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Chromatographic liquid–liquid ternary phase system with permethylated β-cyclodextrin as chiral additive

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ABSTRACT

An enantioselective liquid–liquid high-performance liquid chromatography system based on ternary systems with limited miscibility and chiral additives which are concentrated in the stationary liquid, is proposed, tested and characterized. Enantiomers of different types of compounds were used to probe enantioselectivity of the system. Conditions at which the partition mechanism of the chromatographic separations may be assumed are given and proven experimentally. The water–ethanol–2,2,4-trimethylpentane system at 25°C was used with permethylated β-cyclodextrin as the chiral additive.

INTRODUCTION

The ternary liquid–liquid systems with limited miscibility introduced and developed by Huber and co-workers [1–5] have proved to be of importance in high-performance liquid chromatography (HPLC). In a series of papers [3–6] it has been demonstrated that from the point of view of variation of capacity factor range and selectivity, reproducibility of retention characteristics and long-term stability of chromatographic columns the ternary systems are advantageous over other partition systems.

The principle of the idea may be seen in Fig. 1 representing the phase diagram of the water–ethanol–2,2,4-trimethylpentane system at 25°C. A mixture of a gross composition falling within the range of limited miscibility (L) splits into two liquid phases, one of which is water-rich (and thus "polar") while the other is a hydrocarbon-rich phase ("apolar"). The advantage of using such a system is that by choosing appropriate gross compositions one may modify the "polarity" of the liquid phases being in equilibrium, and in this way one may control the selectivity of the system.

The immiscible ternary phases could be used as liquid phases in partition chromatography. The partition system can be created by one of two methods: by the conventional way [2] or by the "solvent-generated" technique [3–6].

In the conventional method the solid support is coated with the stationary phase before column is packed, or the stationary liquid is injected into the column in the eluent stream.

In the solvent-generated phase technique, one of the liquid phases of the

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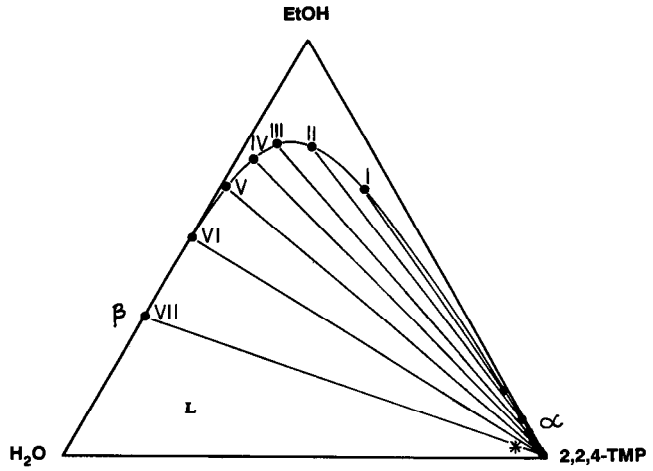


Fig. 1. Triangular phase diagram of the ternary system consisting of water–ethanol–2,2,4-trimethylpentane at 25.0 ± 0.1°C [2]; β = water-rich phase; α = water-poor phase, ✳ = gross composition.

liquid–liquid systems is used as eluent. The corresponding second liquid phase is spontaneously generated on the solid support surface, provided the column used is packed with an appropriate solid support, *i.e.*, the solid support must be better wetted by the stationary liquid phase than by the eluent.

It seemed interesting to extend the use of the ternary HPLC systems to chiral separations. A way to attain this would be to modify the selectivity of the system by means of chiral additives. The main assumption of this approach is that the additives, when present in minute concentrations, do not significantly change miscibility in the ternary system used and that the only important characteristic of the additive is its appropriate enantioselectivity. For this reason cyclodextrins and their derivatives seem to represent a good choice. In a recently reported series of papers, Sybilska and co-workers [7–11] have demonstrated that cyclodextrins may conveniently be used for chiral separations when used as the component of a mobile phase in HPLC.

