalence of complexation-induced chemical shifts of (+)- and (−)-MN with SBE-β-CD. No splitting of signals was observed in ^{13}C NMR spectra of

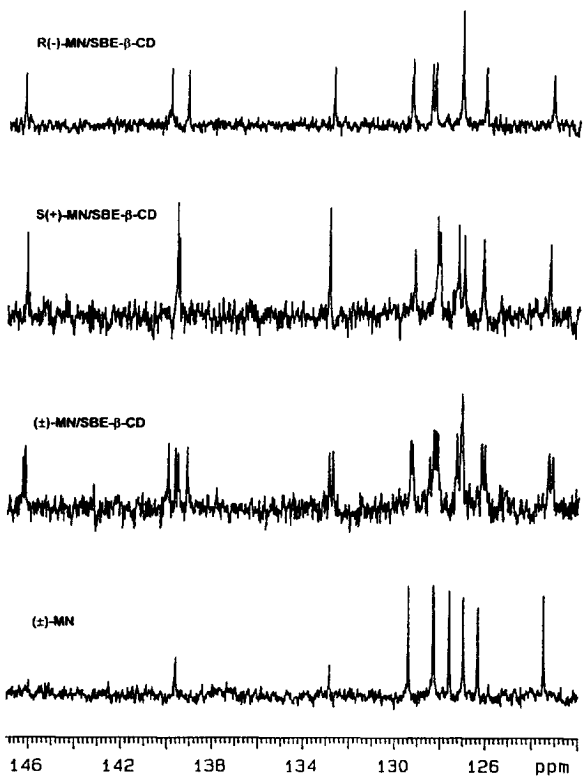

Fig. 3. ^{13}C NMR spectra of (±)-MN and equimolar mixtures (8.5 m*M* each) of (±)-MN/SBE-β-CD, *S*-(+)-MN/SBE-β-CD and *R*-(−)-MN/SBE-β-CD in $^{2}H_2O$.

SBE-β-CD complexes with either (+)- or (−)-MN. This result means that the exchange between complexed and free substance is fast on the NMR time scale, and signal splitting occurs only as a result of enantiomeric composition. Among the aliphatic carbon signals of the (±)-MN molecule, only the carbon signal in position 10 is clearly split, whereas most signals in the aromatic region are duplicated. The split ^{13}C-signal of the aliphatic carbon (position 10) is shifted up-field, whereas both up-field [C(6), C(8), C(9)] as well as down-field shifts [C(6^a)] were observed for the aromatic region.

It is also noteworthy that ^{13}C-signals for *R*-(−)-MN are shifted more than those for *S*-(+)-MN, which is in good agreement with the migration behavior of this species in CE (migration order *S*-(+)-MN in front of *R*-(−)-MN, see Fig. 5c).

Stoichiometry of the (±)-MN complexes with CDs

Another important advantage of the use of ^{13}C NMR in this case is the clear splitting of a number of (±)-MN signals. This enables the use of the racemic mianserine for a study of enantiospecific binding parameters and the stoichiometry of the selector–selectand complexes. The study of the competitive binding of enantiomers to the chiral selector is also made possible.

For a more detailed characterization of the above-mentioned (±)-MN–CD complexes, an attempt was made to determine the stoichiometry of the complexes of (±)-MN with these chiral selectors. This was done by plotting the product of complexation-induced chemical shifts of C(6) carbon atom by molar fraction of (±)-MN versus molar fraction of (±)-MN in solutions of (±)-MN/CD mixtures. According to the theory of the Job plot, this dependence should reach a maximum for stoichiometric selectand–selector mixtures.

The Job plots of the (±)-MN complexes with various CDs given in Fig. 4 show that 1:1 guest–host complexes predominate in all cases. Thus, a different stoichiometry of the selector–selectand complexes does not seem to be the reason for the variation in chiral recognition ability of the chiral selectors under study. Different stoichiometries of naproxen [32] as well as of binaphthyl derivatives [33] with various CDs have been reported recently.

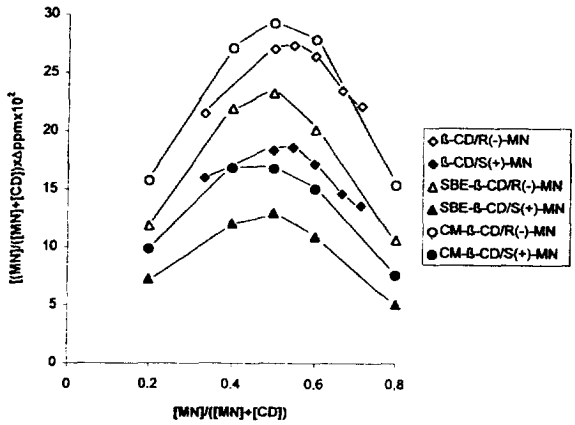

Fig. 4. Job plots for (±)-MN complexes with various CDs.

