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Journal of Chromatography A, 875 (2000) 135–161

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Separation of drug enantiomers by capillary electrophoresis in the presence of neutral cyclodextrins ☆

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Abstract

This is a selected review, highlighting our results obtained in an extended screening program (''The German–Chinese Drug Screening Program''), with a focus on a set of original data obtained with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) as the chiral solvating agent (CSA). The enantioseparation of 86 drugs by capillary zone electrophoresis in the presence of this CSA was successful for 47 drugs. The migration separation factors (α_m) and the migration retardation factors (R_m) were compared with those found for native β-cyclodextrin (β-CD). The patterns thus obtained were also compared with those observed for hexakis(2,3,6-tri-*O*-methyl)-α-CD (TM-α-CD) and octakis(2,3,6-tri-*O*-methyl)-γ-CD (TM-γ-CD), respectively. From the statistical data, it can be concluded that there is a remarkable influence of the analyte structure on the electrophoretic data. A substructure 4H was found in the analyte structure that has a significant influence on the analytes' behaviour. Thus, analytes bearing the substructure 4H do not only have a strong affinity to the CDs but also a high rate of success of chiral separation in all systems reviewed. In light of this, the different ring sizes of native cyclodextrins (α-, β- and γ-CD) readily explain their behaviour towards a limited test set of chiral drugs. Sterical considerations point to the significance of *side-on-binding* versus *inclusion* in the cavity of the host. In addition to the findings from the screening program, numerous references to the literature are given.

Keywords: Reviews; Enantiomer separation; Cyclodextrins; Drugs

Contents

☆Part 11 in a series: Separation of enantiomers of drugs by capillary electrophoresis, heptakis(2,3,6-tri-*O*-methyl)-cyclodextrin as chiral solvating agent.

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01210-8

1. Introduction

1.1. Background information on chiral drugs

Following the guidelines of the Food and Drug Administration (FDA) in the USA in 1992 and similar regulatory guidelines in Europe and Japan, single enantiomers of chiral drugs should be brought to the market wherever this is possible. Prior to the approval of a new drug, the enantiomers must be separated on a preparative scale, and the pharmacological effects as well as the metabolic pathways must be studied separately for each enantiomer, preferably by CE–MS coupling [1]. Only in exceptional cases, a mixture of the enantiomers may be approved, although, the general policy is to promote marketing of single enantiomer drugs. As highlighted in Chem. Eng. News [2,3], this creates an increasing demand for enantiomeric intermediates and enantioselective technologies. Dependent on the availability and cost-effectiveness, asymmetric synthesis by either chemical catalysis or biotechnology are competing with preparative separation of the racemic drug in a final stage. Separation science has taken an important role in all stages of the process: Enantiomer separation on an analytical scale is required for monitoring the enantiomeric purity and for screening chromatographic conditions suitable for preparative liquid chromatography.

Throughout this paper, all drug names used are international nonproprietary names assigned to the pharmaceuticals by the World Health Organization (WHO).

1.2. The role of capillary electrophoresis in the separation of enantiomers

It has been pointed out that capillary electrophoresis (CE) is one of the most convenient and advantageous approaches to the direct analysis of the enantiomer composition in aqueous solution [4,5]. Therefore, the assessment of the enantiomeric composition of chiral drugs has become a prominent application of CE in industry [6]. We maintain that capillary zone electrophoresis (CZE) is by far the most widely used mode of operation in CE: According to our database, Chirbase/CE (commercially available from the authors), capillary zone electrophoresis (CZE) [7] and micellar electrokinetic chromatography (MEKC) [8,9] are the most widely used methods of enantiomer separation by CE, contributing 72% and 21%, respectively, of all original articles published in the field; other modes amount to 8% of all articles, including reports on several modes in the same article, as summarised in Table 1. It is expected for the future that the database Chirbase/CE will provide a useful basis for establishing structure–enantioselectivity relationships; however, there is a definite lack of publications that cover a

Table 1
Methods used in capillary electrophoresis for the separation of enantiomers, as of Feb 1999, according to our database Chirbase/CE [14]

Method used	Abbreviation	Articles	Percentage of total
Capillary zone electrophoresis	CZE	241	71.9
Micellar electrokinetic chromatography	MEKC	69	20.6
Capillary electrochromatography	CEC	10	3.0
Capillary gel electrophoresis	CGE	9	2.7
Capillary isotachophoresis	ITP	6	1.8
Total		335	100

large number of analytes investigated under uniform conditions.

1.3. The Chinese–German chiral drug screening program

To this end, we have established the *Chinese–German Chiral Drug Screening Program*, a long termed project to develop and apply state-of-the-art technology to the analytical and preparative separation of racemic drugs by CE. A present collection of 151 chiral drugs is still growing. A selection thereof consisting of 86 most suitable candidates for the analytical separation with capillary zone electrophoresis is now running through different stages of an extensive screening process. This project has become feasible by the generous donation of fully automated CE equipment by Bio-Rad Labs. (Hercules, CA,USA). Meanwhile, we have obtained a significant number of comparable data [10–20] for neutral cyclodextrins, whereas the utility of charged cyclodextrins will be covered in a forthcoming stage of the project.

1.4. Advantage of cyclodextrins for efficient screening of chiral separation

Cyclodextrins are cyclic glucopyranoses that have a characteristic conical shape with a hydrophobic cavity and a polar exterior. They are able to form inclusion complexes with a wide range of substances, including racemic drugs. Different migration of the two drug enantiomers in the electric field is effected by the different stabilities of the two diastereomeric supramolecular complexes formed upon addition of the cyclodextrin containing buffer solution [the chiral solvating agent, (CSA)]. The complexation constants are primarily determined by the size, geometry, hydrophobicity and hydrogen-bonding properties of the analytes. The differences in complexation constants are often not completely understood. Supplementary investigations on this topic by various spectroscopic and chemometric methods have been identified as an integral part of this screening project and will be published elsewhere.

For efficient screening, the cyclodextrins offer several advantages; they are available at a reasonable price and in sufficient quantity, and their structure is amenable to a large range of chemical variations, including the formation of anionic and cationic species, respectively. Following their successful application in liquid and gas chromatography, as recorded in the molecular database Chirbase [21], they are also widely used in the different modes of CE.

1.5. Original data on heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, and comparative studies

As a core part of this selected review, we report on our original data obtained for 86 drug racemates by CZE in the presence of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD, also known as permethyl-β-cyclodextrin) in the run buffer. Two key figures defined in our previous publications, i.e., the *migration separation factor* (α_m) [11], and the *migration retardation factor* (R_m) [11] were compared with recently published data for native β-cyclodextrin (β-CD) [12,17]. This comparison may allow one to judge the influence of the permethylation on the migration separation factors (α_m) and on the migration retardation factors (R_m), respectively. We also maintained the idea of dividing the analytes into two classes, one where the substructure 4H [16] is present, and one where the substructure 4H is absent. In an overall comparison of four cyclodextrins, i.e., native β-CD, TM-α-CD, TM-β-CD and TM-γ-CD, the significance of this approach may be validated further. In another section of this selected review, the three native cyclodextrins (α-, β- and γ-CD) were compared for a subset of three drugs. Numerous references to CE data found in the literature were quoted separately for each of the 86 drugs compiled in the Tables.

2. Experimental

All experiments were carried out on a Bio-Focus 3000 automatic capillary electrophoresis system (Bio-Rad Labs.) equipped with a variable wavelength detector operated at 200 nm. Operating parameters were as follows: injection: 15 kV for 3 s; analysis: 15 kV $+ \rightarrow -$; capillary temperature: 25°C; thus, migration towards the cathode was applied.

Fused silica capillaries (0.05 μm I.D., 0.375 μm O.D.) were obtained from Yongnian Optical Conductive Fiber Plant (Yongnian, Province Hebei, China). In the laboratory these were coated with polyacrylamide on the inner surface. The total length of each capillary used was 30 cm (25.5 cm to detector window).

The plain run buffer contained 100 m*M* sodium dihydrogenphosphate, adjusted to pH 2.5 with phosphoric acid. TM-β-CD was added to the plain buffer to give a final concentration of 45 m*M*. Only 15 m*M* β-CD was used because of the limitation of its solubility in water. The analytes were dissolved in the run buffer to yield a sample concentration of 0.1 mg/ml.

TM-β-CD was kindly donated by Bio-Rad Labs. Due to the complete methylation of all hydroxy groups, the purity of the CD used was fairly high (analytical grade). The drug samples were donated by the following manufacturers: Allergan, Ankerpharm, Arzneimittelwerk Dresden, ASTA Medica, Astra Chemicals, Bayer, Boehringer Mannheim, Chephasaar, Ciba-Geigy, Dispersa, Dolorgiet, Durachemie, Gödecke, Hexal, Intersan, Jenapharm, Kali-Chemie, Klinge, Knoll, Kreussler, Krewel, Mann, Medice, E. Merck, 3M Medica, Pfizer, Pharmacia, Rhône-Poulenc Rorer, Robugen, Roche, Röhm Pharma, Schering, Sigma, Thiemann, Wellcopharm, and Zyma. Internal purity standards of the manufacturers apply to all drug samples. All other chemicals were analytical grade.

