CHIRALITY 11:635–644 (1999)

Chiral Recognition of Verapamil by Cyclodextrins Studied With Capillary Electrophoresis, NMR Spectroscopy, and Electrospray Ionization Mass Spectrometry

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ABSTRACT Capillary electrophoresis (CE) allows the observation of the opposite affinities of the enantiomers of (±)-verapamil [2-isopropyl-2,8-bis(3,4-dimethoxyphenyl)-6-methyl-6-azaoctannitrile, VP] toward β-cyclodextrin (β-CD) and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD). In addition, in the presence of β-CD in the background electrolyte, longer migration times and lower separation factors were observed compared to TM-β-CD. The binding constants of (+)- and (–)-VP with β-CD and TM-β-CD determined using ^{13}C NMR spectroscopy explain the results observed in CE. Electrospray ionization mass spectrometry (ESI-MS) was used as an alternative technique for the characterization of VP-CD complexes. *Chirality 11:635–644, 1999.*

KEY WORDS: chiral CE; NMR spectroscopy; ESI-MS; cyclodextrins; (±)-verapamil/cyclodextrin complexes; stoichiometry; binding constants

Capillary electrophoresis (CE) is rapidly being established as one of the major techniques for analytical-scale enantioseparations.[1] The most important advantage of this technique is the high peak efficiency that allows the detection of very weak intermolecular solute/selector interactions or very low stereoselectivities in these interactions. Further advantages of CE include small amounts of samples, selectors and buffers, short analysis times, flexibility, low costs, etc. The immobilization of the selector is also not required in CE. On the other hand, CE does not provide direct information on the molecular mechanisms of chiral recognition. Other instrumental techniques, such as nuclear magnetic resonance (NMR) spectroscopy, mass-spectrometry (MS), and X-ray crystallography may complement CE well from this viewpoint.

Cyclodextrins (CD) are the most widely used chiral selectors in CE.[1–4] Despite many efforts,[5–7] the study of their chiral recognition mechanisms still remains challenging.[8,9] One of the key issues in understanding how CDs work stereoselectively is the enantiomer recognition pattern. It is known that the affinity of the enantiomers of some chiral compounds is opposite toward differently modified CDs.[10] For instance, D-alanine naphthylamide is preferentially complexed with native β-CD whereas the L-enantiomer is more strongly bound by heptakis(2,3-diacetyl)-β-CD.[11] β-CD prefers D-oxamniquine as a guest, whereas the L-enantiomer is preferentially bound by randomly substituted 2-hydroxypropyl-β-CD.[12] Further examples are described in the literature.[13–15] It is difficult to predict how a given chemical modification affects the multiple forces involved in the intermolecular interactions between CDs and their guests. This knowledge may contribute to the understanding of the nature of the major forces contributing to solute/CD binding and those responsible for stereoselectivity.

Chiral recognition of the enantiomers of the calcium channel blocker verapamil (VP, 2-isopropyl-2,8-bis(3,4-dimethoxyphenyl)-6-methyl-6-azaoctannitrile) by various cyclodextrins was studied in this work using CE, NMR spectroscopy, and ESI-MS spectrometry.

MATERIALS AND METHODS

Materials

Racemic VP (Fig. 1) was obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany). The enantiomers of VP were obtained by diastereomeric crystallization with optically pure (+)- or (–)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate as described in Ref. 19. α-, β-, and γ-CD, carboxymethyl-β-CDs (CM-β-CD) with average molecular substitution degrees (DS) of 2.1 and 3.5, succinyl-β-CD (SUC-β-CD) with a DS of 3.5, sulfoethyl-β-CD (SEE-β-CD) with a DS of 2.8, and methyl-β-CDs (ME-β-CD) with a DS of 4.2 and 12.6 were a gift from Wacker-Chemie (Munich, Germany). Heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), 2-hydroxypropyl-β-CD (HP-β-CD) with a DS of 4.2, and 2-hydroxy-

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Received for publication 20 October 1998; Accepted 22 February 1999

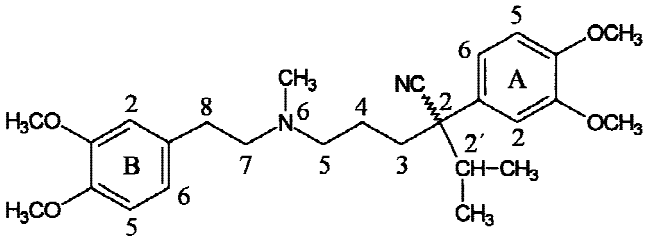

Fig. 1. Structure of verapamil (VP).

propyl-γ-CD (HP-γ-CD) with a DS of 4.8 were from Fluka (Buchs, Switzerland). β-CD-sulfate (SU-β-CD) DS of 7–11, was from Aldrich (Steinheim, Germany), and sulfobutyl-β-CDs (SBE-β-CD) with DS 4.0 and 7.0 were from CyDex, LC (Kansas). Schematic representation of all the CDs used in this study are shown in Fig. 2. Analytical grade ^2H_2O, NaH_2PO_4, H_3PO_4, NaOH, HCl, and triethanolamine were purchased from Merck (Darmstadt, Germany).

Methods

CE separations were performed using a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA) equipped with a diode array detector. The samples (0.1 mg/mL in the background electrolyte) were injected by a pressure of 0.5 psi for 3 s. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ) with 50 μm ID, 31.2 cm total length, and 21 cm effective length was used. Other experimental conditions are given in the figure legends. To increase the solubility of β-CD, urea (2 M) was added to the buffer solutions.

^1H NMR, ^{13}C NMR, homonuclear correlated spectroscopy (HOMCOR), heteronuclear chemical shift correlation (HETCOR), and distortionless enhancement polarization transfer (DEPT) spectral analyses used for the signal assignments of VP were performed using 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 stoichiometry of the VP-CD complexes was determined by the continuous variation method[1,14–16] based on the ^{13}C NMR chemical shifts. The same signal were used for the calculation of the binding constants according to Scott's method.[1,14,15,17,18]

Electrospray ionization mass spectra (ESI-MS) of α-CD, β-CD, TM-β-CD, and their complexes with (±)-VP were obtained using an ion trap mass spectrometer (LCQ, Finnigan, Branford, CT) equipped with an electrospray interface. The aqueous solutions of 0.6 mg/mL (±)-VP, an equimolar amount of the CDs, and a solution of their 1:1 mixture were introduced into the ion source of the mass spectrometer at a flow rate of 5 μL/min using a syringe pump. The ionization voltage was 6 kV for the sample of (±)-VP and CD and 4 kV for their mixture. The temperature of the inlet capillary was 200°C. Detection was performed in the positive ion mode.

