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Separation of enantiomers of drugs by capillary electrophoresis I. γ-Cyclodextrin as chiral solvating agent

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Abstract

Enantiomer separation was studied for a set of 57 chiral drugs. With γ-cyclodextrin as chiral solvating agent in capillary zone electrophoresis, seven enantiomeric pairs could be separated without recoursing to an optimization procedure. Possible interaction mechanisms between selector and selectand molecules are briefly discussed.

1. Introduction

The separation of enantiomers of chiral drugs deserves particular attention in view of the recent changes in regulation, by Japanese, Chinese, European and American institutions (e.g., Food and Drug Administration) [1]. Thus, in order to bring a new drug to the market, the main activity and possible side effects of both enantiomers of the drug must be established. This requires both preparative and analytical methods for the separation of enantiomers.

Due to its high resolution power, easy sample preparation and short analysis times, capillary electrophoresis has turned out as a favourable method in this field, in addition to high-performance liquid chromatography (HPLC) and gas chromatography (GC). As recorded in our molecular graphical database Chirbase/CE, different separation principles have been successfully applied. These include complexation by crown ethers [2,3], micellar electrokinetic chromatography (MEKC) with bile salts [4–6] and amino acid derivatives [7,8], ligand exchange [9,10], and affinity interaction with proteins [11–13]. By far the most convenient approach, however, involves the addition of cyclodextrins and derivatives thereof, respectively, as complexing agents to the running buffer [14,15]; this method was applied in 120 out of 247 papers quoted in our database. Whereas most experiments quoted in the literature were performed with β-cyclodextrins, γ-cyclodextrin (Fig. 1) was less frequently used, basically because of its higher price. In this paper we report first results of an extensive screening of chiral drugs with regard to separability with cyclodextrins in CE under different conditions, starting with native γ-cyclodextrin.

2. Experimental

All experiments were carried out on a Bio-Focus 3000 automatic capillary electrophoresis

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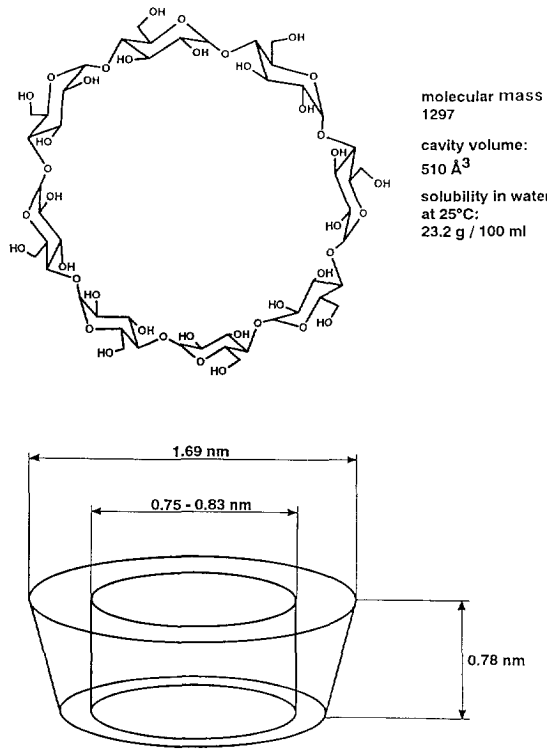


Fig. 1. γ-Cyclodextrin: structure, shape, properties.

system (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a variable-wavelength detector operated at 200 nm and an acrylamide-coated capillary from the same manufacturer. γ-Cyclodextrin was obtained from Wacker Chemie (Munich, Germany). Analytical samples of the chiral drugs were supplied by different pharmaceutical companies (3M Medica, Ankerpharm, Astra Chemicals, Arzneimittelwerk Dresden, Bayer, Boehringer Mannheim, Ciba, Durachemie, Gödecke, Hexal, Jenapharm, Klinge, Knoll, Krewel, Mann, Medice, Merck, Pfizer, Röhm Pharma, Rhône-Poulenc Rorer, Roche). All other chemicals were analytical grade.

The run buffer was prepared from a 0.1 *M* solution of sodium dihydrogenphosphate (NaH_2PO_4) and adjusted to pH 2.5 with phosphoric acid (H_3PO_4). As a chiral solvating agent (CSA), γ-cyclodextrin was dissolved in the plain phosphate buffer, to give a 15 mmol/l solution.