In this work the ternary partition system water–2,2,4-trimethylpentane–ethanol with chiral additives was used for chiral separation. A hydrophilic solid support was coated with the more polar liquid phase of the ternary phase system containing as a chiral additive different amounts of permethylated β-cyclodextrin, a compound successfully used for enantiomeric resolution in liquid–solid chromatography (LSC) [12,13].

Enantioselectivity of the partition system is shown and column stability and efficiency are discussed.

EXPERIMENTAL

Apparatus

Chromatographic experiments were carried out with a Type 310 high-pressure microbore liquid chromatograph (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a UV detector (254 nm) containing a 1-μl

flow cell and a 0.5-μl injector. The column was kept at within ±0.1°C of the desired temperature using a water thermostat (Typ U3, MLW, Germany).

Partition coefficients were determined by a Specord (Carl Zeiss, Jena, Germany) UV–VIS absorption spectrometer equipped with quartz cells with a light path of 30 mm. Magnetically stirred glass vessels kept in the water bath were used for the determination of the static partition coefficients. The water bath (Typ U10, MLW) was used to control the temperature within ±0.1°C.

Reagents

Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) was supplied by Chinoin (Budapest, Hungary). D-Mandelic and L-mandelic acid enantiomers were supplied by E. Merck and Fluka, respectively. The pure enantiomers of methyl mandelate were prepared in simple esterification reactions from D- and L-mandelic acids in methanol solutions with sulphuric acid as catalyst. 2,2′-Dihydroxy-1,1′-binaphthol was synthesized and purified according to Vogel [14].

All other reagents and solvents were of analytical/reagent grade and were used without purification. The formulae of the investigated compounds are given in Table I.

LiChrosorb Si 60, 5 μm (E. Merck) was used as column packing. The test substances, because of their poor solubility in the eluent, were dissolved in a 2,2,4-trimethylpentane (2,2,4-TMP)–chloroform (2:1, v/v) mixture.

Procedure

The ternary liquid–liquid system consisting of 2,2,4-TMP–ethanol–water was used for static and chromatographic experiments: 95% 2,2,4-TMP, 2% ethanol and 3% water (w/w) (system VII in Fig. 1); the position of the tie line of system VII was constructed according to ref. 15. The less polar phase, α, was used as eluent and the more polar phase, β, as the liquid stationary phase (Fig. 1). The chromatographic column packed with the solid support LiChrosorb Si 60 was loaded with the stationary liquid by pumping the water-rich phase through the column, either without TM-β-CD or containing different amounts of TM-β-CD ($c_{\text{TM-}\beta\text{-CD}}$ = 65 and 130 mg/ml), until the column was filled. The non-stationary part was removed by pumping the corresponding water-poor phase through the column until column bleeding was finished and a steady state had been reached. In further experiments (chiral partition system) the eluent was saturated with TM-β-CD. TM-β-CD is poorly soluble in the water-poor phase used and its concentration in the eluent was about 0.05 mg/ml.

For comparison, chromatographic experiments were carried out in the LSC mode. In this case the composition of the ternary eluent was chosen from the homogeneous region of the phase diagram (above the equilibrium line): 95% 2,2,4-TMP, 3% ethanol and 2% water (w/w).

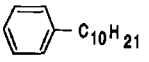
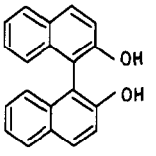
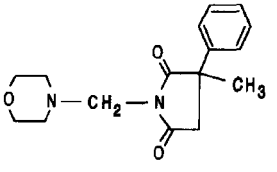
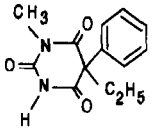
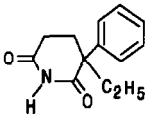
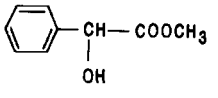
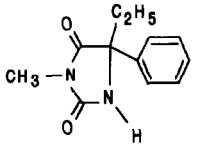
Liquid–liquid partition coefficients, K_i^{L-L}, for the distribution of test compounds between two coexisting liquid phases of the ternary liquid–liquid system were determined at 25.0 ± 0.1°C by absorption spectrometry as described previously [2]:

$$K_i^{L-L} = \frac{c_i^{\beta}}{c_i^{\alpha}} = K_i^{\beta/\alpha}$$

where c_i^{β} and c_i^{α} are the concentration of component i in the more polar phase (β) and the less polar phase (α), respectively.