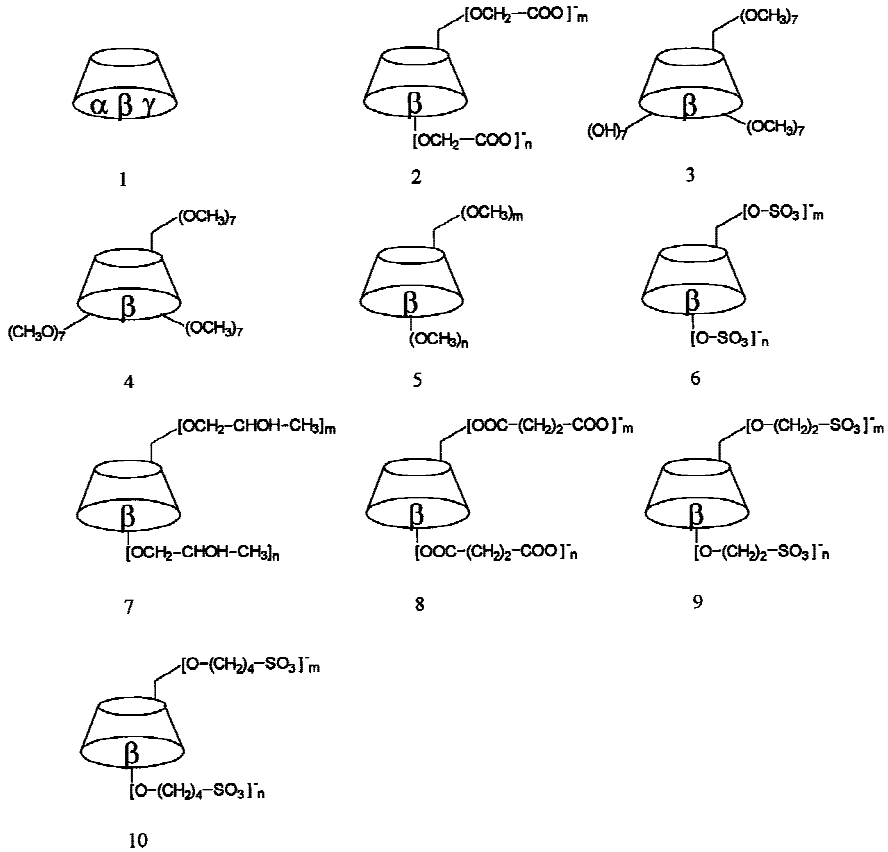

Fig. 2. Structure of CDs: (1) α-, β-, γ-CD; (2) CM-β-CD; (3) DM-β-CD; (4) TM-β-CD; (5) ME-β-CD; (6) β-CD sulfate; (7) HP-β-CD; (8) succinyl-β-CD; (9) SEE-β-CD; (10) SBE-β-CD.

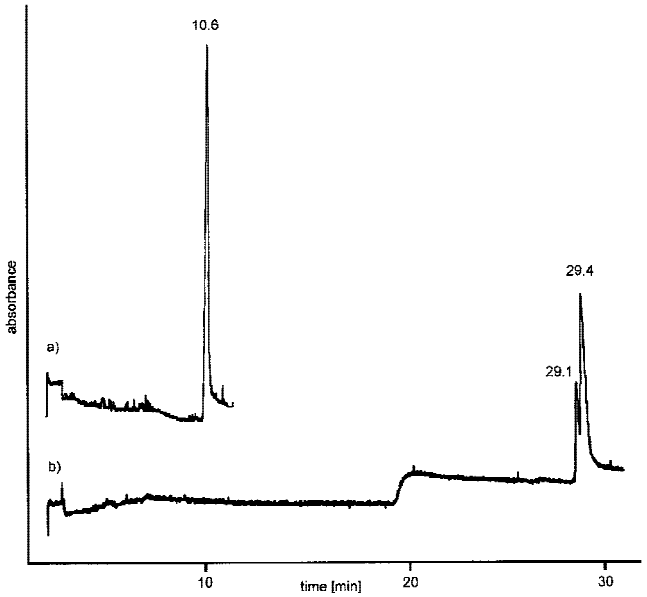

Fig. 3. Electropherogram of the mixture of the enantiomers of VP [(+)/(–) = 2/1] in the presence of 18 mg/mL of β-CD in 50 mM sodium dihydrogenphosphate (pH = 3.0) (a) and in 100 mM triethanolamine phosphate (pH = 3.0) (b) buffers. Capillary: fused-silica (50 μm ID × 60 cm long, 43 cm to the detector). Applied voltage: 24 kV.

RESULTS AND DISCUSSION

CE

Enantioseparation of (±)-VP in CE has been the subject of several previous studies.[20–37] However, the migration order of the enantiomers is addressed only in few of them. Ohara et al.[23] determined the migration order with TM-β-CD to be *R*-(+)- before *S*-(–)-VP. Clothier and Tomellini reported the migration order with chiral surfactant sodium deoxycholate to be (–)-VP before (+)-VP.[24] The enantioseparation of (±)-VP with native CDs was impossible in most of buffer systems used.[20,30] However, Bechet et al.[21] achieved a partial enantioseparation of (±)-VP in a phosphoric acid–triethanolamine buffer at pH 3.0 in the presence of native β-CD. Our preliminary experiment confirmed the advantage of this buffer compared to sodium dihydrogenphosphate for the enantioseparation of (±)-VP (Fig. 3).

The separation of (±)-VP using various CDs are summarized in Table 1. The affinity of the enanatiomers of VP varies strongly from CD to CD. In addition, charged CD derivatives possess a self-electrophoretic mobility that changes depending on the type of the ionic groups. Therefore, it was impossible to examine all CDs in this study at the same or similar concentrations. The concentrations given in Table 1 resulted in the optimal enantioseparation with the particular CD under the separation conditions of this study. Among the native CDs, enantioseparation was only observed with β-CD. Only a slight increase of the migration time was observed in the presence of α-CD compared to selector-free buffer. The increased viscosity of the buffer containing α-CD might be responsible for this minor effect. Longer migration times were observed in the presence of γ-CD. Thus, (±)-VP may have a marked affinity towards γ-CD. However, even if this is the case, the intermolecular interactions between (±)-VP and γ-CD lack stereoselectivity.