Stock solutions of the bulk drug samples (1 mg/ml) were prepared in deionized and distilled water. These were diluted ten-fold with a 1:1 mixture of purified water and the run buffer to give the sample solutions, which were transferred to the capillary by electrokinetic injection. The injection was carried out by applying a voltage of 8 kV for 6 s.

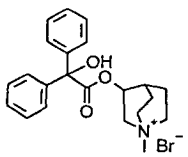
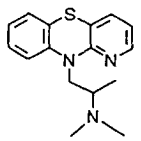
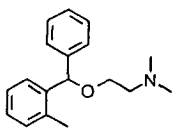
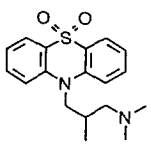
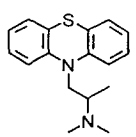
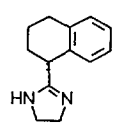
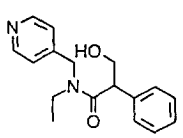
3. Results

Under standard conditions, kept as constant as possible throughout this study, seven enantiomeric pairs could be separated without recoursing to a time-consuming optimization procedure. In Table 1, these analytes are listed in alphabetical order of their names. All drug names used are international nonproprietary names of the pharmaceuticals assigned by the World Health Organization (WHO). The migration times in plain phosphate buffer were compared to the migration times in cyclodextrin-containing phosphate buffer. For all of the analytes separated, migration times were remarkably prolonged by the addition of γ-cyclodextrin to the running buffer, indicating a significant interaction between analyte and selector molecules.

The ratio of the migration times of the enantiomers, tm_2/tm_1, was taken as a measure of separation selectivity since no endoosmotic flow was observed [16]. The separation factors obtained range from 1.046 to 1.009. The enantiomers of oxomemazine, with a separation factor of 1.046, were completely resolved, as shown in Fig. 2. As demonstrated for tetryzoline in Fig. 3, a separation factor of 1.028 was still almost sufficient for baseline separation. Tropicamide with a separation factor of 1.009 was only partially resolved. This and other separations may be optimized further by adjusting both CSA concentration and pH individually for each analyte.

Table 2 compiles 50 racemates, in alphabetical order of their names, which failed to separate under standard conditions. Again, the molecular structures of all analytes are listed, along with their migration times in the CSA-containing

Table 1
Compounds successfully separated into the enantiomers, under standard conditions

Compound structure	Pharmaceutical name	tm_p [a] (min)	tm_1 [b] (min)	tm_2 [b] (min)	tm_2/tm_1 [c]
	Clidinium bromide	10.05	11.98	12.23	1.021
	Isothipendyl	8.83	11.17	11.33	1.014
	Orphenadrine	12.05	13.60	13.80	1.015
	Oxomemazine	8.98	11.95	12.50	1.046
	Promethazine	8.73	12.19	12.44	1.028
	Tetryzoline	7.57	8.35	8.58	1.028
	Tropicamide	8.60	10.46	10.55	1.009

[a] Migration time in plain phosphate buffer.
[b] Migration times in cyclodextrin containing phosphate buffer; first and second detected enantiomer, respectively.
[c] Separation factor of the enantiomers.
Conditions: instrument, Bio-Rad Bio-Focus 3000; capillary, fused-silica, coated, 44.5 cm × 50 μm; sample concentration, 0.1 mg/ml; buffer, phosphate, 100 mmol/l, pH 2.5; CSA, γ-cyclodextrin, 15 mmol/l; injection, 6 s, 8 kV; run, 14 kV +→−; detection, 200 nm/0.005 AUFS; capillary temperature, 30°C.

buffer. Although most analytes showed prolonged migration times as compared to plain buffer, some were not clearly retarded. In these cases, the limits of long-term reproducibility of the absolute migration times did not permit a quantitative treatment.