3. Results and discussion

3.1. Theoretical considerations

The experimental conditions were comparable to our previous investigations; however, trimethyl-β-cyclodextrin was used as the CSA. In view of the strong impact of the CSA concentration on the degree of separation in CZE [22,23] at a given pH [23], a constant CSA concentration of 45 m*M* and pH 2.5 was chosen throughout this study. According to our experience in the chiral drug screening program, the applied conditions are relatively optimum for most analytes although they may be less appropriate for a few analytes. Because of the limited solubility of β-CD, the concentration was set at 15 m*M* in this particular case [12,17], rendering the comparison not as appropriate as in previous papers of this series [19,20].

Following the suggestion by Heuermann and Blaschke [24], we defined a *migration separation factor* (α_m), by analogy with the separation factor (α) frequently used in chromatography. The factor (α_m) was derived from the migration times of the first ($t_{m(1)}$) and second ($t_{m(2)}$) eluted enantiomer, see Eq. (1) [11].

$$\alpha_m = t_{m(2)}/t_{m(1)} \quad (1)$$

α_m is equivalent to the ratio (α_μ) of the electrophoretic mobilities of the enantiomers [24], see Eq. (2),

$$\alpha_\mu = \mu_1/\mu_2 \quad (2)$$

where μ_1 and μ_2 are the electrophoretic mobilities of the first and second eluted enantiomer, respectively. Apparently, the enantiomer separation factor α_m is not dependent on the capillary performance.

It is beyond the scope of this article to outline the relationship between α_m and the complex formation constants (K_1) and (K_2) of the two drug enantiomers with the cyclodextrin host. The determination of *K* by CE has been reported elsewhere [25,26].

Similar to the effective mobility difference of the enantiomers $\Delta\mu$ calculated by Wren and Rowe [22,27], see Eq. (3),

$$\Delta\mu = \mu_{\mathrm{eff}(1)} - \mu_{\mathrm{eff}(2)} \quad (3)$$

α_m is expected to have a maximum at a certain CSA concentration, namely at

$$c_{\mathrm{opt}} = (\mu_u/\mu_c)^{1/2} \cdot (K_1 \cdot K_2)^{-1/2} \quad (4)$$

where c_{opt} is the CSA concentration producing the largest α_m value, and μ_u and μ_c are the electrophoretic mobilities of the uncomplexed and complexed forms of the analyte, respectively. The optimisation of the separation is possible for particular enantiomeric pairs [28]; however, for a set of 86 drugs we prefer to treat all compounds under similar conditions. In a previous paper, we have shown that no resolution was missed that way even though some racemates have a different optimum CD concentration [13]. A maximum difference in total mo-

bilities of the enantiomers can be reached if about half of each enantiomer is bound to the CSA [28]. Thus, high complex formation constants, as indicated by strong retardation, can be balanced out by lower CSA concentration and vice versa. In Ref. [13], 23 selected analytes were measured with both 45 m*M* and 30 m*M* HP-γ-CD as the CSA. The lower concentration seemed close enough to the initial value of 45 m*M* to avoid the occurence of minima and maxima, respectively, of α_m between the two points. The verification of that hypothesis is displayed in Fig. 1 [13]. While all racemates not resolved at 45 m*M* CSA were not influenced ($\Delta\alpha_m = 0$), most of those with strong retardation profited from the concentration decrease ($\Delta\alpha_m > 0$). On the other hand, many of the less strongly retarded analytes showed a better separation at higher CSA concentration, resulting in negative values of $\Delta\alpha_m$ for a decrease of the CSA concentration [13].

It has been pointed out that $t_{m(2)}/t_{m(1)}$ is directly related to the ratio of electrophoretic mobilities (μ), see Eq. 5 [11].

$$t_{m(2)}/t_{m(1)} = \mu_{(1)}/\mu_{(2)} \quad (5)$$

As emphasized previously, the electroosmotic flow (EOF) may be neglected under these conditions, based on the observation that the neutral compound dimethylsulfoxide could not be detected within 2 h under the conditions applied. Notably, this definition of α_m bears the advantage of not being influenced by the peak shape, thus revealing the degree of enantiodiscrimination, regardless of the peak resolution achieved. Furthermore, α_m is deemed to be independent of capillary length, electric field strength etc., and therefore a good measure for the comparison of the chiral recognition of different racemates by the CSA [14].

As a preliminary tool to elucidate the impact of the chiral solvating agent on the migration velocity,

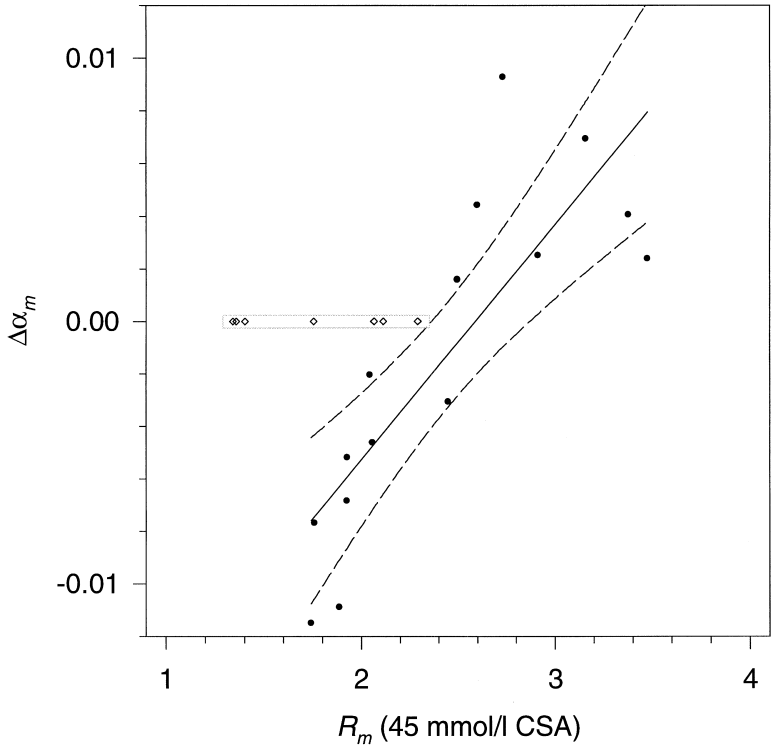


Fig. 1. Change of the HP-γ-CD concentration from 45 mmol/l to 30 mmol/l may lead to a drift in the migration separation factor $\Delta\alpha_m$, plotted versus retardation R_m at 45 mmol/l. $\Delta\alpha_m = \alpha_{m(30\ \mathrm{mmol/l})} - \alpha_{m(45\ \mathrm{mmol/l})}$. (◇), $\alpha_{m(45\ \mathrm{mmol/l})} = 1$ and $\Delta\alpha_m = 0$; (●) $\alpha_{m(45\ \mathrm{mmol/l})} > 1$ and $\Delta\alpha_m \neq 0$ (linear regression and 95% confidence interval shown).

we defined a *migration retardation factor* (R_m) as the ratio of the migration time (if resolved, of the second eluted enantiomer) in the CSA-containing buffer ($t_{m(CSA)}$) and the migration time in plain phosphate buffer ($t_{m(plain)}$), as an approximate parameter for measuring the strength of the host–guest interaction, see Eq. (6) [11].

$$R_m = t_{m(CSA)}/t_{m(plain)} \quad (6)$$

It should be noted that changes in the viscosity of the buffer upon CSA addition may cause a small, yet undetermined uncertainty to a comparison of two different hosts.

3.2. Original data obtained for 86 drugs with TM-β-CD

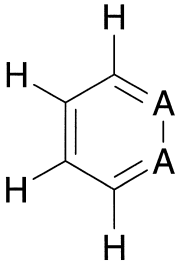
Based on the impact of different substructures on the electrophoretic data [26], we choose to divide the total of 86 chiral drugs into two classes, according to their chemical structure, one class where the substructure 4H [16] (see Fig. 2) is present, and another class where 4H is absent. In Table 2, the retardation and separation data are compiled for the 43 analytes containing the substructure 4H, while in Table 3, the data are provided for the 43 analytes lacking the substructure 4H.