The most significant differences were observed between the chiral recognition properties of native β-CD and its permethylated analog, heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) (Fig. 4). The addition of equal amounts (w/v) of these two CDs to the background electrolyte resulted in significantly longer migration times but less resolved peaks of (±)-VP using β-CD as compared to TM-β-CD. Moreover, the migration order of the enantiomers were opposite to each other when used β-CD and TM-β-CD as chiral selectors. Among the other CDs studied, most of the charged β-CD derivatives afforded moderate enantioseparations of (±)-VP with the same migration order of the enantiomers as native β-CD. It was impossible to achieve enantioseparations of (±)-VP at moderate concentrations of randomly substituted neutral β-CD derivatives. Heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) gave very poor enantioseparation. Quite surprisingly, the migration order of the VP enantiomers in the last case was the same as with native β-CD.

NMR Spectroscopy

(±)-VP possesses two substituted phenyl moieties that may serve as the alternative binding sites with the CDs. Therefore, complexes may be formed having a different stoichiometry and structure between (±)-VP and the CDs. The goal of the NMR studies was to investigate the stoichiometry and binding constants of (+)- and (–)-VP complexes with β-CD and TM-β-CD, in order to provide a plausible explanation for the markedly different affinity of the enantiomers of VP toward these two CDs.

^{13}C NMR spectra of a nonracemic mixture [(+)/(–) = 1/2] of VP enantiomers in the absence and in the presence of β-CD (molar ratio VP/β-CD = 2/1) and TM-β-CD (molar ratio VP/TM-β-CD = 2/1) are shown in Fig. 5. The ratio of all splitted signal intensities (approximately 1:2) definitely indicated that this effect was caused by the nonequivalence of the complexation-induced chemical shifts (CICS) for the enantiomers. This was confirmed also by ^{13}C NMR experiments on the pure enantiomers under identical conditions. The different recognition patterns of (±)-VP by these CDs is evident from the spectra. For instance, the ^{1}H resonance signals (data not shown) of CH_3 of the isopropyl groups are split in the presence of TM-β-CD but not in the presence of native β-CD. Remarkable differences were observed also in the ^{13}C NMR spectra. In the presence of β-CD, the ^{13}C resonance signals at 17.41 ppm (CH_3 group of the isopropyl residue) and 36.69 ppm (C-3) were split. In the case of TM-β-CD the ^{13}C resonance signals at 17.41, 17.97, 109.57, 119.87, 122.54 (C≡N), and 149.19 ppm were split. Chemical shifts were observed upfield as well as downfield in the presence of both CDs. The chemical shifts were always larger with β-CD. However, CICS differences between the enantiomers were always higher in the case of TM-β-CD. This is a first indication for a stronger but less stereoselective interaction of (+)- and (–)-VP with β-CD compared to TM-β-CD.

Another interesting effect observed in the NMR spectra

TABLE 1. Migration times and the migration order of (+)- and (–)-VP in presence of CDs

Cyclodextrin	Concentration (mg/mL)	Migration time (min)		Migration order
		t_1	t_2	
Without CD		12.01	12.01	–
SU-β-CD	1	12.09	12.25	(–) before (+)
SEE-β-CD	0.8	15.30	15.56	(–) before (+)
SBE-β-CD (4.0)[a]	1	15.90	15.90	–
SBE-β-CD (7.0)	0.2	12.48	12.48	–
SUC-β-CD	2	15.02	15.39	(–) before (+)
CM-β-CD (3.5)	0.3	14.08	14.30	(–) before (+)
CM-β-CD (2.1)	1	16.15	16.77	(–) before (+)
β-CD	36	22.51	22.85	(–) before (+)
ME-β-CD (4.2)	36	32.37	32.37	–
ME-β-CD (12.6)	36	28.97	28.97	–
DM-β-CD	18	28.80	29.04	(–) before (+)
TM-β-CD	36	13.30	13.70	(+) before (–)
α-CD	18	13.60	13.60	–
	50	15.58	15.58	–
γ-CD	18	14.99	14.99	–
	50	21.96	21.96	–
	100	26.98	26.98	–
HP-β-CD	18	24.52	24.52	–
	60	29.59	29.59	–
HP-γ-CD	18	14.41	14.41	–
	36	16.61	16.61	–

[a]Average degree of substitution is shown in the parentheses.

was that in the presence of β-CD the CICSs were more pronounced for the signals of (+)-VP, whereas the opposite was observed in the presence of TM-β-CD. CICS may serve as a preliminary indication for the binding pattern of enantiomers. Thus, β-CD and TM-β-CD may possess opposite recognition patterns toward the enantiomers of VP. However, as shown in our previous studies[14,38] CICS may not always provide reliable information on the binding priority of the enantiomers. Therefore, the stoichiometry and the binding constants of the intermolecular complexes were determined using Job's[1,14,15,16,38] and Scott's[1,14,15,17,18,38] techniques, respectively. Multiple sets of the data were used in both cases.

In the case of β-CD (Fig. 6) for both enantiomers of VP and for all three ^{13}C resonance signals used (17.41, 17.97, and 36.69 ppm), clear maxima were observed at a [VP]/[VP]+[CD] ratio equal to 0.5. This unambiguously indicates that this complex has 1:1 stoichiometry.

