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USE OF CYCLODEXTRINS IN ISOTACHOPHORESIS

VII. RESOLUTION OF STRUCTURALLY RELATED AND CHIRAL PHENOTHIAZINES

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SUMMARY

Commercially available phenothiazine derivatives were used for the study of cyclodextrin complex formation by cationic isotachophoresis with α-, β- and γ-cyclodextrin and methylated analogues of β-cyclodextrin as leading electrolyte additives. The relationships between the type of solute substituent in the 10- and/or 2-position and the stability of the created cyclodextrin complex were studied and the results were utilized for the optimization of isotachophoretic conditions suitable for the resolution of the studied phenothiazine derivatives. Successful resolution of three racemic solutes was achieved.

INTRODUCTION

Phenothiazine and its derivatives are an important group of neuroleptic and anti-allergic drugs. More than 100 compounds derived from the fundamental phenothiazine skeleton have been synthesized and pharmacologically tested during the past few decades[1]. The most important from the clinical point of view proved to be derivatives substituted in the 10- and/or 2-position.

From the analytical point of view, commercially produced phenothiazine derivatives (approximately 50 registered drugs) represent a readily available set of structurally related compounds that could be utilized as model solutes for the study of various separation mechanisms and for testing the capabilities of proposed analytical systems to give a precise correlation between structure and retention data.

The favourable mass-to-charge ratio and suitable molecular structure of most phenothiazine derivatives substituted in the 10-position make it possible to utilize

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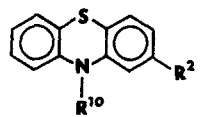
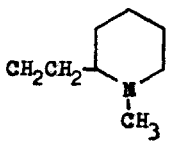
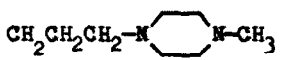
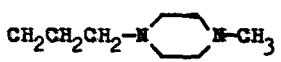
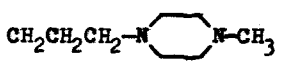
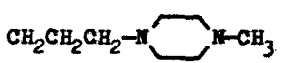
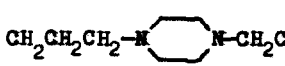
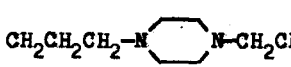
TABLE I

STRUCTURAL FORMULAE OF THE COMPOUNDS INVESTIGATED



Compound	R^2	R^{10}	*Name*
1 [(+)- and (−)-]	SCH_3		Thioridazine = 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylthiophenothiazine
2 [(+)- and (−)-]	H	$CH_2CH(CH_3)N(CH_3)_2$	Promethazine = 10-[2-(dimethylamino)propyl]phenothiazine
3 [(+)- and (−)-]	CN	$CH_2CH(CH_3)CH_2N(CH_3)_2$	Cyamepromazine = 10-[3-(dimethylamino)-2-methylpropyl]-phenothiazine-2-carbonitrile
4	H	$CH_2CH_2CH_2N(CH_3)_2$	Promazine = 10-[3-(dimethylamino)propyl]phenothiazine
5	Cl	$CH_2CH_2CH_2N(CH_3)_2$	Chlorpromazine = 2-chloro-10-[3-(dimethylamino)propyl]-phenothiazine
6	Cl		Prochlorperazine = 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine
7	CF_3		Trifluoperazine = 10-[3-(4-methyl-1-piperazinyl)propyl]-2-trifluoromethylphenothiazine
8	SCH_2CH_3		Thiethylperazine = 2-ethylthio-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine
9	$SO_2N(CH_3)_2$		Thioproperazine = N,N-dimethyl-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine-2-sulfonamide
10	Cl		Perphenazine = 4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazineethanol
11	CF_3		Fluphenazine = 4-[3-(2-trifluoromethylphenothiazin-10-yl)propyl]-1-piperazineethanol

them as a model compounds for the study of cyclodextrin (CD) inclusion complex formation using cationic isotachophoresis (ITP). Previous experiments showed that cyclodextrins, behaving as specific selectors, may substantially improve the separation of structurally related compounds and various types of isomers[2–7]. The aim of this work was to study the interactions of cyclodextrins with structurally related phenothiazine derivatives and to propose ITP conditions suitable for their effective resolution. Attempts have been made to obtain relationships between the type of solute substituent in the 10- and/or 2-position and the stability of the CD complex formed. Particular attention has been paid to the optimization of ITP conditions suitable for the resolution of three racemic solutes.