3.3. Comparison of TM-β-CD and β-CD for 86 drugs

In contrast to the findings with other permethylated CDs [19,20], chiral recognition with TM-β-CD looks quite promising. Whereas only 20 racemates can be separated in the presence of β-CD at 15 m*M* concentration, a total of 47 drug racemates could be separated into the enantiomers in the presence of TM-β-CD, see Tables 2 and 3. The electropherograms are not shown here, as they did not reveal any remarkable aberrations from those published with other CSAs. A detailed presentation of the fraction of successful enantiomer separations (''success rate R_{suc}'') for analytes of the two structure classes is given in Fig. 3. With both β-CD and TM-β-CD, racemates containing the substructure 4H have a slightly better chance to be separated than those where 4H is absent. With TM-β-CD, R_{suc} is significantly higher than with β-CD, for both classes of analytes, although, a direct comparison of the two CSAs is hindered by the low solubility of β-CD in the running buffer.

Likewise, analytes containing the substructure 4H have a higher affinity, as judged from the fraction of $R_m > 1.5$, to the two cyclodextrins than analytes lacking 4H, see Fig. 4. However, the fraction of analytes with $R_m > 1.5$ is significantly higher for β-CD than for TM-β-CD, if 4H is present in the analyte structure, thus indicating specific binding of the 4H region to β-CD, as discussed below. For analytes not containing the substructure 4H, the R_m values are slightly higher with TM-β-CD. The chiral separation status with β-CD and TM-β-CD is depicted in Fig. 5. It is interesting to note that 14 analytes had been separated with both CSAs, six drugs bearing the substructure 4H and eight drugs lacking 4H. Among the drugs separated with TM-β-CD but not with β-CD (vertical line in Fig. 5), there are many that do not contain 4H (white dots), but there are also two with fairly high separation factors α_m with TM-β-CD that do contain 4H (black dots). Further studies may reveal the specific modes of binding to TM-β-CD for these two entries.

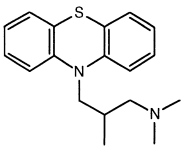
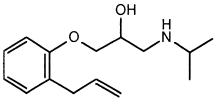
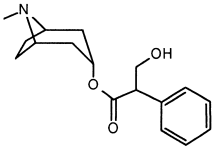
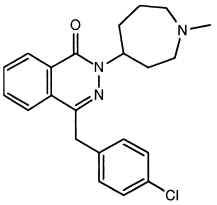
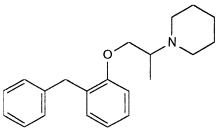
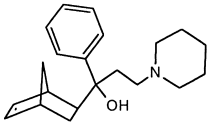
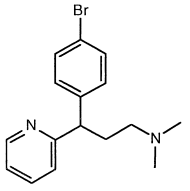
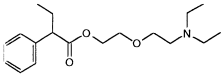
A plot of the migration retardation factor R_m with TM-β-CD versus R_m with β-CD is shown in Fig. 6. In contrast to the TM-α-CD [19], the majority of the black dots (4H present) is located below the diagonal line, i.e., in the lower right part of the diagram, while the majority of the white dots (4H absent) is found in the upper left region of the diagram. This discrimination may work even better when the two selector



Substructure 4H

Fig. 2. A = ''any atom'' (C, N, O, etc.).

Table 2
Migration times and parameters derived thereof, for the 43 analytes where the substructure 4H is present

Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Alimemazine	13.34	–	1.000	2.590	10.00	–	1.000	1.942	[10,15]
	Alprenolol	10.21	–	1.000	1.731	9.43	–	1.000	1.598	[43,15]
	Atropine	11.37	–	1.000	2.174	6.93	7.08	1.022	1.325	[44,15]
	Azelastine	13.81	–	1.000	2.067	15.56	15.74	1.012	2.329	[45,15]
	Benproperine	12.86	–	1.000	2.364	11.13	11.23	1.009	2.046	[30,15]
	Biperiden	16.46	–	1.000	2.348	13.33	15.29	1.147	1.902	[10,15]
	Bromphenamine	6.57	6.66	1.014	2.424	5.46	5.51	1.009	2.015	[46,15]
	Butamirate	14.16	–	1.000	2.617	13.27	13.59	1.024	2.453	[13,15]

(*Continued on next page*)

Table 2 (*continued*)

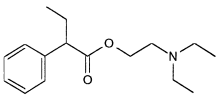
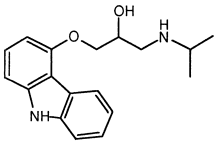
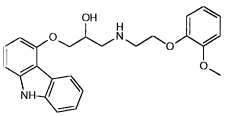
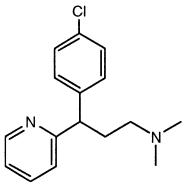
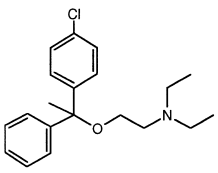
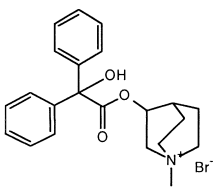
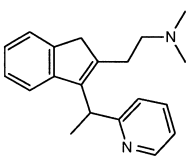
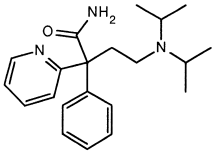
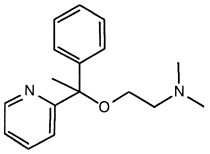
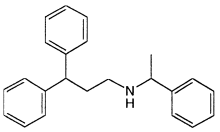
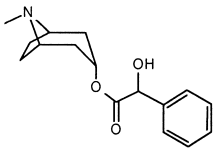
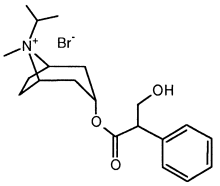
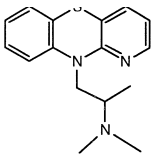
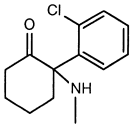
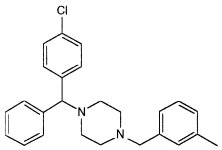
Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Butetamate	13.33	–	1.000	2.645	9.26	9.30	1.004	1.837	[10,15]
	Carazolol	7.71	–	1.000	1.367	10.28	10.41	1.013	1.823	[10,15]
	Carvedilol	13.43	13.81	1.028	1.886	14.25	–	1.000	2.001	[47,15]
	Chlorphenamine	5.34	5.39	1.009	2.086	4.49	4.51	1.004	1.754	[48,15]
	Chlorphenoxamine	12.14	–	1.000	2.716	12.23	12.51	1.023	2.736	[13,15]
	Clidinium bromide	15.34	–	1.000	2.389	8.17	–	1.000	1.273	[10,15]
	Dimetindene	5.29	5.47	1.030	1.696	3.73	–	1.000	1.196	[49,15]

Table 2 (*continued*)

Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Disopyramide	6.06	–	1.000	1.530	6.18	–	1.000	1.561	[50,15]
	Doxylamine	4.08	4.17	1.022	1.613	3.53	–	1.000	1.395	[51,15]
	Fendiline	13.27	–	1.00	2.215	1.32	11.46	1.012	1.890	[30,15]
	Homatropine	8.78	9.15	1.040	1.756	6.25	6.30	1.008	1.250	[44,15]
	Ipratropium bromide	11.59	–	1.000	2.146	6.95	7.09	1.020	1.287	[10,15]
	Isothipendyl	10.87	11.00	1.010	2.265	7.61	7.91	1.039	1.585	[10,15]
	Ketamine	7.42	7.51	1.012	1.973	5.27	–	1.000	1.402	[52,15]
	Meclozine	18.41	–	1.000	2.781	19.26	–	1.000	2.870	[11,15]

(*Continued on next page*)

Table 2 (*continued*)

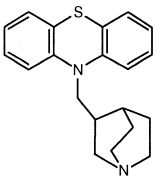
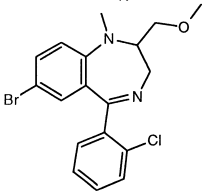
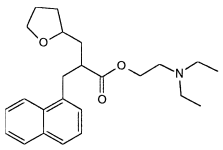
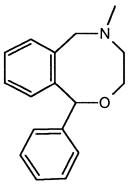
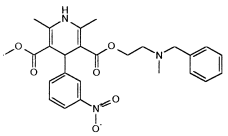
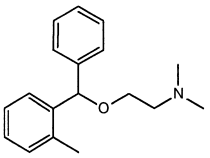
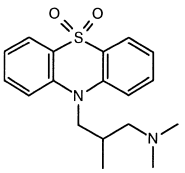
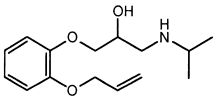
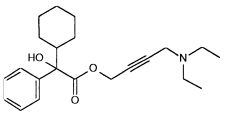
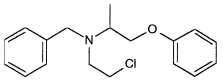
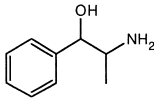
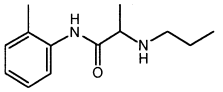
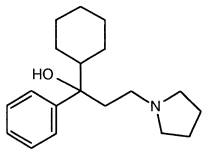
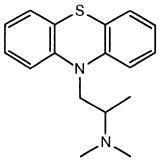
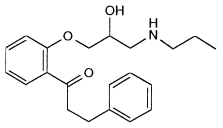
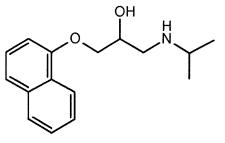
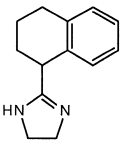
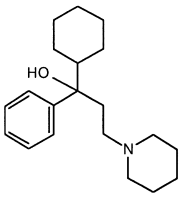
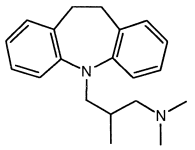
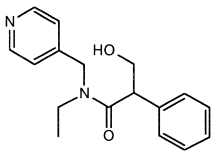
Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Mequitazine	17.01	–	1.000	2.761	11.40	11.57	1.015	1.851	[10,15]
	Metaclazepam	9.73	–	1.000	1.719	9.13	9.84	1.078	1.613	[30,15]
	Naftidrofuryl	12.71	12.84	1.010	2.080	9.53	–	1.000	1.560	[10,15]
	Nefopam	13.09	–	1.000	2.716	7.41	7.92	1.069	1.537	[24,15]
	Nicardipine	9.26	–	1.000	1.374	13.83	13.97	1.010	2.052	[45,15]
	Orphenadrine	11.79	–	1.000	2.643	7.93	8.12	1.024	1.778	[10,15]
	Oxomemazine	10.77	10.97	1.020	2.075	7.51	–	1.000	1.447	[53,15]