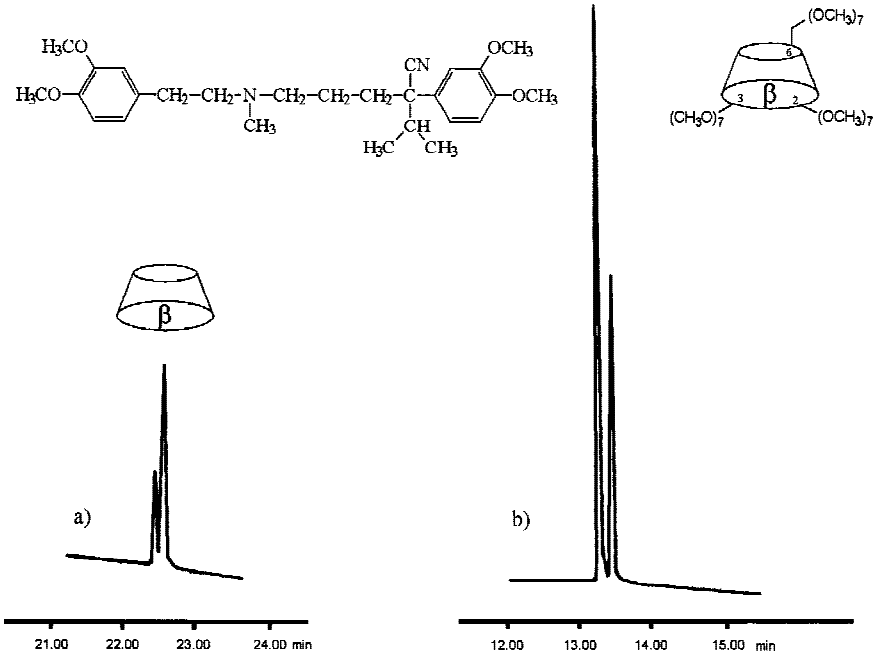

Fig. 4. Electropherogram of the mixture of VP enantiomers [(+)/(–) = 2/1] in the presence of 36 mg/mL of β-CD (a) and 36 mg/mL TM-β-CD (b). In the case of β-CD 2M urea was added to the buffer. Capillary: fused-silica (50 μm × 31.2 cm long, 21 cm to the detector). Applied voltage; 20 kV. Buffer: 100 mM triethanolamine phosphate (pH = 3.0).

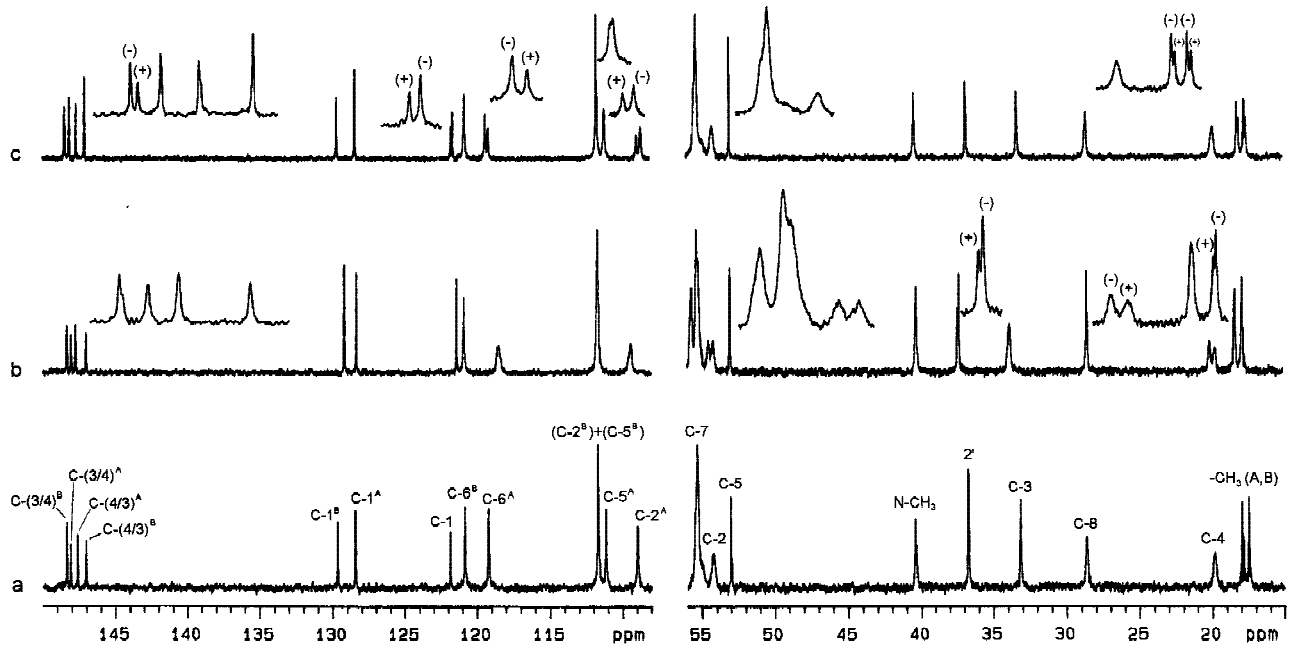

Fig. 5. ^{13}C NMR spectra of the mixture of VP enantiomers [(+)/(−) = 1/2] in the absence (a) and in the presence of β-CD (b) and TM-β-CD (c), in a VP/CD ratio of 2/1. Only parts of the spectra are depicted.

Job's plots constructed for the (±)-VP/TM-β-CD complex (Fig. 7) based on the resonance signals at 17.41 and 17.97 ppm also showed that the transient diastereomeric complexes have predominantly 1:1 stoichiometry. However, for two other split resonance signals with TM-β-CD, a scattered pattern (data not shown) was obtained which did not allow us to construct a Job's plot. In addition, downfield as well as upfield shifts of these resonance signals