The complexation-induced chemical shift differences of (±)-MN with various CDs

The complexation-induced chemical shift differences between enantiotopic signals were found to be strongly dependent on the type of chiral selector, which means qualitatively that in addition to mobility, these CD derivatives differ also in terms of the enantiospecific binding. As an illustrative example of the correlation between ^{13}C NMR spectra and CE results, the electropherograms of the enantioseparation of racemic (±)-MN using various CD chiral selectors, each in a comparable concentration of 0.06 m*M*, are depicted parallel to the fragments of the ^{13}C NMR spectra of the equimolar mixtures of the same species in Fig. 5. A correlation between the separation selectivity and complexation-induced chemical shift differences is obvious. It should be noted that only the difference in binding enantioselectivity with (±)-MN is responsible for the marked variation in efficiencies of CM-β-CD and SBE-β-CD, as no substantial difference exists between the mobilities of these two chiral selectors under the same conditions [18]. Thus, one can conclude that the

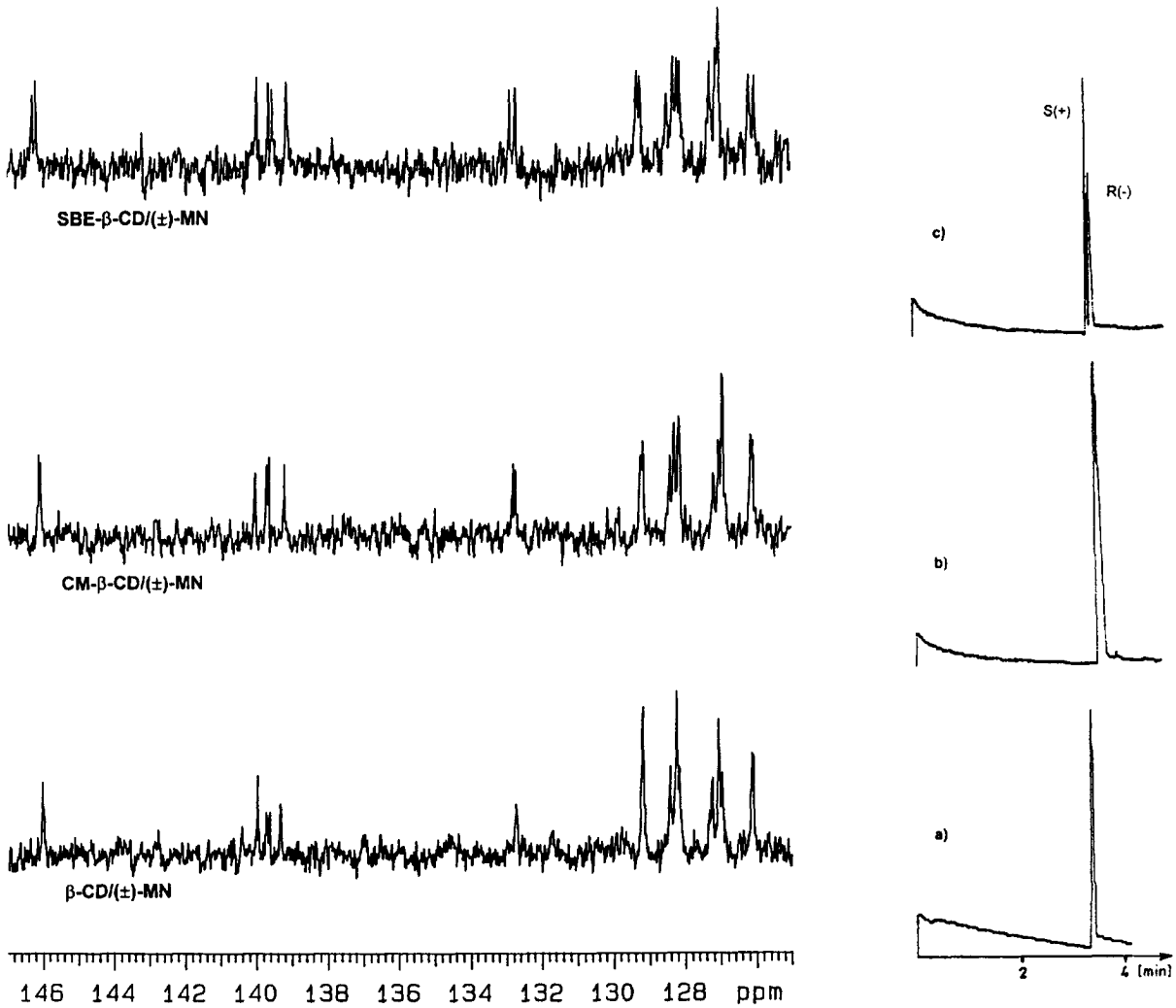

Fig. 5. Correlation between selectivity of the enantioseparation (α) and complexation-induced chemical shift differences of the enantiotopic signals of the (±)-MN molecule. CE conditions: GROM, 50 m*M* phosphate buffer, pH 6.0: (a) 0.06 m*M* β-CD, (b) 0.06 m*M* CM-β-CD and (c) 0.06 m*M* SBE-β-CD. ^{13}C NMR spectra were taken in equimolar (8.5 m*M* each) solutions of CDs and (±)-MN in $^{2}H_2O$. TMS was used as the external standard.