Table 2 (*continued*)

Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Oxprenolol	7.07	–	1.000	1.368	8.93	8.99	1.007	1.727	[54,15]
	Oxybutynin	12.89	–	1.000	2.374	14.24	–	1.000	2.622	[13,15]
	Phenoxybenzamine	8.86	–	1.000	1.914	7.40	–	1.000	1.598	[10,15]
	Phenylpropanolamine	5.46	–	1.000	1.386	5.72	–	1.000	1.452	[55,15]
	Prilocaine	6.64	–	1.000	1.105	6.53	6.59	1.009	1.087	[10,15]
	Procyclidine	16.68	–	1.000	4.244	15.05	18.28	1.215	3.830	[30,15]
	Promethazine	12.86	–	1.000	2.582	8.63	8.75	1.014	1.733	[56,15]
	Propafenone	11.67	–	1.000	2.153	12.00	12.00	1.000	2.214	[10,15]
	Propanolol	9.03	–	1.000	1.909	9.84	10.00	1.016	2.080	[57,15]

(*Continued on next page*)

Table 2 (*continued*)

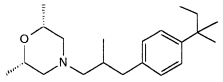
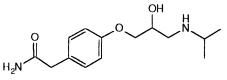
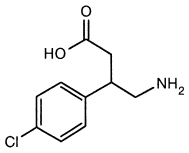
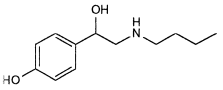
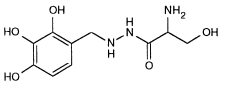
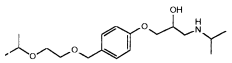
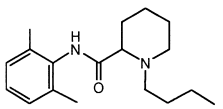
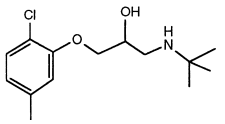
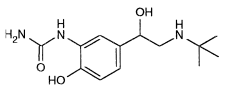
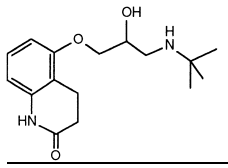
Analyte structures 4H present		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Tetryzoline	6.99	7.27	1.040	1.668	5.22	5.35	1.025	1.246	[10,15]
	Trihexyphenidyl	13.91	–	1.000	2.254	12.44	13.33	1.072	2.016	[10,15]
	Trimipramine	13.69	–	1.000	2.535	10.89	–	1.000	2.017	[10,15]
	Tropicamide	9.65	10.09	1.046	2.040	6.51	6.70	1.029	1.376	[10,15]

[a] The first and the most recent references are given.

concentrations are properly adjusted. In principle, this finding indicates that a two-phase-system of any kind (e.g. two sensors), where one phase would be loaded with β-CD and the other would be charged with TM-β-CD, should be able to significantly discriminate or even separate drugs according to their molecular structure. In a similar fashion, suitable CD derivatives could be used to divide combinatorial chemistry libraries into sublibraries. To our knowledge, these ideas have not yet been put in practice. Apparently, the interaction of the analytes with β-CD on average is stronger than with TM-β-CD despite of the lower concentration of β-CD, in good agreement with the pattern displayed in Fig. 4. The mean R_m value of analytes containing 4H is 1.821 with TM-β-CD and 2.146 with β-CD, whereas the mean R_m value of analytes lacking 4H is 1.665 with TM-β-CD and 1.566 with β-CD. For analytes with substructure 4H (black dots), the maximum R_m values are approximately 4.0 with TM-β-CD and 4.2 with β-CD, respectively.

Likewise, plots of the migration separation factor (α_m) versus the retardation factor (R_m) reveal distinct differences for the two CSAs. For β-CD (Fig. 7), most data points are distributed in the medium range of R_m, between 1 and 3. The dots corresponding to successful separations ($\alpha_m > 1$) are evenly distributed over a medium range $1.1 < R_m < 2.5$. For TM-β-CD (Fig. 8), the overall R_m range is slightly wider, and so is the distribution of the successful separations ($\alpha_m > 1$), with a higher population density in the region around $R_m \approx 1.5$. From the two black entries with $\alpha_m = 1.15$ and 1.22, respectively, in the presence of TM-β-CD, the first one is in the medium region

Table 3
Migration times and parameters derived thereof, for the 43 analytes where the substructure 4H is absent

Analyte structures 4H absent		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Amorolfine	13.20	–	1.000	2.370	16.81	–	1.000	3.018	[13,15]
	Atenolol	8.49	–	1.000	1.383	7.59	–	1.000	1.236	[58,15]
	Baclofen	6.09	–	1.000	1.171	7.42	7.58	1.022	1.427	[10,15]
	Bamethan	6.99	–	1.000	1.290	8.02	8.34	1.040	1.480	[59,15]
	Benserazidine	5.48	–	1.000	1.054	6.67	–	1.000	1.283	[10,15]
	Bisoprolol	14.05	–	1.000	2.063	11.54	–	1.000	1.695	[10,15]
	Bupivacaine	6.25	–	1.000	1.299	7.47	7.68	1.028	1.553	[47,15]
	Bupranolol	10.60	–	1.000	1.931	10.07	–	1.000	1.834	[10,15]
	Carbuterol	6.46	–	1.000	1.112	8.50	–	1.000	1.463	[60,15]
	Carteolol	6.97	–	1.000	1.367	7.45	–	1.000	1.461	[13,15]

(*Continued on next page*)

Table 3 (*continued*)

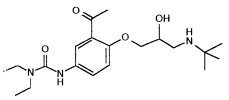
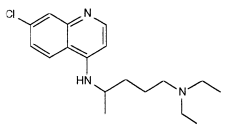
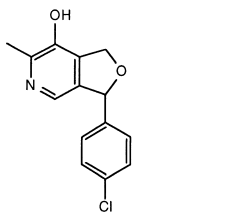
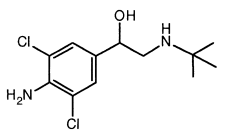
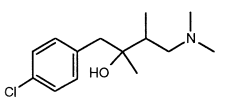
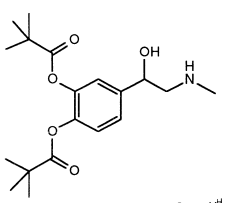
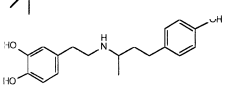
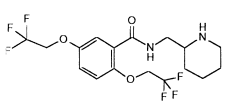
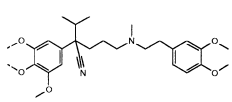
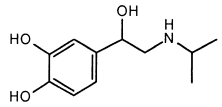
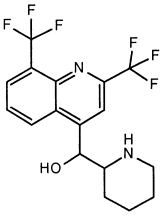
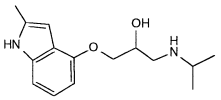
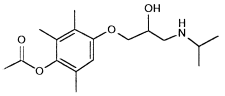
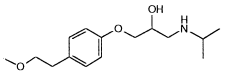
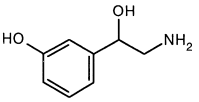
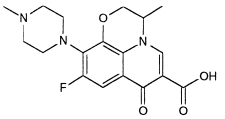
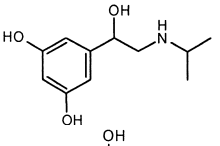
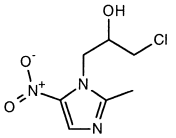
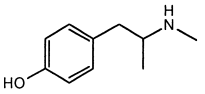
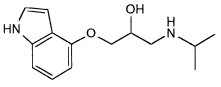
Analyte structures 4H absent		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Celiprolol	8.59	–	1.000	1.218	11.36	–	1.000	1.611	[53,15]
	Chloroquine	3.37	–	1.000	1.199	3.97	–	1.000	1.413	[61,15]
	Cicletanine	12.80	–	1.000	2.388	9.82	10.09	1.027	1.832	[62,15]
	Clenbuterol	7.04	7.24	1.028	1.109	8.37	8.53	1.019	1.318	[63,15]
	Clobutinol	12.74	–	1.000	2.627	12.02	12.16	1.012	2.478	[10,15]
	Dipivefrine	15.47	–	1.000	2.536	19.88	–	1.000	3.259	[58,15]
	Dobutamine	11.58	–	1.000	2.169	9.96	–	1.000	1.865	[10,15]
	Flecainide	8.88	–	1.000	1.685	9.15	–	1.000	1.736	[64,15]
	Gallopamil	10.20	–	1.000	1.627	9.89	–	1.000	1.577	[53,15]