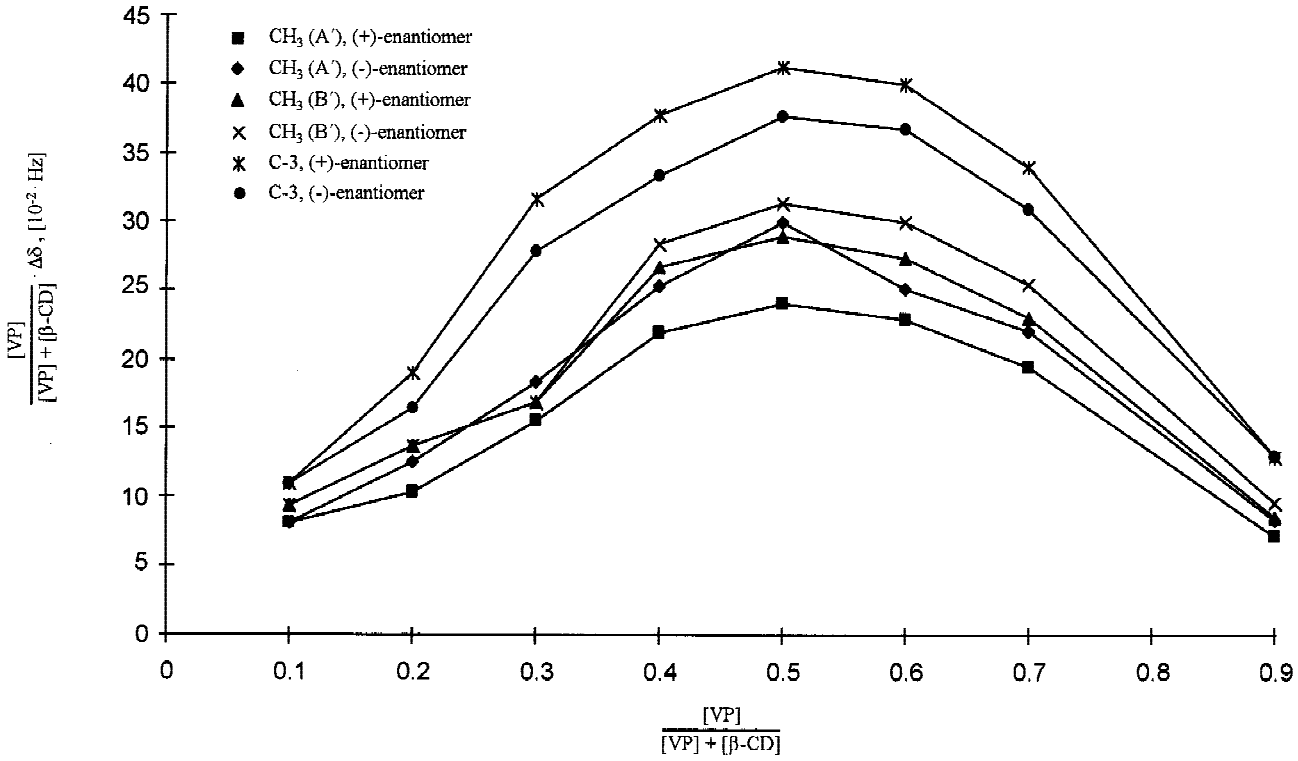

Fig. 6. Job's plots for (±)-VP/β-CD complex.

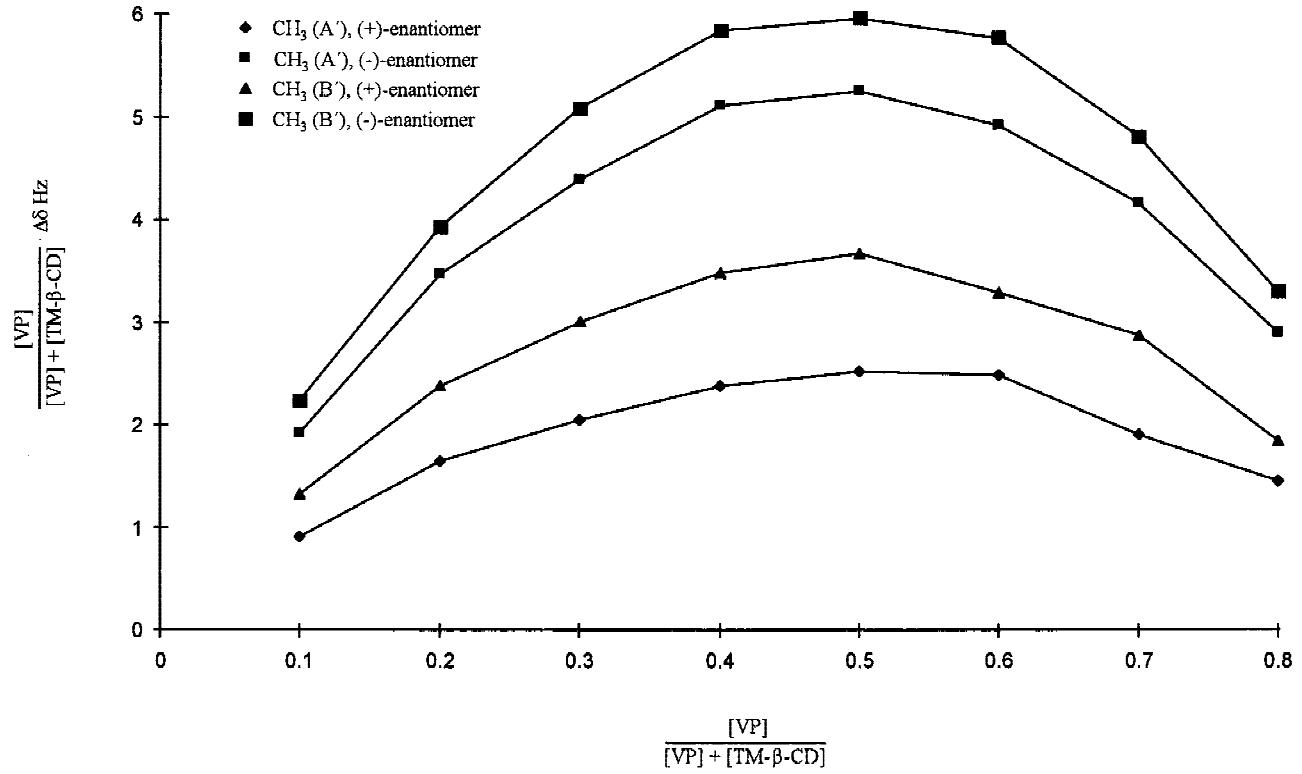

Fig. 7. Job's plots for (±)-VP/TM-β-CD complex.

were observed, depending on the ratio of the components in the mixture. ESI-MS was used (see data below) in order to confirm the stoichiometry of the complexes given by NMR.