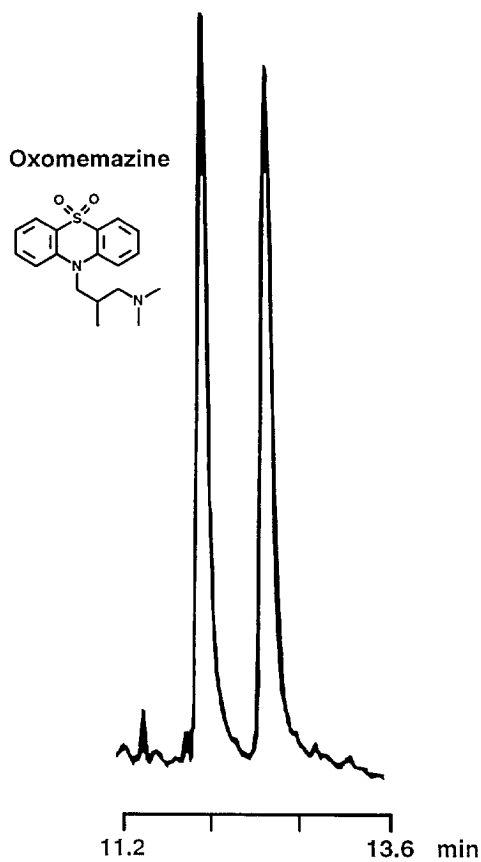


Fig. 2. Electropherogram of oxomemazine enantiomers. Conditions as quoted in Table 1. Detection at 200 nm, 0.005 AUFS.

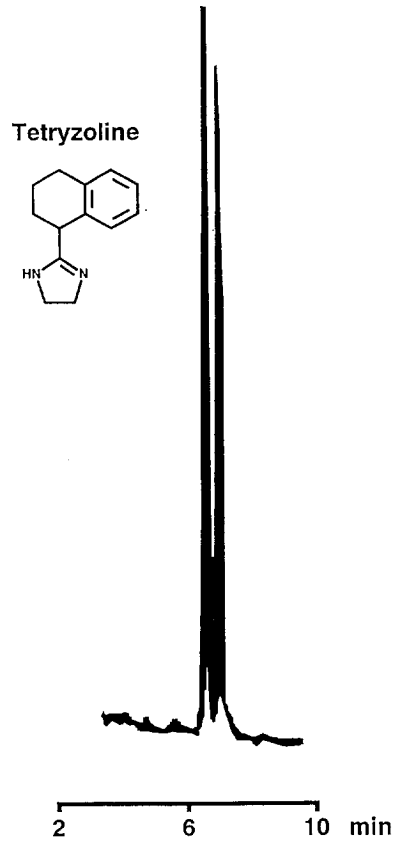


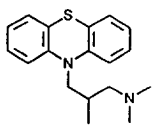
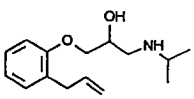
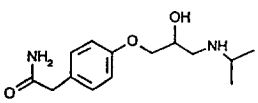
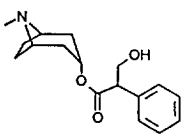
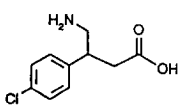
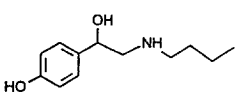
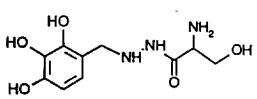
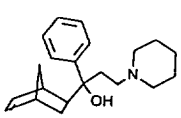
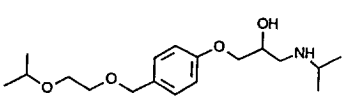
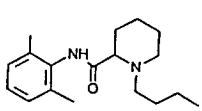
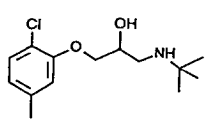
Fig. 3. Electropherogram of tetryzoline enantiomers. Conditions as quoted in Table 1. Detection at 200 nm, 0.005 AUFS.

4. Discussion

As illustrated in Fig. 1, the γ-cyclodextrin molecule has a cavity of a diameter of 0.75–0.83 nm [17]. In α- and β-cyclodextrin, the diameter is approximately 0.30 and 0.15 nm smaller, respectively. Although the cavity in γ-cyclodextrin was occasionally considered too large for a significant enantiomer discrimination, there was a definite lack of experimental proof for this view. Indeed, an open mind might expect even improved recognition properties for the γ-cyclodextrin selector at least for large, bulky selectands. It should be mentioned that there are biological receptors with active sites larger than 80 nm, apt to include guests as large as a steroid molecule [18].