Table 3 (*continued*)

Analyte structures 4H absent		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Isoprenaline	6.48	6.59	1.017	1.268	7.65	7.79	1.018	1.497	[24,15]
	Mefloquine	7.44	7.58	1.020	1.187	14.86	–	1.000	2.370	[24,15]
	Mepindolol	7.31	–	1.000	1.562	7.28	–	1.000	1.556	[30,15]
	Metipranolol	7.14	–	1.000	1.169	10.75	–	1.000	1.759	[10,15]
	Metoprolol	12.27	–	1.000	2.203	10.22	–	1.000	1.835	[54,15]
	Norfenefrine	4.93	–	1.000	1.248	5.79	5.95	1.028	1.466	[65,15]
	Ofloxacin	8.35	–	1.000	1.301	8.13	8.28	1.018	1.266	[66,15]
	Orciprenaline	7.94	8.25	1.039	1.536	8.85	9.42	1.064	1.712	[65,15]
	Ornidazole	9.88	–	1.000	0.738	20.18	20.40	1.011	1.508	[67,15]
	Pholedrine	9.09	9.40	1.034	2.217	6.39	6.71	1.050	1.559	[24,15]
	Pindolol	6.93	–	1.000	1.523	7.07	–	1.000	1.554	[47,15]

(*Continued on next page*)

Table 3 (*continued*)

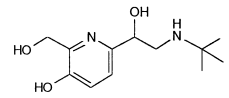
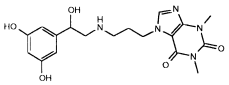
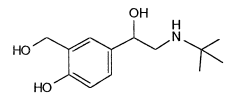
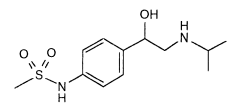
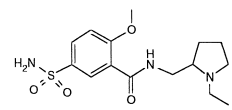
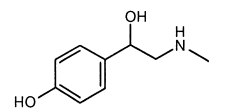
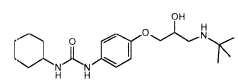
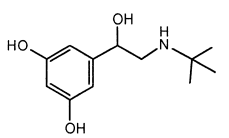
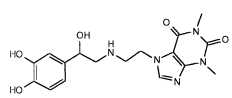
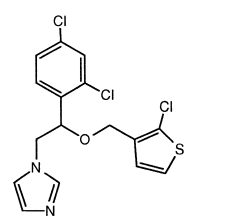
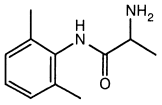
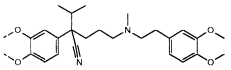
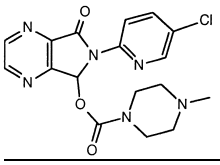
Analyte structures 4H absent		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Pirbuterol	3.46	–	1.000	0.903	4.59	–	1.000	1.198	[68,15]
	Reproterol	9.80	9.96	1.020	1.382	9.13	9.24	1.012	1.288	[30,15]
	Salbutamol	6.62	–	1.000	1.221	8.15	–	1.000	1.504	[24,15]
	Sotalol	8.77	–	1.000	1.645	7.30	–	1.000	1.370	[24,15]
	Sulpiride	6.81	–	1.000	1.446	6.91	–	1.000	1.467	[69,15]
	Synephrine	6.12	–	1.000	1.437	6.23	6.38	1.024	1.462	[24,15]
	Talinolol	13.60	–	1.000	1.946	14.87	–	1.000	2.127	[13,15]
	Terbutaline	8.72	9.20	1.055	1.591	8.57	8.80	1.027	1.564	[57,15]
	Theodrenaline	8.73	–	1.000	1.274	8.57	8.65	1.009	1.251	[30,15]
	Tioconazole	13.28	13.38	1.010	2.450	13.98	14.24	1.019	2.579	[70,15]

Table 3 (*continued*)

Analyte structures 4H absent		Separation data with β-CD				Separation data with TM-β-CD				See also Ref.[a]
		$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	$t_{m(1)}$ (min)	$t_{m(2)}$ (min)	α_m	$R_{m(2)}$	
	Tocainide	5.02	–	1.000	1.116	6.55	6.73	1.027	1.456	[71,15]
	Verapamil	12.43	–	1.000	1.844	10.20	11.01	1.079	1.513	[47,15]
	Zopiclone	10.72	11.42	1.065	1.536	8.30	8.36	1.007	1.189	[49,15]

[a] The first and the most recent references are given.

($R_m = 1.9$), and the second one has an extreme R_m ($R_m = 3.83$). As stated before, this particular behaviour deserves further attention in future studies. In contrast, the TM-α-CD [19] showed a more uniform behaviour, in terms of both affinity and enantioselectivity.

Frequency functions of the analytes in various ranges of R_m are shown in Figs. 9 and 10. The

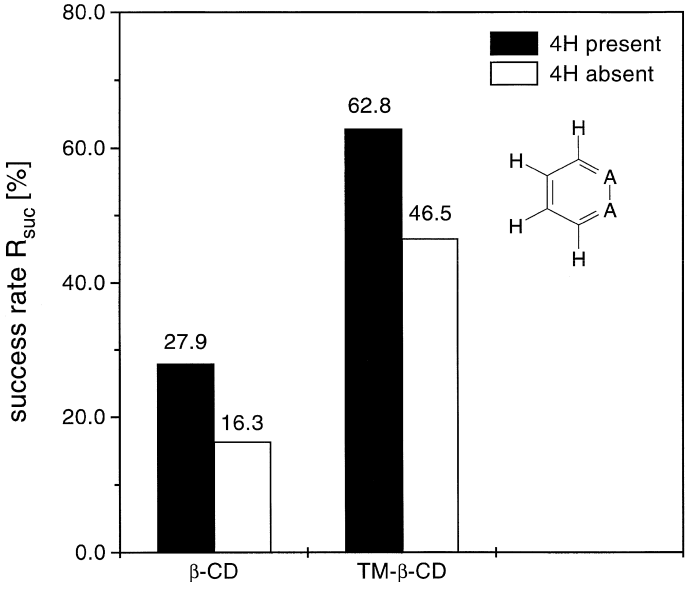


Fig. 3. Fraction (%) of analytes with separation factors $\alpha_m > 1.0$ (''success rate R_{suc}'') in relation to all analytes of the same structure class; *black colour*: Analytes containing the substructure 4H; *white colour*: Analytes not containing the substructure 4H.

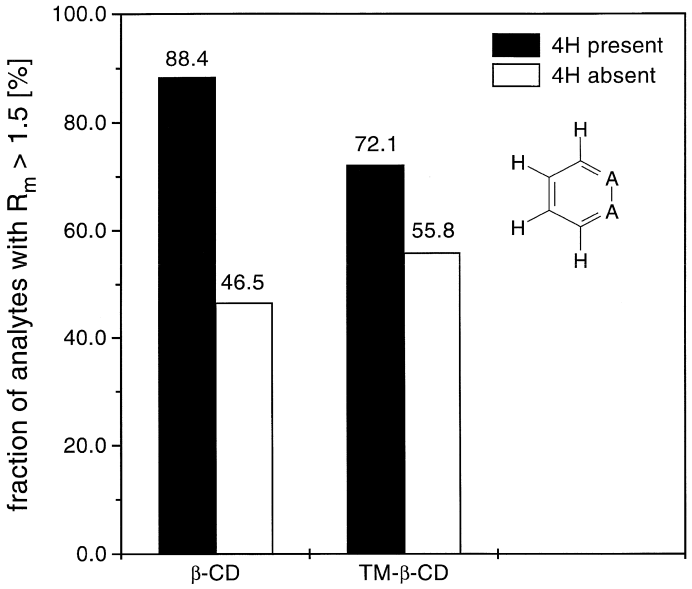


Fig. 4. Fraction (%) of analytes with separation retardation factors $R_m > 1.5$ in relation to all analytes of the same structure class; *black colour*: Analytes containing the substructure 4H; *white colour*: Analytes not containing the substructure 4H.