EXPERIMENTAL

Chemicals

Redistilled water was used in the preparation of the solutions of the electrolytes and compounds investigated. All chemicals were of the highest quality commercially available: sodium hydroxide, 4-morpholinoethanesulphonic acid (MES), β-alanine (β-ALA), 37% hydrochloric acid, acetic acid, sodium acetate, L(+)-tartaric acid (Merck, Darmstadt, F.R.G.); 6-aminocaproic acid (EACA) (Sigma, St.Louis, MO, U.S.A.); Natrosol 250 HR (hydroxyethylcellulose; HEC) (Hercules, Wilmington, U.S.A.); Zerolit DM-F (indicator) (BHD, Poole, U.K.); α- and γ-cyclodextrins (α- and γ-CD) (Astec, Whippany, U.S.A.); β-cyclodextrin (β-CD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (diMe-β-CD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (triMe-β-CD) (Chinoin, Budapest, Hungary). Natrosol 250 HR solutions were purified by using Zerolit DM-F mixed-bed ion-exchange resin.

The solutes investigated (Table II) were provided by laboratories of the State Institute for the Control of Drugs (Prague, Czechoslovakia). Sample solutions (2 mg/ml) were prepared by dissolving each substance in 50 m*M* hydrochloric acid and were stored in dark bottles in a refrigerator.

Methods

Isotachophoretic experiments were performed using a Tachopor 2127 instrument (LKB, Bromma, Sweden), equipped with a conductivity detector and a poly(tetrafluoroethylene) capillary. The sample solutions were injected with a 10-μl microsyringe (Hamilton, Bonaduz, Switzerland). The operating conditions are given in Table II.

The optical rotation of optically enriched mixtures of enantiomeric phenothiazines (compounds **1**, **2** and **3**) were measured with a Model 241 polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.).

The ITP separation pattern of the enantiomers was determined indirectly by comparison of ITP and optical rotation measurements for optically enriched samples (obtained by selective precipitation of racemates with γ-CD (compound **1**) or with L(+)-tartaric acid (compounds **2** and **3**).

RESULTS AND DISCUSSION

The preliminary ITP experiments, aimed at selecting suitable ITP conditions,

showed that the optimum pH of the leading electrolyte (LE), ensuring sufficient ionization and optimum ITP resolution of the solutes in the form of uncomplexed ions (unmodified LE) or inclusion complexes (CD-modified LE), lies between 5.2 and 5.7. Two electrolyte systems with MES or acetate counterions were chosen for the following experiments.

To test the influence of the types of CDs used on the effective mobility and the possible resolution of the compounds investigated, the relative step heights, $(h_i)_{rel} = h_S - h_L / h_T - h_L$ [h_S, h_L and h_T = step heights of the sample, LE and terminating electrolyte (TE), respectively] were measured.

The effectiveness of the isotachophoretic resolution was determined on the basis of $(h_i)_{rel}$ differences $[\Delta(h_i)_{rel}]$ of the solutes. According to the given experimental conditions determining the accuracy and reproducibility of the measurements, $(h_i)_{rel}$ differences lower than 0.01 were considered to be negligible and the solute pairs involved to be unresoluted. A value of 0.02 was specified as the lowest $\Delta(h_i)_{rel}$ limit in order to achieve effective and practically significant solute resolution. Intermediate $\Delta(h_i)_{rel}$ values in the range 0.01–0.02 are characteristic of solute pairs that could hardly be separated under given ITP conditions. A low resolution, resulting from closely similar effective mobilities of the solutes, requires the use of long capillary in order to obtain a sufficient maximum load capacity[8] acceptable in practice.

All important data obtained from the experiments with single solutes are summarized in Table III. For a clearer understanding of the experimental data and an easier description of the role of CDs in structural differentation, the compounds studied were divided into several subsets in which only one structural parameter of the molecule was changed. With such an approach, the solutes (**6**, **7**, **8**, **9**), (**4**, **5**) and **10**, **11**) can be included in subset A (with a common alicyclic or heterocyclic-containing 10-substituent but varying 2-substituent). Analogously, the solutes (**5**, **6**, **10**) and (**7**, **11**) form subset B (with a common 2-substituent but varying 10-substituent). The