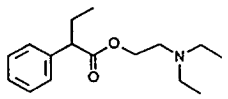
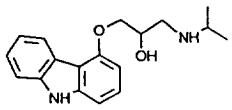
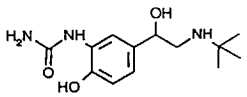
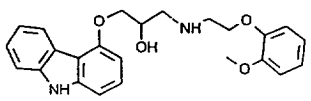
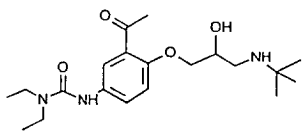
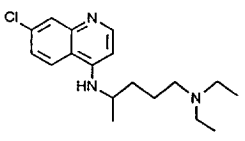
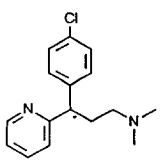
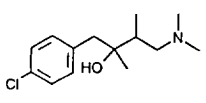
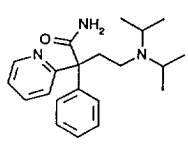
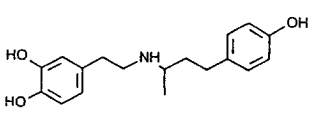
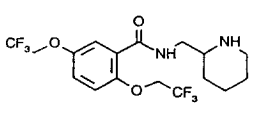
From these considerations, we expected to find several of the large tricyclic antihistaminic drugs among the seven at least partially separated analytes, as listed in Table 1. On the other hand, bicyclic and monocyclic sub-structures are also present among the molecules separated. Still these molecular structures are dominated by an extended, flat region. It may be assumed that these regions form an alignment with the upper rim of the cyclodextrin. The two enantiomers then differ significantly in their ability to stick their basic tail into the partially acidic cyclodextrin cavity. This model takes into consideration that possible interaction mechanisms do not only involve internal inclusion complex formation, but also external association [19]. An alternative model suggests to include the aromatic and

Table 2
Compounds not separated into the enantiomers, under standard conditions

Compound structure	Pharmaceutical name	tm [a] (min)
	Alimemazine	14.49
	Alprenolol	11.52
	Atenolol	9.61
	Atropine	11.59
	Baclofen	8.31
	Bamethan	9.14
	Benserazide	7.89
	Biperiden	14.04
	Bisoprolol	10.90
	Bupivacaine	8.75
	Bupranolol	13.97

(Continued on p. 186)

Table 2 (*continued*)

Compound structure	Pharmaceutical name	tm [a] (min)
	Butetamate	9.96
	Carazolol	10.77
	Carbuterol	9.55
	Carvedilol	14.13
	Celiprolol	11.48
	Cloroquine	5.79
	Chlorphenamine	5.85
	Clobutinol	8.54
	Disopyramide	8.80
	Dobutamine	11.12
	Flecainide	9.99

(*Continued on p. 188*)

Table 2 (*continued*)

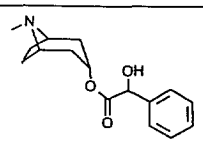
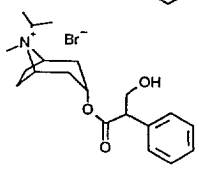
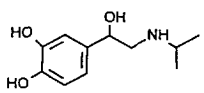
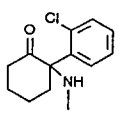
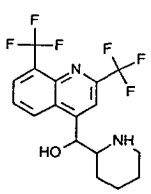
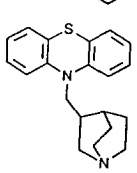
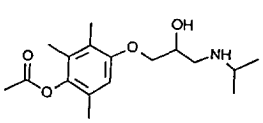
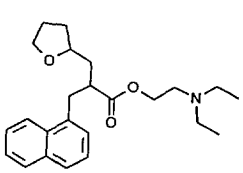
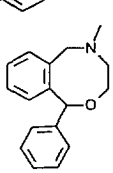
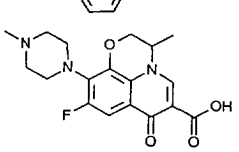
Compound structure	Pharmaceutical name	tm [a] (min)
	Homatropine	8.54
	Ipratropium bromide	10.97
	Isoprenaline	8.50
	Ketamine	10.01
	Mefloquine	10.10
	Mequitazine	14.49
	Metipranolol	10.35
	Naftidrofuryl	14.12
	Nefopam	9.38
	Ofloxacin	9.04