TABLE I

STRUCTURES OF INVESTIGATED COMPOUNDS

Compound	Structure
1. Decyl benzene	
2. 2,2′-Dihydroxy-1,1′-binaphthol	
3. Morsuximide	
4. Methylphenobarbital	
5. Glutethimide	
6. Methylmandelate	
7. Mephenytoin	

The less polar phase containing appropriate amounts of the dissolved test compounds and the more polar phase with or without chiral additive ($c_{TM\text{-}\beta\text{-}CD}$ = 65 and 130 mg/ml) were used in the static partition experiments.

RESULTS AND DISCUSSION

Verification of retention mechanism

The retention of a substance in a chromatographic system is given by

$$V_{Ri} = V_m + V_s K_i$$

where V_R is the retention volume of solute, V_m is the volume of the mobile phase, V_s is the volume of the stationary phase, and $K_i = c_i^s/c_i^m$, the partition coefficient in the chromatographic system. If the retention of a solute is caused by pure liquid–liquid distribution, the retention volumes, V_{Ri}, of the different substances depend linearly upon their static partition coefficients, K_i^{L-L}. The results of the static partition experiments using two coexisting liquids of the ternary phase system with and without the chiral additive are given in Table II.

The data in Table II show that the values of static partition coefficients depend very strongly on the concentration of TM-β-CD in the water-rich liquid phase. For most of the test compounds the estimated K^{L-L} values increase with increasing concentration of the chiral agent added to the more polar phase indicating that complexation of the investigated solutes by the β-CD derivative takes place. The only exception was found for solute **4**, where the K_i^{L-L} value goes through a maximum point.

Fig. 2 shows the correlation of the static partition coefficients and the chromatographic retention data. The plot in Fig. 2a is for the system without chiral additive and contains data obtained in the LSC mode and the liquid–liquid chromatography (LLC) mode. One can see a shift of the retention data towards the

TABLE II

LIQUID–LIQUID PARTITION COEFFICIENTS OF RACEMIC TEST COMPOUNDS FOR LIQUID–LIQUID SYSTEMS WITHOUT CHIRAL ADDITIVE AND WITH DIFFERENT CONCENTRATIONS OF TM-β-CD IN THE STATIONARY PHASE

Substance	K_i^{L-L}		
	0 mg/ml	65 mg/ml	130 mg/ml
1. Dodecylbenzene	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
2. 2,2'-Dihydroxy-1,1'-binaphthol[a]	1.10 ± 0.06	3.46 ± 0.06[b]	5.88 ± 0.24[b]
3. Morsuximide	1.42 ± 0.18	1.80 ± 0.03	1.85 ± 0.51
4. Methylphenobarbital[a]	4.34 ± 0.98	8.59 ± 0.36[b]	6.98 ± 0.45[b]
5. Glutethimide[a]	5.21 ± 0.19	6.53 ± 0.28[b]	8.37 ± 0.24[b]
6. Methylmandelate	6.48 ± 0.22	6.98 ± 0.21	10.20 ± 0.28
7. Mephenytoin	7.86 ± 0.36	7.87 ± 0.21	14.49 ± 0.28

[a] Racemic mixtures resolved in chiral LL systems.

[b] K_i^{L-L} represent "average" values for racemic mixtures.