Scott's plots constructed based on the same ^{13}C-NMR signals obtained in another set of experiments were linear for both β-CD and TM-β-CD (Figs. 8 and 9). This, on the one hand, further supports the 1:1 stoichiometry of the

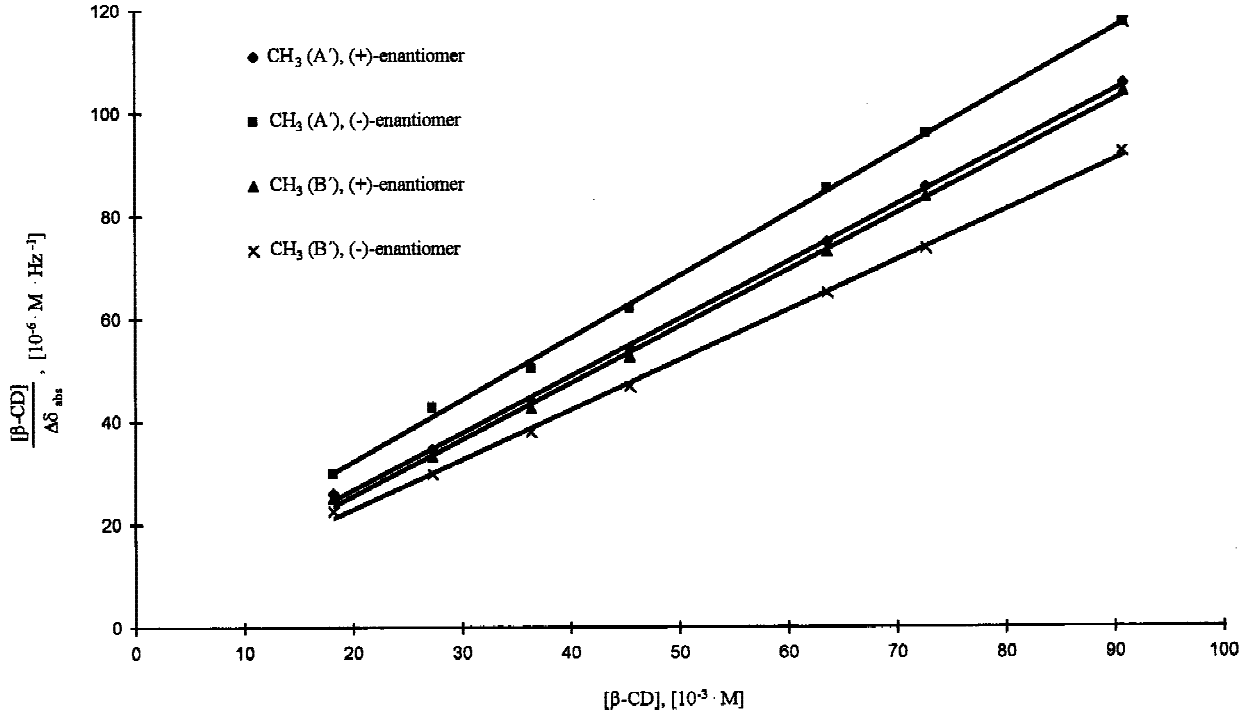

Fig. 8. Scott's plots for (±)-VP/β-CD complex.

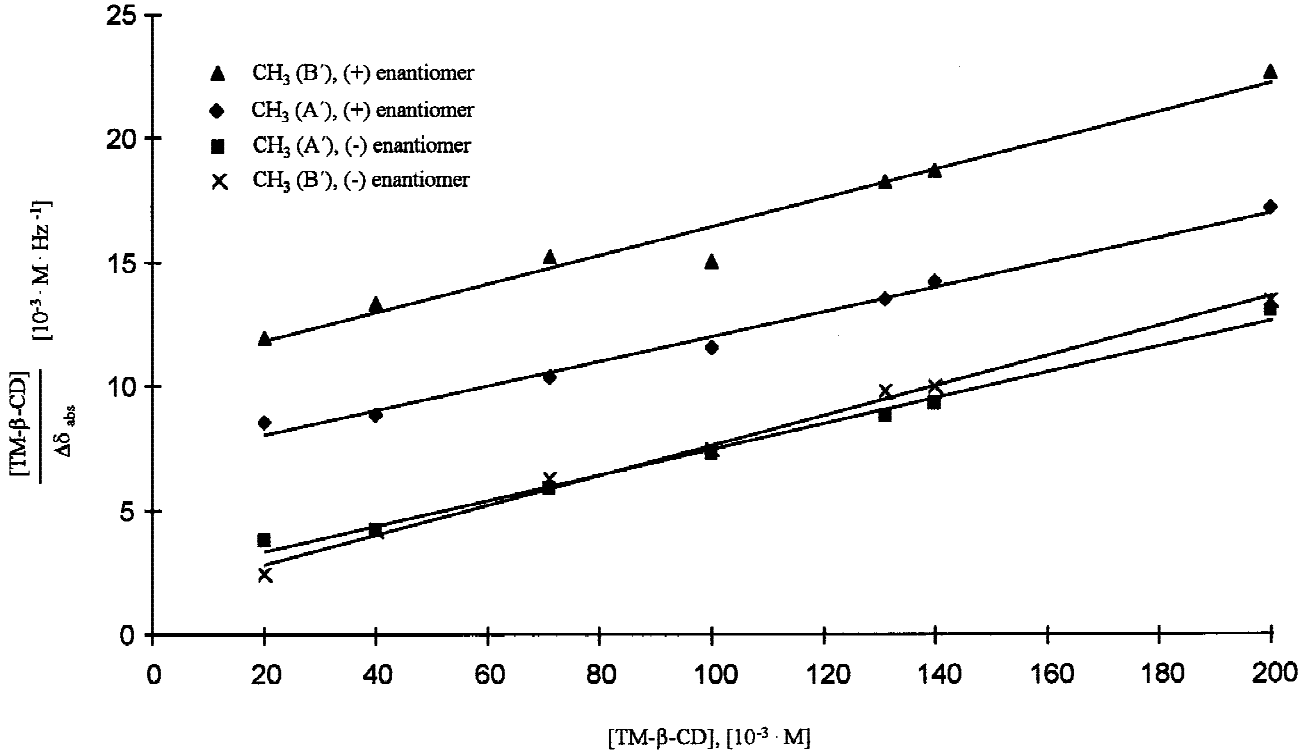

Fig. 9. Scott's plots for (±)-VP/TM-β-CD complex.

complexes mentioned above and on the other hand, allows to calculate the averaged apparent binding constants of (+)- and (–)-VP to β-CD and TM-β-CD. These data are summarized in Table 2. The binding data correlate well with the results observed in CE. Thus, the longer migration time of (±)-VP in the presence of β-CD compared to TM-β-CD (Fig. 4) may be attributed to the relatively high affinity of (±)-VP to β-CD. In contrast to the high affinity towards β-CD, the enantiomers of VP are better resolved with TM-β-CD. The reason for this is the significantly higher binding selectivity of (+)- and (–)-VP to TM-β-CD compared to β-CD (α_{bind} = 5.0 in the case of TM-β-CD compared to 1.3 in the case of β-CD). Thus, the low affinity of (±)-VP toward TM-β-CD is very well counteracted by a high binding selectivity that makes TM-β-CD the better chiral selector for the separation of the enantiomers of VP. The reason for the opposite migration order of the enantiomers of VP in the presence of β-CD and TM-β-CD is the opposite affinity of (+)- and (–)-VP toward these CDs (Table 2).