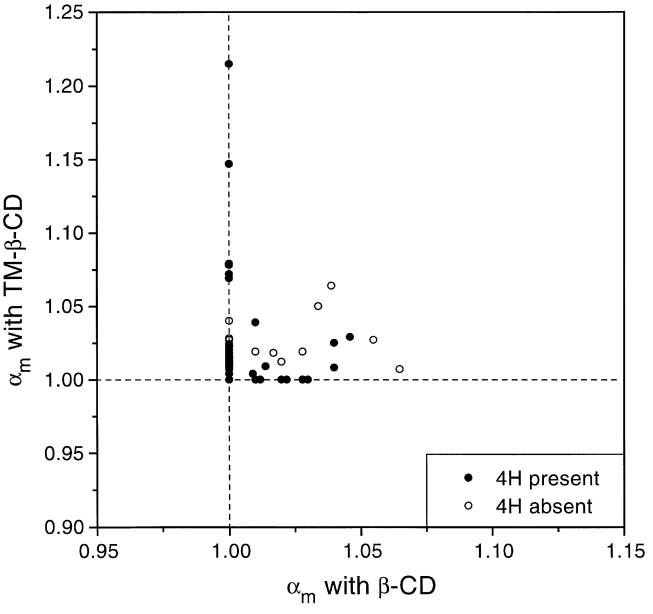


Fig. 5. Separation factors α_m in the presence of β-CD versus α_m in the presence of TM-β-CD, for analytes where the substructure 4H is present (*black colour*), and analytes where the substructure 4H is absent (*white colour*). In the origin (1/1), the white dot is omitted for clarity, only the black dot is depicted.

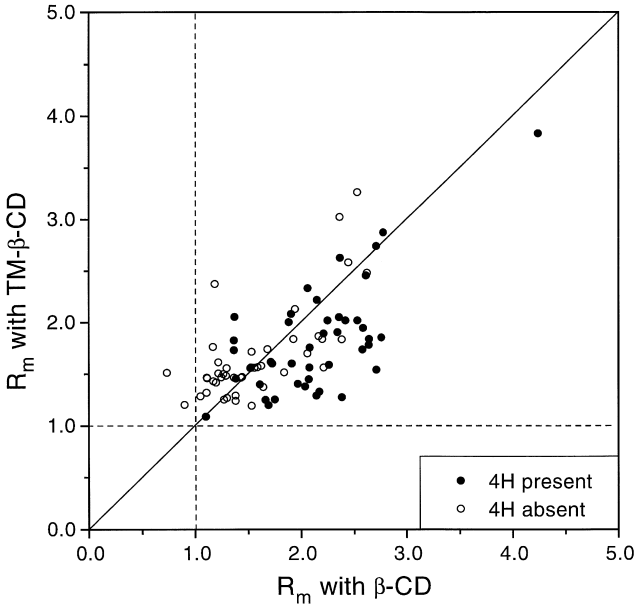


Fig. 6. Separation retardation factors R_m in the presence of β-CD versus R_m in the presence of TM-β-CD, for analytes where the substructure 4H is present (*black colour*), and analytes where the substructure 4H is absent (*white colour*).

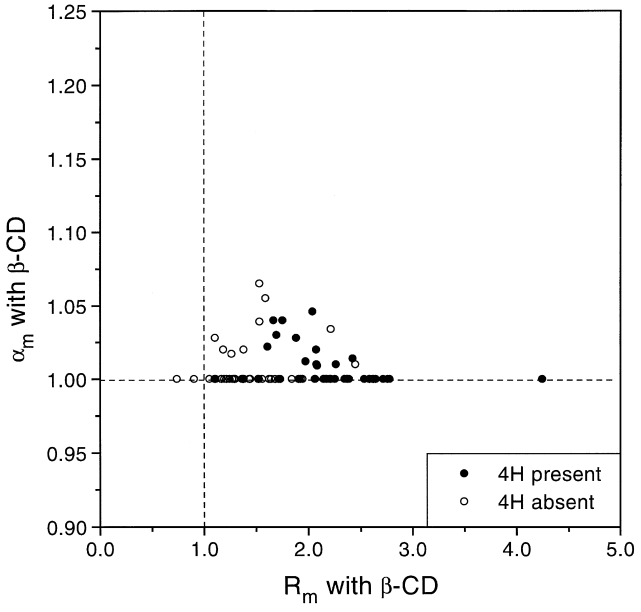


Fig. 7. Separation factors α_m versus separation retardation factors R_m in the presence of β-CD, for analytes of the two structure classes (*black and white colour*).

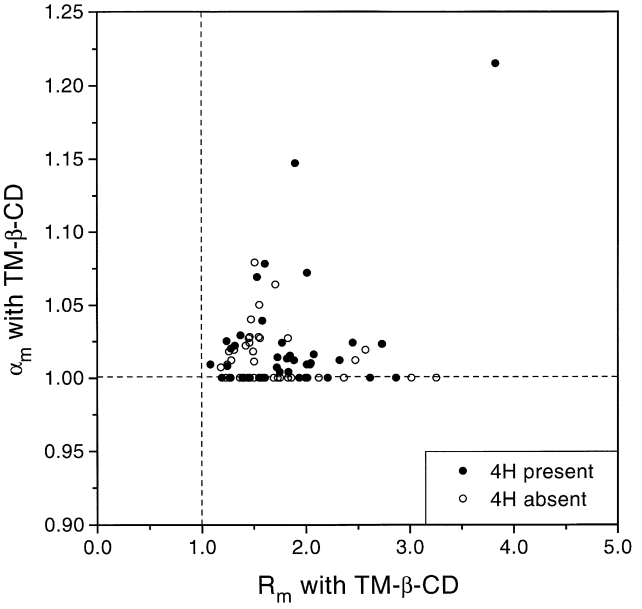


Fig. 8. Separation factors α_m versus separation retardation factors R_m in the presence of TM-β-CD, for analytes of the two structure classes (*black and white colour*).

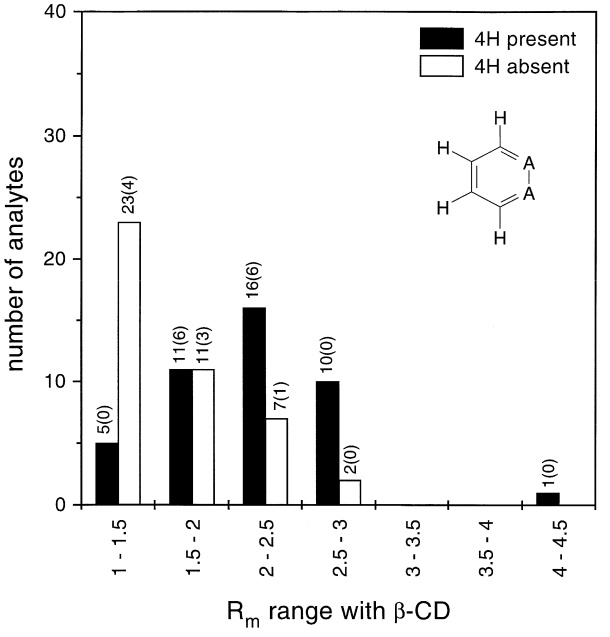


Fig. 9. Number of analytes found in different R_m ranges with β-CD, for analytes of the two structure classes (*black and white colour*). The number in brackets is the number of analytes which can be separated.

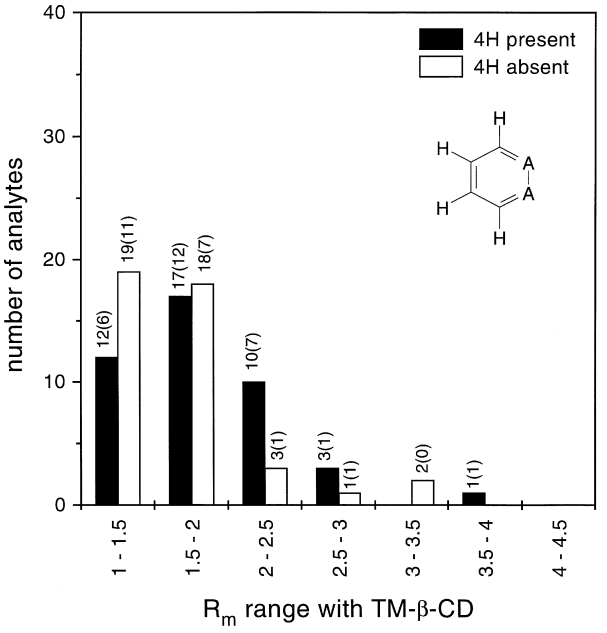


Fig. 10. Number of analytes found in different R_m ranges with TM-β-CD, for analytes of the two structure classes (*black and white colour*). The number in brackets is the number of analytes which can be separated.

number of analytes is indicated on top of the bars, the number of successful separations appended in brackets. The behaviour of analytes with the two CSAs is similar for the highest chance of success, found in the second and third range ($1.5 < R_m < 2.5$). A closer look, however, reveals subtle differences. A maximum in the frequency function is observed for the black bars at $1.5 < R_m < 2$ for TM-β-CD; the maximum for the white dots is located at $1 < R_m < 1.5$ for both CSAs, but the skew is more smooth for β-CD. The chance of success of enantiomer separation is generally higher for TM-β-CD; this is especially true for the entries where 4H is absent, and also for small R_m values for both classes of analytes.