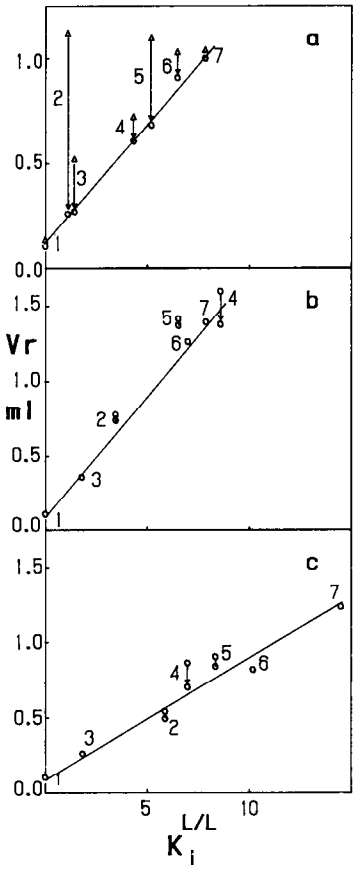


Fig. 2. Correlation of static partition coefficients and chromatographic retention data. Test compounds as in Table I. Column (250 × 1 mm); solid support LiChrosorb Si 60 (5 μm); liquid–liquid phase system VII (Fig. 1). (a) Shift of the retention volume data in the linear regression with the partition coefficients at the transition from LSC to LLC, $c_{TM\text{-}\beta\text{-}CD} = 0$. (b) $c_{TM\text{-}\beta\text{-}CD} = 65$ mg/ml. (c) $c_{TM\text{-}\beta\text{-}CD} = 130$ mg/ml.

linear regression line if the mechanism is changed from LSC to LLC. The plots in Fig. 2b and c give the correlation between retention data and static partition coefficients for liquid–liquid systems with different amounts of TM-β-CD dissolved in the water-rich stationary phase. The investigated liquid–liquid chiral systems show enantioselectivity towards three of the racemic mixtures tested. The retention data of these compounds are presented in the diagrams Fig. 2b and c, but their values are not included in the regression analysis. The parameters of linear regression data for unresolved racemic mixtures are given in Table III. As can be seen from the regression data and the plots in Fig. 2, an excellent linrar correlation has been found for the investigated compounds subsisting a liquid–liquid retention mechanism. The volume of the mobile phase, V_m^{cal}, determined from the intercept of the regression lines and the V_R axis, is the same as that determined by measuring the retention of decylbenzene (*i.e.*, a substance with negligible retention in the liquid–liquid normal-phase systems investigated).

TABLE III

REGRESSION PARAMETERS FOR RETENTION VOLUMES AND PARTITION COEFFICIENTS (OBTAINED FOR UNSEPARATED RACEMATES) ACCORDING TO $V_{Ri} = V_m + K_i^{L-L}V_s$

n, Number of measurements; r, regression coefficient; V_m^{calc}, V_s^{calc}, data calculated by regression; s_V, standard deviation; V_m^{exp}, retention volume for decylbenzene.

$c_{TM-\beta-CD}$ (mg/ml)	Regression parameters				
	n	r	$V_m^{calc} \pm s_V$ (ml)	$V_s^{calc} \pm s_V$ (ml)	V_m^{exp} (ml)
0	7	0.9942	0.113 ± 0.019	0.115 ± 0.004	0.106
65	4	0.9988	0.089 ± 0.021	0.167 ± 0.004	0.110
130	4	0.9971	0.105 ± 0.036	0.075 ± 0.004	0.10

The V_s values which characterize the amount of the stationary liquid phase depend on the phase system used (see Table III). However, no systematic trend is observed when the composition of the liquid is changed. Similar conclusions were reported previously for other systems [3,4].

As the next step in the verification of the distribution mechanism a partition system was used in which only the stationary liquid phase contained TM-β-CD. The eluent in this case was not saturated with the chiral additive. Such systems display exactly the same enantioselectivity as the system with the eluent saturated with TM-β-CD. A loss of enantioselectivity was observed after rather a long time (more then 250 dead volumes, Fig. 3). This stable enantioselectivity may be attributed to the very low solubility of TM-β-CD in the water-poor phase used as eluent.

The results presented above show that the retention mechanism of the chiral system investigated is actually a partition between the two liquid phases; the recognition of enantiomers occurring in the liquid stationary phase.