ESI-MS

Several recent papers have shown that fast atom bombardment mass spectrometry (FAB-MS) and ESI-MS may be used to study the stoichiometry and relative binding strengths in noncovalent complexes.[15,39–44] MS techniques provide m/z ratios and thus direct information about the stoichiometry of the complex based on a single experiment. In addition, MS techniques are faster and less expensive than laborious NMR measurements. The most important advantage of MS is that it detects complexes with different stoichiometries which is difficult using NMR spectroscopy. As mentioned above, not all resonance signals in the NMR spectra provided unambiguous data on the formation of 1:1 complexes of VP with TM-β-CD.

TABLE 2. Complexation-induced chemical shift (CICS) differences at saturation ($\Delta\delta_c$) and the apparent binding constants (K_a) of (±)-VP with β-CD and TM-β-CD

	Chemical shift at saturation $\Delta\delta_s$, Hz		Apparent binding constants K_a, M^{-1}		$K_a \pm \Delta K_a$, M^{-1}		
Cyclodextrin	(+)-VP	(–)-VP	(+)-VP	(–)-VP	(+)	(–)	α^a
β-CD	0.903	0.830	238	148	272 ± 34	207 ± 59	1.30
	0.911	1.033	306	266			
TM-β-CD	20.2	19.3	7	23	6 ± 1	30 ± 7	5.00
	17.3	16.6	5	37			

[a] α was determined as the ratio of the higher apparent binding constant and the lower constant.

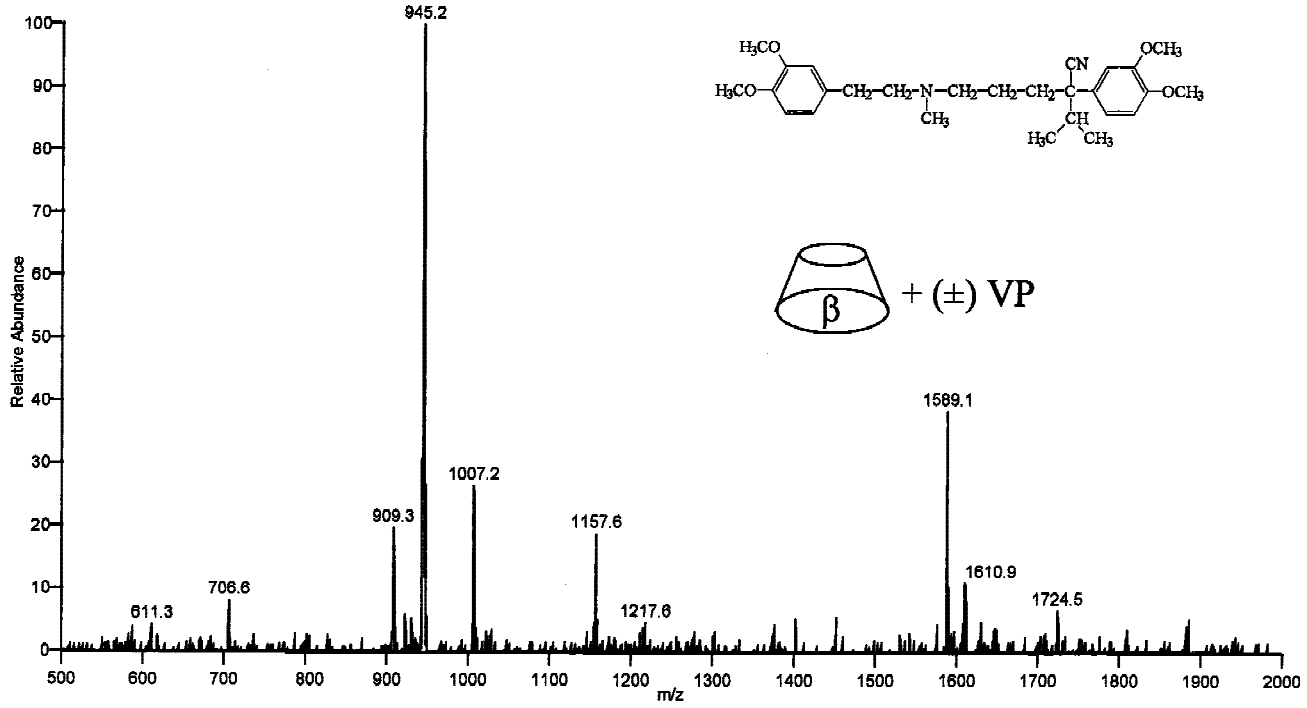

Fig. 10. ESI-MS spectra of 5.0 mg/mL solution of β-CD and an equimolar amount of (±)-VP in double-distilled water.

Therefore, ESI-MS was used as an alternative technique in order to obtain an independent information on the stoichiometry of these complexes.

Besides the ion corresponding to the sodium adduct of native β-CD (*m/z* 1157.6), an ion with *m/z* 1589.1 was observed in the ESI-MS spectra of equimolar mixtures of (±)-VP and β-CD (Fig. 10). This corresponds to the 1:1 complex of (±)-VP and β-CD.

In the ESI-MS spectra of equimolar mixtures of (±)-VP and TM-β-CD (Fig. 11), the ion with *m/z* 1883.6 was detected with a relatively high abundance besides the ions corresponding to the ammonium (*m/z* 1446.7, minor peak)