3.4. Comparison of TM-β-CD with TM-α-CD and TM-γ-CD for 86 drugs

The influence of the ring size of the cyclodextrin is demonstrated in the following two charts for a comparison of TM-α-CD, TM-β-CD and TM-γ-CD. As stated, the presence of the substructure 4H (black bars) is beneficial for the separation; hence, the black bars are always higher than the corresponding white bars. Moreover, the superiority of the β-derivative over both the α- and γ-homologues is striking (see Fig. 11). The chance for a particular enantiomeric pair to be separated with TM-α-CD is approximately half of that with TM-β-CD, and with TM-γ-CD only one third to one fourth. Affinity is not the main reason. Although it is true for all three permethylated cyclodextrins that the analytes containing the substructure 4H are bound more strongly (Fig. 12), the absolute R_m data for different ring sizes do not reflect the pattern found for α_m. TM-α-CD yields the overall highest binding constants, expressed by the fraction of analytes with $R_m > 1.5$, but not the highest rates of success R_{suc} (cf. Fig. 11). We conclude that a medium affinity is sufficient for a successful enantiomer separation, but not the only prerequisite. Apart from peculiar details for individual drugs, the odd number of glucose units for β-CD and derivatives thereof (7 units) may give rise to a lower local symmetry, compared with the even-membered

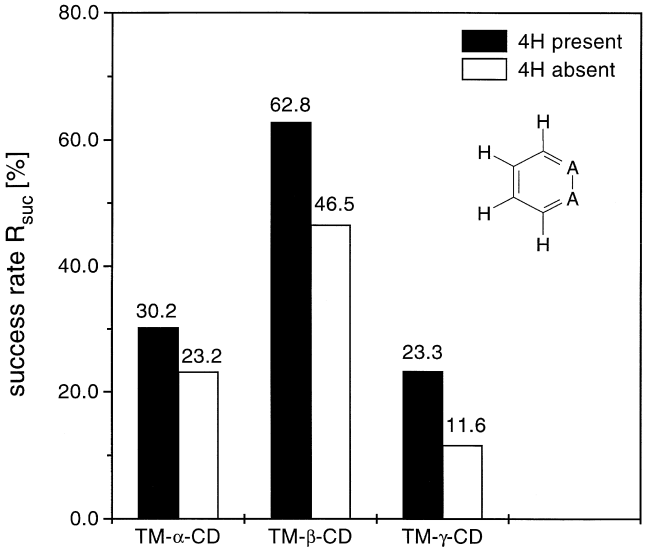


Fig. 11. Fraction (%) of analytes with separation factors $\alpha_m > 1.0$ (''success rate R_{suc}'') in relation to all analytes of the same structure class; *black colour*: Analytes containing the substructure 4H; *white colour*: Analytes not containing the substructure 4H, influence of ring size.

homologous α-CD (6 units) and γ-CD (8 units), respectively [30]

3.5. A highlight on the native cyclodextrins

As expected from these considerations, the effect of the ring size is also quite striking for the native cyclodextrins. These have a characteristic conical shape with a hydrophobic cavity and a polar exterior. A few guidelines may serve a better understanding of the possible separation mechanism. Total or at least partial inclusion of hydrophobic parts of a wide

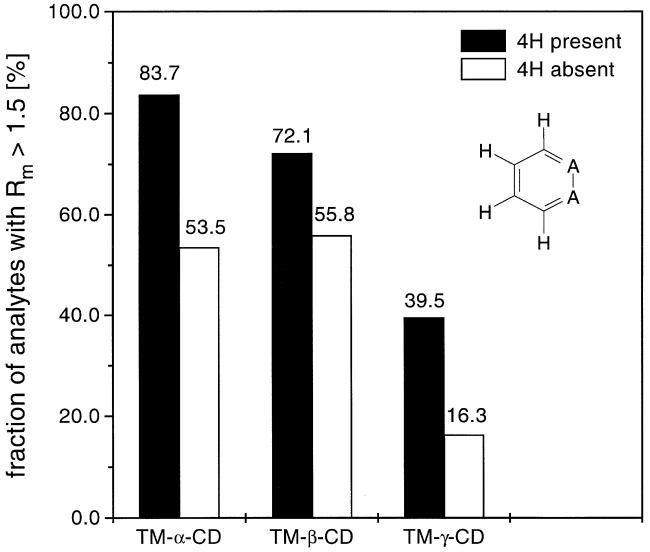
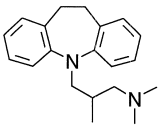


Fig. 12. Fraction (%) of analytes with separation retardation factors $R_m > 1.5$ in relation to all analytes of the same structure class; *black colour*: Analytes containing the substructure 4H; *white colour*: Analytes not containing the substructure 4H, influence of ring size.

range of substances is believed to occur in aqueous solution [31]. Apparently, the complexation constants are primarily determined by the size, geometry, hydrophobicity and hydrogen-bonding properties of the analytes. After extended screening with different cyclodextrins and derivatives thereof, we started to recognise certain motifs in the drug structures that most probably direct the course of the separation experiments [29], the motif 4H being one with a striking discriminatory effect, as outlined above for the permethylated cyclodextrins. A few examples are given in the following for the native cyclodextrins. Trimipramine (electropherogram shown in Fig. 13), alimemazine and oxomemazine may serve to illustrate the possible role of this structural element that in this context is only a small part of an extended tricyclic system, besides the crucial aliphatic amine side-chain. The three closely related structures (Fig. 14) allow to identify systematic changes in the electrophoretic behaviour, dependent on the ring size of the cyclodextrin added, see Table 4 [16–18].

The cavity diameter is known as 5.7, 7.8, and 9.5 Å for α-, β-, and γ-cyclodextrin, respectively [32]. Hence, a benzene ring with a total diameter of ca. 7.3 Å along the H–C···C–H axis does not fit in a horizontal orientation (as depicted in Fig. 15) into the α-cyclodextrin cone; instead, a partial inclusion (''*side-on-binding*'') is deemed the most likely possibility [16]. The cavity of β-cyclodextrin is just sufficiently large to accommodate a whole benzene ring, but the substituents in ortho-position may sterically restrict the degree of inclusion. Although tricyclic structures have been suggested to fit especially well into γ-cyclodextrin [33,34], the cavity is presumably far too narrow for a complete inclusion of the tricyclic system. Despite the increased cavity diameter, *side-on-binding* of the benzene ring is still the most likely possibility, although this may be paralleled by pi–pi stacking of two drug molecules in a 2:1 complex. A crude model on *side-on-binding* of Trimipramine by γ-CD in a 1:1 complex is illustrated in Fig. 16.

The separation and retardation data outlined in Table 4 are also meaningful. In all nine combinations, the R_m values that are closely related to the complex formation constants K_S show a uniform trend: α-CD>β-CD>γ-CD. Hence, the small region of four C–H units of a benzene ring that we have



Trimipramine

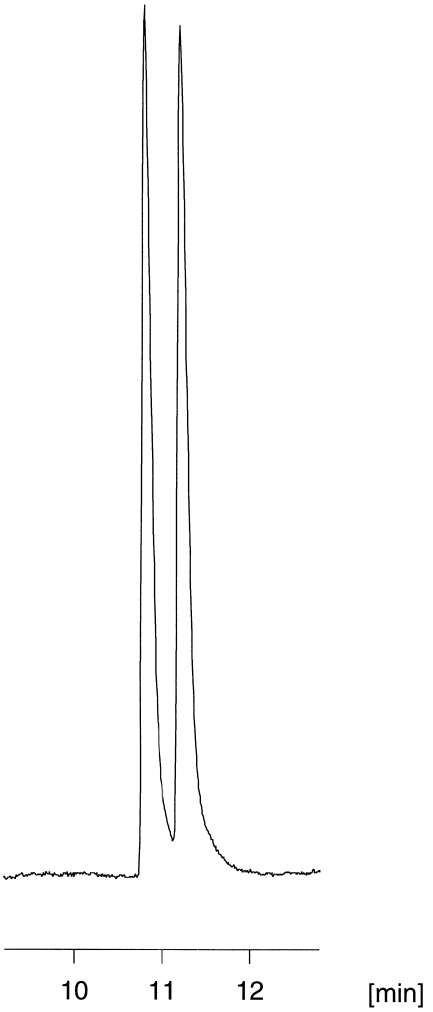


Fig. 13. Electropherogram of Trimipramine with 45 mmol/l γ-CD (Taken from Ref. [16]). Separation conditions: Fused-silica capillary, 30 cm (25.5 cm to detector)×50 μm I.D.; buffer, 100 mmol/l phosphate (pH 2.5); applied voltage 15 kV; UV detection at 200 nm.