TABLE II

ELECTROLYTE SYSTEMS AND CONDITIONS FOR ITP

Parameter	*Conditions*
Leading electrolytes:	
LE I	10 m*M* sodium hydroxide including 0.04% HEC adjusted with MES to pH 5.57
LE II	10 m*M* sodium acetate including 0.04% HEC adjusted with acetic acid to pH 5.55
Terminating electrolytes:	
TE I	10 m*M* EACA
TE II	10 m*M* β-Ala
Capillaries:	
I	250 mm × 0.55 mm I.D.
II	520 mm × 0.55 mm I.D.
Current	For capillary I, LE I and II and TE I: 150 μA (5 min), 50 μA for detection
	For capillary II, LE II and TE II: 100 μA (15 min), 50 μA for detection
Thermostat temperature	18°C
Detection	Conductivity

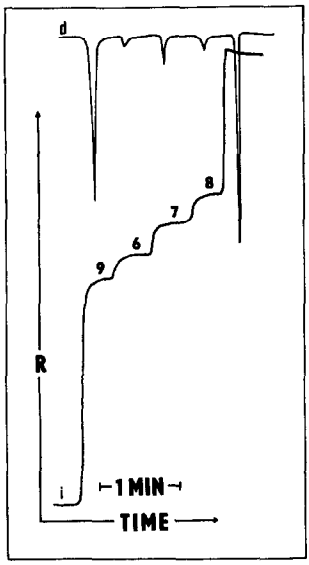


Fig. 1. ITP separation of a mixture of compounds **6–9**. Electrolyte system: LE I with 10 m*M* triMe-β-CD and TE I; capillary I. Injected volume: 2 μl (concentration of each compound = 0.5 mg/ml). *R* = detector response; *i* = conductivity signal; *d* = differentiated conductivity signal.

most interesting from the analytical point of view is probably subset C, containing racemic solutes (**1**, **2**, **3**).

ITP resolution of subset A solutes.

The $(h_i)_{rel}$ difference of solutes **6** and **9** with the unmodified electrolyte system LE I, TE I is not significant and the system does not resolve them. Solute **8** is not able to form a stable zone owing to its low solubility under the given ITP conditions.

The addition of α-CD to LE I significantly improves the solubility of solute **8** and makes it possible to differentiate **8** and **9**, with bulky $-SC_2H_5$ and $-SO_2N(CH_3)_2$ substituents, from 6 and 7, with small Cl and CF_3 groups in the 2-position. Increasing the α-CD concentration results in a significantly stronger retardation of solutes 6 and 7. It can be assumed that the mentioned bulky substituents protect one of the aromatic rings of phenothiazine against complexation with the relatively small α-CD cavity whereas Cl and CF_3 substituents are still dimensionally acceptable for complexation.

The $(h_i)_{rel}$ differentiation of solutes **6**, **7**, **8** and **9** is not achieved with β-CD- and diMe-β-CD-modified LE I, even though the retardation effects are much more significant than with α-CD. This result does not conflict with the mentioned concept of blockage of α-CD binding positions by the phenothiazine 2-substituent. An increase in the diameter of the CD cavity (α-CD < diMe-β-CD < β-CD) makes the differences in the dimensions of the 2-substituents not so critical for inclusion complex formation.

The measurements with γ-CD-modified LE I demonstrated the possibility of the resolution of solute **6**, which is less retarded from the others. Such a reappearance

TABLE III

$(h_i)_{rel}$ VALUES OF COMPOUNDS INVESTIGATED

Values measured in capillary I.