Table 2 (*continued*)

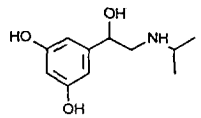
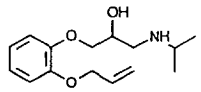
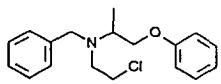
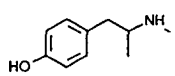
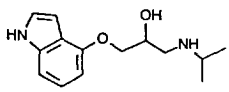
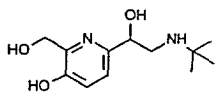
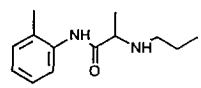
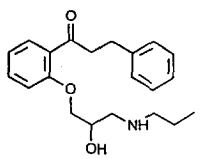
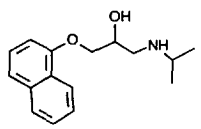
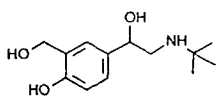
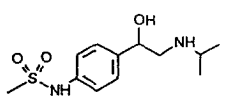
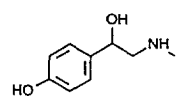
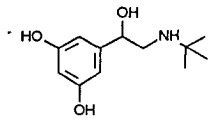
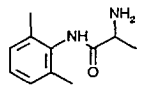
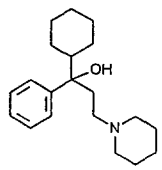
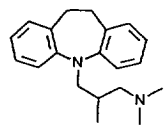
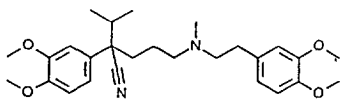
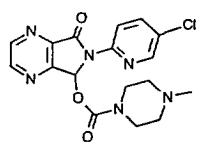
Compound structure	Pharmaceutical name	tm [a] (min)
	Orciprenaline	9.45
	Oxprenolol	9.09
	Phenoxybenzamine	11.17
	Pholedrine	7.45
	Pindolol	9.28
	Pirbuterol	6.74
	Prilocaine	8.70
	Propafenone	13.54
	Propranolol	10.75
	Salbutamol	9.01
	Sotalol	9.29
	Synephrine	7.57

Table 2 (*continued*)

Compound structure	Pharmaceutical name	tm [a] (min)
	Terbutaline	9.63
	Tocainide	8.53
	Trihexyphenidyl	12.79
	Trimipramine	20.13
	Verapamil	13.76
	Zopiclone	10.04

[a] Migration time in CSA containing buffer.
Conditions as quoted in Table 1.

heterocyclic portions, respectively, leaving the amino groups to external association. One may favour the first over the second model in view of the small retention increase of tetryzoline, bearing a bulky imidazoline side-chain, as compared to oxomemazine, promethazine and isothipendyl, each having an open chain side-chain (all structures are depicted in Table 1). On this basis, it may be readily explained why the even more bulky structure of mequitazine was not separated at all (see Table 2); however, the lack of separation of alimemazine (see Table 2), with a close similarity to both oxomemazine and promethazine (found in Table 1), appears rather puzzling on first glance. This indicates clearly that further experimental evidence is required to decide on this question.

Moreover, not only the spatial conformation of the selectands should be considered, but also other properties, such as polarity and hydrogen-bonding capability. It will be one of our future aims to gather independent information on these questions by physicochemical studies of well-defined host–guest complexes of this type.

Throughout the study, the experimental conditions had been kept as constant as possible within the limits of this methodology. The given pair of CSA concentration and buffer pH is a

priori not optimal in this survey, because different complex formation constants and pK_a values of different selectands are expected to cause significant variations in the individual optimum separation conditions.

Such an individual optimization of the separation conditions for each analyte will certainly yield further progress in peak resolution, at least for the analytes listed in Table 1. The method development will also include the evaluation of reproducibility and detection limits. Last but not least, due to the higher solubility of γ-cyclodextrin compared to β-cyclodextrin, there is still a chance to find separation conditions for up to now unresolved enantiomeric pairs by choosing higher concentrations of γ-cyclodextrin as chiral solvating agent.

Acknowledgements

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