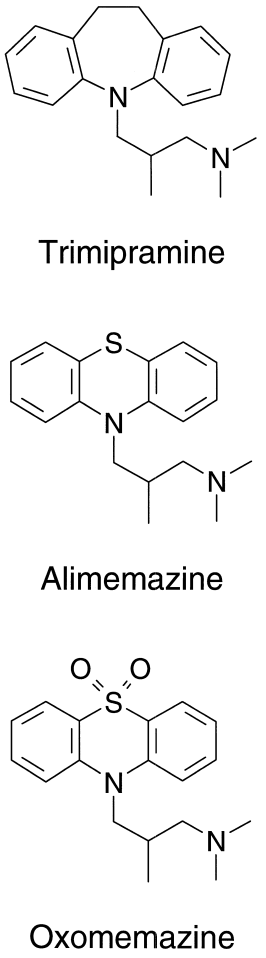


Fig. 14. Structure comparison of the tricyclic drugs trimipramine, alimemazine, and oxomemazine (Taken from Ref. [16]).

addressed as the ''substructure 4H'' throughout this paper is indeed sufficient for hydrophobic binding of the drug. Polar groups in the analyte structure, in particular the protonated tertiary amine located in the side chain of all three entries, will always stay hydrated in aqueous solution, but they are expected to interact with the hydroxyl groups at the upper rim of the CD. Dependent on stereoelectronic details in a given complex, subtle differences in interaction strength of the two drug enantiomers with the chiral cyclodextrin host may bring about a small yet observable enantiodiscrimination.

The comparison of the sulfone oxomemazine and the mercapto analogue alimemazine is also straightforward. Oxomemazine is bound less strongly, due to steric hindrance by the extra sulfone oxygen atoms; however, the binding is more enantioselective, as a consequence of an additional dipole dipole interaction of the type O–H···O=S. Moreover, the degree of enantiodiscrimination is decreased as the complexes gain conformational freedom, again in the order: α-CD>β-CD>γ-CD. In view of the peculiar conformation of the seven-membered ring, it is no surprise that trimipramine shows a different trend: Enantioseparation is only observed with γ-CD.

Table 4
CZE data of selected tricyclic drugs in the presence of native cyclodextrins: correlation with the inner diameter of the host cavity (taken from Ref. [16])[a]

	CSA					
	α-CD		β-CD		γ-CD	
	Inner diameter					
	5.7 Å		7.8 Å		9.5 Å	
	α_m	R_m	α_m	R_m	α_m	R_m
Trimipramine	1.000	2.756	1.000	2.494	1.037	2.042
Alimemazine	1.000	2.734	1.000	2.575	1.000	2.048
Oxomemazine	1.063	2.127	1.019	2.078	1.000	1.576

[a] α_m, migration separation factor; $\alpha_m = t_{m(2)}/t_{m(1)}$; R_m, migration retardation factor; $R_m = t_{m(2)}/t_{m(\text{plain})}$; α-, β-, and γ-CD were applied in concentrations of 45, 15, and 45 mmol/l, respectively.

The impact of *side-on-binding* was further corroborated on a broad statistical basis for all 86 drugs and γ-CD as the host. Again, an investigation of the success rate of enantiomer separation (R_{suc}) reveals a significant preference for analytes bearing this substructure; in this case, no more than three C–H units are just sufficient to produce a similar effect [16].

4. Conclusion

Side-on-binding of an aryl group at the border of the cyclodextrin cavity is a new, straightforward concept that readily explains the binding strength and the rate of success for enantioseparation of a great number of chiral drugs. A comparison of the size of the substructure 4H (generally an aryl group or a heterocycle) with the inner diameter of β-CD serves to explain the high chance of a successful enantioseparation. Likewise, a high rate of success may be

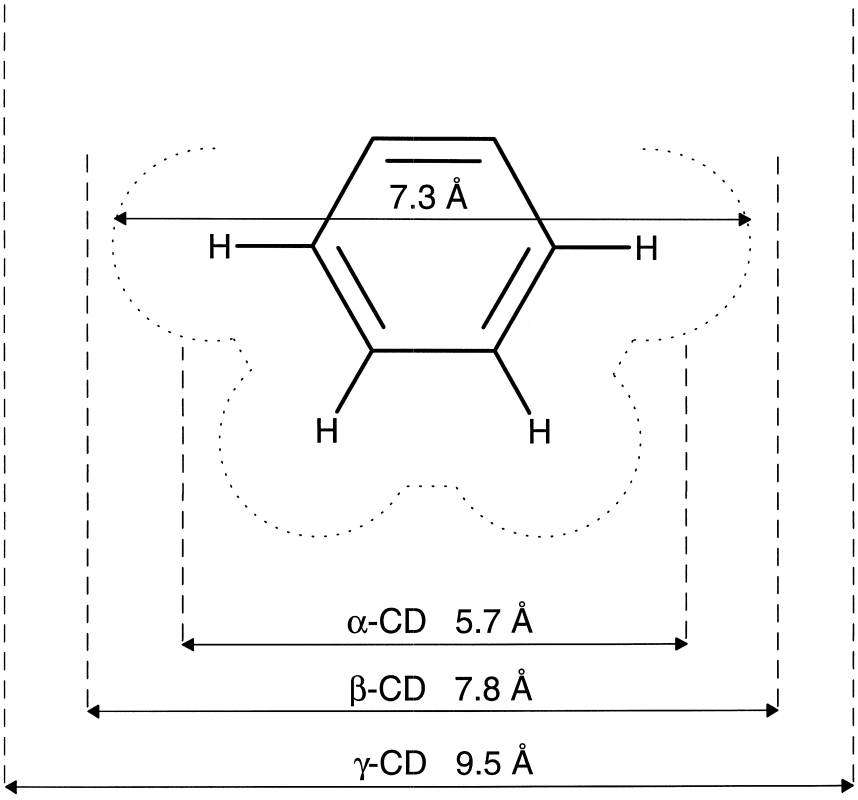


Fig. 15. Comparison of the benzene geometry with the cavity diameter of α-, β-, and γ-cyclodextrin (Taken from Ref. [16]).

expected for TM-β-CD and also for heptakis-6-*O*-hydroxypropyl-β-CD (HP-β-CD) [15]. As side-on-binding is also frequently encountered for other ring sizes, α- [11,18] and γ-cyclodextrin [10,16], as well as derivatives like HP-α-CD [14], HP-γ-CD [13], TM-α-CD [19], and TM-γ-CD [20] have their own merits. Other neutral cyclodextrins such as 2,6-di-*O*-methyl-β-CD, hydroxyethyl-β-CD, 2,3-di-*O*-acetyl-β-CD, carboxymethyl-β-CD, 6-*O*-α-D-glucosyl-α-cyclodextrin or 6-*O*-α-D-maltosyl-β-cyclodextrin [35–37] have been described (see also Tables 2 and 3 for numerous literature quotations), but they have not yet been investigated on a broad basis. Further studies on this topic by NMR-spectroscopy [38–40], microcalorimetry [41,42] and chemometric methods, including artificial neural networks [29], have been identified as an integral part of this screening project and will be reported in a different context.

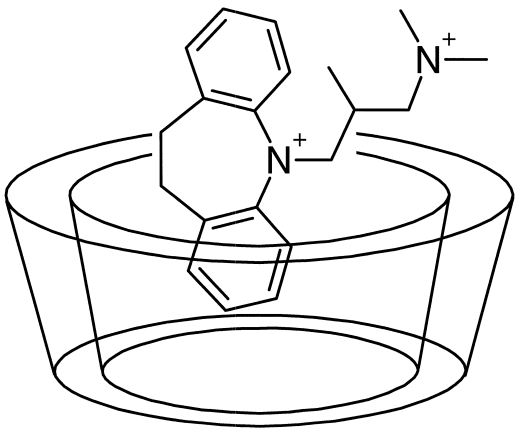


Fig. 16. Proposed model of interaction between trimipramine and γ-CD (Taken from Ref. [16]).

Acknowledgements

We would like to thank Fonds der Chemischen

Industrie, Deutsche Forschungsgemeinschaft and National Natural Science Foundation of China for grants (Project 29635020). Furthermore, we are indebted to Bio-Rad Labs. (Hercules, CA, USA) for support, and to various pharmaceutical companies for samples of drug racemates.

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