counter-current mobility of anionic CD-derivatives is one of their important advantages [12–17]. Further, the higher stereoselectivity of binding of the racemic compounds in comparison with native and other nonionic CD-derivatives, proved on the basis of direct ^{13}C NMR measurements, can also contribute to the higher separation efficiency of charged CD-derivatives.

Acknowledgements

The authors thank Heinrich-Hertz-Stiftung for a stipend (B.Ch.), the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie for financial support, Dr. F.A. van der Vlugt (Organon, Netherlands) for samples of mianserine and analogues, Prof. J.F. Stobaugh and

Prof. V.J. Stella, University of Kansas (Lawrence, KS, USA), for a sample of SBE-β-CD, and Wacker Chemie (Munich, Germany) for a sample of SEE-β-CD and CM-β-CD.

References

[1] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295.
[2] H. Engelhardt, W. Beck and Th. Schmitt, Kapillarelektrophorese, Methoden und Möglichkeiten, Vierfweg, 1994, p. 134.
[3] S. Fanali, J. Chromatogr., 474 (1989) 441.
[4] S. Fanali and P. Bocek, Electrophoresis, 11 (1990) 757.
[5] M. Heuermann and G. Blaschke, J. Chromatogr., 648 (1993) 505.
[6] Th. Schmitt and H. Engelhardt, J. High Resolut. Chromatogr. Chromatogr. Commun., 16 (1993) 525.
[7] S. Terabe, Trends Anal. Chem., 8 (1989) 129.
[8] A. Nardi, A. Eliseev, P. Bocek and S. Fanali, J. Chromatogr., 638 (1993) 247.
[9] B. Pahlen and J.F. Stobaugh, Fourth International Symposium on Pharmaceutical and Biomedical Analysis, April 18–21, 1993, Baltimore, MD, USA, Poster TP-E-12.
[10] N.W. Smith, J. Chromatogr. A, 652 (1993) 259.
[11] Th. Schmitt and H. Engelhardt, Chromatographia, 37 (1993) 475.
[12] B. Chankvetadze, G. Endresz and G. Blaschke, Electrophoresis, 15 (1994) 804.
[13] S. Mayer and V. Schurig, Electrophoresis, 15 (1994) 835.
[14] C. Dette, S. Ebel and S. Terabe, Electrophoresis, 15 (1994) 799.
[15] R.J. Tait, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013.
[16] I.S. Lurie, R. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen and R.E. Weinberger, Anal. Chem., 66 (1994) 4019.
[17] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr. A, 700 (1995) 43.
[18] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr. A, 704 (1995) 234.
[19] B. Chankvetadze, G. Endresz and G. Blaschke, unpublished results.
[20] P.V. Dermarco and A.W. Thakkar, J. Chem. Soc. Chem. Commun., (1970) 2.
[21] J.A. Ripmeester and A. Majid, in O. Huber and J. Szejitli (Editors), Proceedings of the fourth International Symposium on Cyclodextrins, 1988, Kluwer Academic Publisher, Dordrecht, p. 165.
[22] P. Job, Ann. Chem., 9 (1928) 113.
[23] M. Cotta Ramusino and S. Pichini, Carbohydr. Res., 259 (1994) 13.
[24] C. van Rij and D. Feil, Tetrahedron, 29 (1973) 1891.
[25] D.T. Witte, J.P. Franke, F.J. Bruggeman, D. Dijksta and R.A. De Jeeuw, Chirality, 4 (1992) 389.
[26] B. Chankvetadze, E. Yashima and Y. Okamoto, J. Chromatogr. A, 670 (1994) 39.
[27] K.-H. Gahm and A.M. Stalcup, Anal. Chem., 67 (1995) 19.
[28] J.W. Jaroszewski and A. Olsson, J. Pharm. Biomed. Anal., 12 (1994) 295.
[29] D.D. MacNicol and D.S. Rycroft, Tetrahedron Lett., (1977) 2173.
[30] A.F. Casy, Trends Anal. Chem., 12 (1993) 185.
[31] J.P. Behr and J.M. Lehn, J. Am. Chem. Soc., 98 (1976) 1743.
[32] G. Bettinetti, F. Melani, P. Mura, R. Monnanni and F. Giordano, J. Pharm. Sci., 80 (1991) 1162.
[33] K. Kano, Y. Tamiya, C. Otsuki, T. Shimomura, T. Ohno, O. Hayashida and Y. Murakami, Supramol. Chem., 2 (1993) 137.