Conditions	*Compound*										
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***
LE I, TE I	0.300	0.284	0.261	0.279	0.286	0.291	0.325	–	0.292	0.357	0.365
LE I, TE I with 5 m*M* α-CD	0.365	0.309	0.299	0.311	0.344	0.400	0.403	0.345	0.353	0.434	0.436
LE I, TE I with 10 m*M* α-CD	0.439	0.347	0.354	0.356	0.410	0.456	0.453	0.370	0.387	0.498	0.460
LE I, TE I with 20 m*M* α-CD	0.517	0.400	0.417	0.419	0.496	0.549	0.528	0.443	0.450	0.588	0.570
LE I, TE I with 5 m*M* β-CD	0.750	0.600(+) 0.612(−)	0.705(+) 0.711(−)	0.682	0.727	0.745	0.752	0.755	0.737	0.767	0.765
LE I, TE I with 5 m*M* γ-CD	0.558(+) 0.618(−)	0.369	0.396	0.409	0.409	0.602	0.695	0.670	0.672	0.575	0.671
LE I, TE I with 10 m*M* γ-CD	0.637(+) 0.707(−)	0.420	0.441	0.471	0.456	0.683	0.777	0.755	0.756	0.669	0.764
LE I, TE I with 20 m*M* γ-CD	0.700(+) 0.756(−)	0.457	0.480	0.525	0.483	0.728	0.780	0.783	0.786	0.711	0.786
LE I, TE I with 5 m*M* diMe-β-CD	0.776	0.711	0.758	0.758	0.754	0.777	0.789	–	0.777	0.790	0.790
LE I, TE I with 10 m*M* diMe-β-CD	0.794	0.771	0.779	0.780	0.786	0.792	0.806	–	0.824	0.816	0.831
LE I, TE I with 10 m*M* triMe-β-CD	0.514	0.314	0.317	0.344	0.434	0.570	0.633	0.685	0.508	0.574	0.626
LE II, TE I	0.497	0.483	0.442	0.471	0.472	0.553	0.608	–	0.532	0.594	0.668
LE II, TE I with 2.5 m*M* γ-CD	0.849(+) 0.940(−)	0.609	0.648	0.668	0.688	–	1.094	–	1.034	0.899	1.075

of structurally based complexation selectivity, which was unexpected owing to the large size of the γ-CD cavity, indicates a fundamental difference in the structure of the γ-CD–phenotiazine complexes.

Only triMe-β-CD proved to be an efficient LE I additive for complete $(h_i)_{rel}$ differentiation of the solutes studied. It ensures both solubilization and the effect of structural differentiation of the solutes depending on the type of 2-substituent. An example of the successful ITP separation of solutes **6**, **7**, **8** and **9** with a triMe-β-CD-modified LE I is shown in Fig. 1.

Solutes **4** and **5** are able to migrate isotachophoretically in the unmodified LE I, TE I electrolyte system, but they could not be resolved here. The studied CDs added to LE I, except diMe-β-CD, improve the resolution of these compounds. The cyclodextrins could be arranged in the order γ-CD < α-CD < β-CD < triMe-β-CD with regard to their $(h_i)_{rel}$ differentiation effect. The separation pattern of solutes **4** and **5** with α-, β- and triMe-β-CD in LE I is in agreement with our previous experiments; solute **5**, containing a covalently bonded halogen substituent on the aromatic ring, forms stronger inclusion complexes than the unsubstituted solute **4**. A reversed separation pattern was observed at higher γ-CD concentrations (20 m*M*), which supports the idea that the phenotiazine molecule binding sites for γ-CD differ from those for α-, β- and triMe-β-CD.

Similar conclusions could be drawn for solutes **10** and **11**. The $(h_i)_{rel}$ differences in the unmodified and β-CD-containing electrolyte systems is negligible. The addition of α-CD to the LE causes a stronger retardation of solute **10**; γ-CD and triMe-β-CD reverse the separation pattern.

ITP resolution of subset B solutes

From the experiments with subset B solutes it can be generally concluded that complex formation with the CDs studied depends significantly on the structure of the 10-substituent. The weakest retardation was observed for solute **5**, with an alicyclic 10-substituent. Introduction of a piperidine ring into the 10-substituent (solute **6**) had an important effect, supporting inclusion complex formation with the CDs studied. The cyclodextrins could be arranged into the order β-CD < diMe-β-CD < α-CD < triMe-β-CD < γ-CD with respect to their influence on the $(h_i)_{rel}$ differentiation of solutes **5** and **6**.

Extension of the hydrocarbon chain in the 10-substituent and its termination with an OH group [solute **10** (**11**)] is a structural change that alters especially the complex formation with γ-CD. The addition of γ-CD to LE I results in a strong retardation of solute **6** (**7**) and inversion of the separation pattern of solutes **6** and **10** (**7** and **11**) compared with the unmodified elctrolyte system. The other CDs tested did not show any positive resolution effect, their addition resulting in a decrease in the $(h_i)_{rel}$ differences.