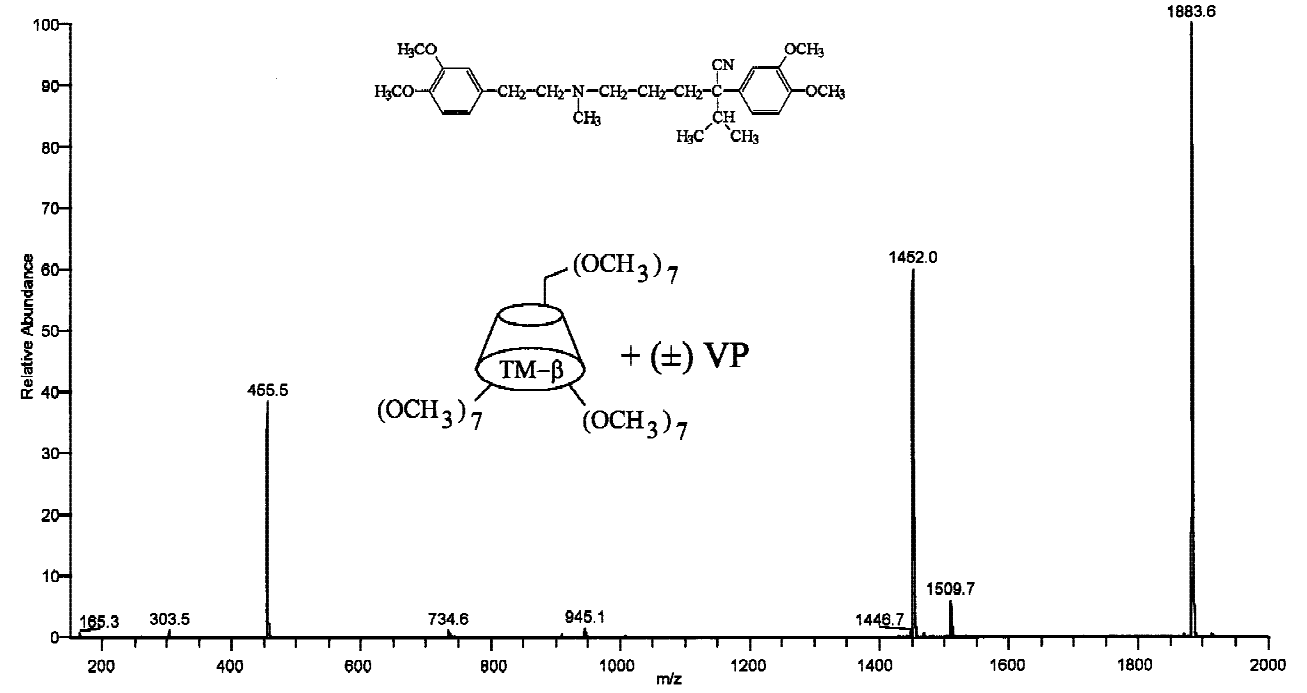

Fig. 11. ESI-MS spectra of 5.0 mg/mL solution of TM-β-CD and an equimolar amount of (±)-VP in double-distilled water.

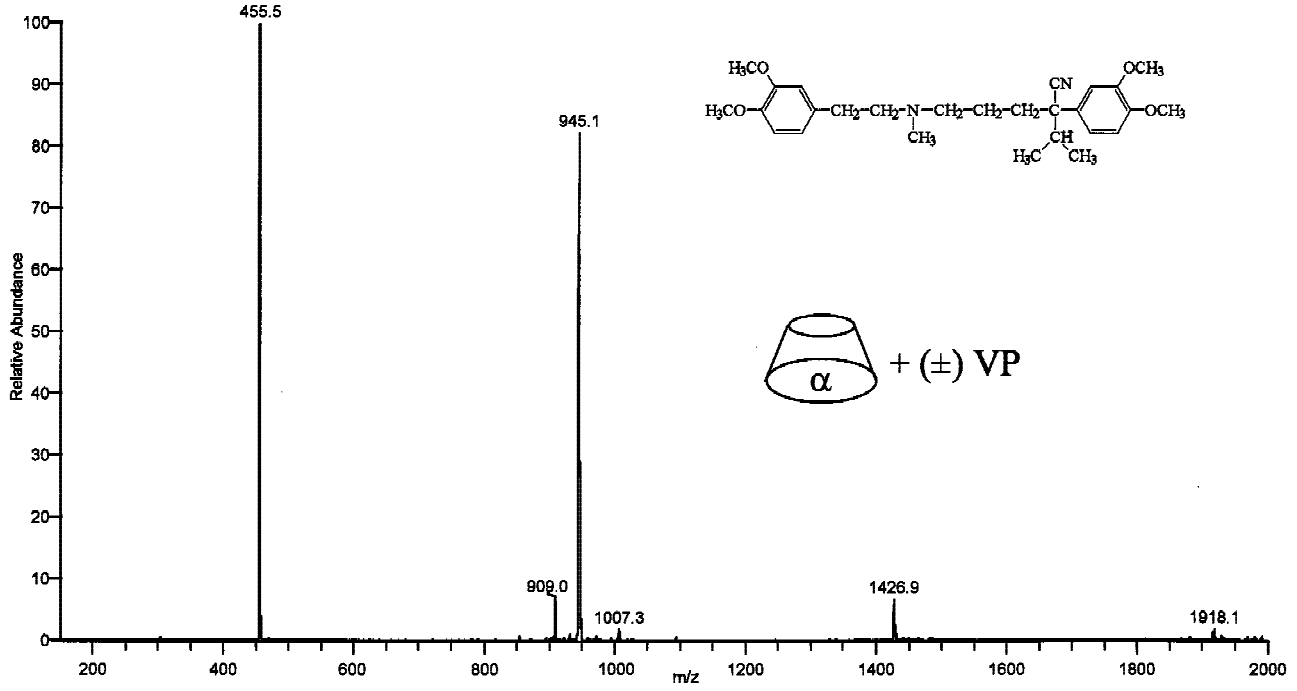

Fig. 12. ESI-MS spectra of 5.0 mg/mL solution of α-CD and an equimolar amount of (±)-VP in double-distilled water.

and sodium (*m/z* 1452.0, major peak) adducts of TM-β-CD. The peak with *m/z* 1183.6 corresponds to the 1:1 complex of (±)-VP and TM-β-CD. The data provided by ESI-MS in both cases supported the 1:1 stoichiometry of these complexes derived from NMR titration experiments.

As mentioned in previous studies, ESI-MS data of noncovalent complexes should be used with some care[15,41] considering the possibility of the formation of false ions. In order to evaluate the probability of false-ion formation under the present experimental conditions the complexation of (±)-VP by the *a priori* weak complexing agent α-CD (see Table 1) was studied under identical experimental conditions. The relative abundance of the ion with *m/z* 1426.9 due to a 1:1 complex between (±)-VP and α-CD was very low (Fig. 12). This result indicates that ESI-MS when used in combination with other techniques can provide additional information about the stoichiometry of inclusion complexes of CDs.

ACKNOWLEDGMENTS

The authors thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemische Industrie for financial support and Wacker-Chemie for a gift of some CDs used in this study.

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