Chiral resolution of subset C solutes

Two electrolyte systems, with MES (LE I) and acetate (LE II) as counterions, were used. The experiments showed that the chiral resolution of solute **1** enantiomers could be achieved with γ-CD-modified LE I or LE II only. The other CDs tested proved to be enantiospecifically ineffective. Although it is not possible to compare directly the $(h_i)_{rel}$ differences for the two enantiomers obtained with LE I and LE II, it

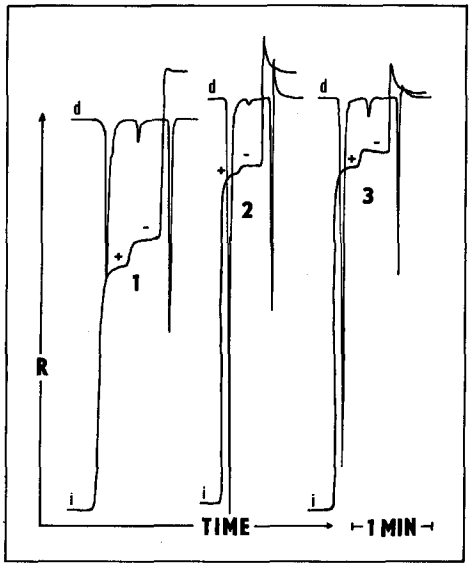


Fig. 2. ITP separation of enantiomers of compounds **1**, **2** and **3**. Electrolyte systems: LE I with 5 m*M* γ-CD for **1**, LE II with 3 m*M* β-CD for **2** and LE II with 1 m*M* β-CD for **3**; TE I for **1** and TE II for **2** and **3**; capillary I for **1** and II for **2** and **3**. Volume injected: 2 μl for **1** and 1 μl for **2** and **3** of racemate solutions. + = Dextrorotatory and − = laevorotatory isomer. *R*, *i* and *d* as in Fig. 1.

can be concluded that the effective chiral resolution of solute 1 with LE II could be achieved with substantially lower γ-CD concentration than with LE I. The possible explanation of this result is based on differences in the stability of the competitive counter-ion inclusion complexes. The small acetate ion, in comparison with the heterocycle-containing MES, is not able to block effectively the CD cavity and thus compete against the CD–solute complex formation.

The choice of ITP conditions suitable for chiral resolution of solutes 2 and 3 is more complicated. β-CD, which seems to be the only enantioselective additive suitable for the resolution of both racemates, provides only a slight differentiating effect and makes the optimization of other ITP conditions critical. It is not possible to achieve chiral resolution with electrolyte system LE I, TE I, probably owing to the above-mentioned counter-ion complex formation which cancels completely the eniantioselectivity of β-CD. Strong retardation effects of β-CD added to LE II requires the use of the slower TE II, which makes it possible to work at optimum β-CD concentrations without the risk of losing the solute in the terminator zone. To improve the maximum racemate load, $(n_r)_{max}$[6], it was necessary to use the longer capillary II.

A practical example of the resolution of racemic solutes **1**, **2** and **3** under optimum ITP conditions is demonstrated in Fig. 2.

CONCLUSIONS

Cyclodextrins, as LE additives, were successfully applied to the improvement of the resolution of various commercially available phenothiazine derivatives. The im-

portant characteristics of the CD-based separation process were studied. The solubilization effect of CDs permits the analysis of poorly soluble phenothiazine derivatives in aqueous electrolyte systems. Structurally related phenothiazine derivatives could be resolved effectively with CD-modified electrolyte systems, depending on the differences in their 2- and 10-substitution.

The chiral resolution of racemic phenothiazine derivatives could be achieved with β- or γ-CD-modified electrolyte systems. Some additional parameters, such as the type of counter ion used, play a crucial role in the quality of the chiral resolution and must be involved in the optimization procedure.

The method developed is very advantageous for practical monitoring of phenothiazine synthesis or drug analysis and broadens the applicability of CD inclusion pseudo-phases in isotachophoresis.

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REFERENCES

1 E. Usdin and D. H. Efron, *Psychotropic Drugs and Related Compounds*, Department of Health, Education and Welfare, Health Services and Mental Health Administration, National Institute of Mental Health, Washington, DC, 1972.
2 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 405 (1987) 379.
3 J. Snopek. I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 411 (1987) 153.
4 I. Jelínek, J. Dohnal, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 435 (1988) 496.
5 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
6 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 439 (1988) 386.
7 J. Snopek, E. Smolková-Keulemansová, I. Jelínek, J. Dohnal, J. Klinot and E. Klinotová, *J. Chromatogr.*, 450 (1988) 373.
8 F. E. P. Mikkers, F. M. Everaerts and J. A. F. Peek, *J. Chromatogr.*, 168 (1979) 293.