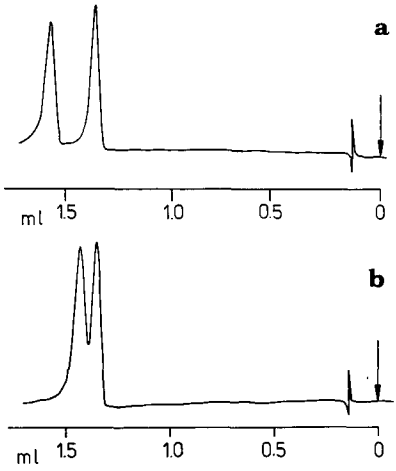


Fig. 3. Enantiomeric resolution of methylphenobarbital in partition system VII with 65 mg/ml TM-β-CD in the stationary phase. Column (250 × 1 mm) packed with LiChrosorb Si 60 (5 μm); flow-rate, 40 μl/min; temperature, 25.0 ± 0.1°C. (a) Eluent saturated with TM-β-CD. (b) Eluent without chiral additive, separation was obtained after more than 250 dead volumes pumped through the column.

TABLE IV

CAPACITY AND SEPARATION FACTORS FOR INVESTIGATED RACEMATES IN LIQUID–LIQUID SYSTEMS WITHOUT CHIRAL ADDITIVE AND WITH DIFFERENT CONCENTRATIONS OF TM-β-CD IN THE STATIONARY PHASE

For the unseparated test racemates $\alpha = 1.0$ and there is only one k' value representing one peak of racemate. For the separated racemates $\alpha > 1.0$ and there are two k' values.

Substance	0 mg/ml	65 mg/ml		130 mg/ml	
	k'	k'	α	k'	α
2. 2,2'-Dihydroxy-1,1'-binaphthol	1.45	5.91	>1.0	3.71, 4.14	1.12
3. Morsuximide	1.55	2.50	1.0	1.50	1.0
4. Methylphenobarbital	4.83	11.51, 13.51	1.17	5.68, 7.2	1.27
5. Glutethimide	5.46	11.44, 11.85	1.07	7.00, 7.59	1.08
6. Methylmandelate	7.58	10.49	1.0	6.81	1.0
7. Mephenytoin	8.43	11.68	1.0	10.76	1.0

Selectivity and column efficiency

Table IV contains capacity factors (k') and separation factors (α) for the test racemic mixtures in the investigated liquid–liquid system. The partition system containing TM-β-CD exhibits enantioselectivity with compounds **2**, **4**, **5** (Figs. 3–5). The enantiomers of **4** and **5** were also separated in the solvent-generated adsorption system with TM-β-CD in the stationary phase [12,16]. However, the latter one also

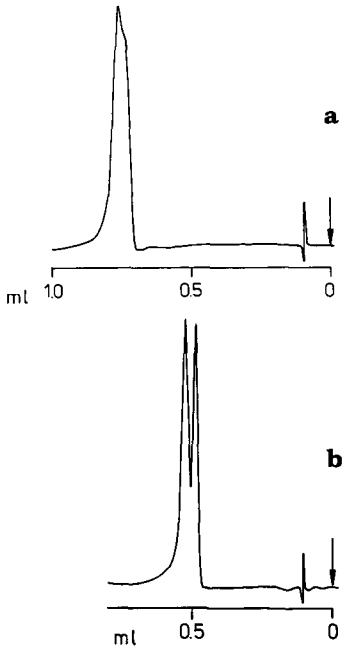


Fig. 4. Change of the enantioseparation of 2,2'-dihydroxy-1,1'-binaphthol with the concentration of chiral agent in the stationary phase. (a) $c_{TM\text{-}\beta\text{-}CD} = 65$ mg/ml. (b) $c_{TM\text{-}\beta\text{-}CD} = 130$ mg/ml. Conditions as in Fig. 3.

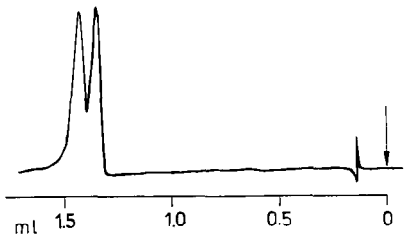


Fig. 5. Enantiomeric resolution of glutethimide in partition system VII with 65 mg/ml TM-β-CD in the stationary phase. Conditions as in Fig. 3.

enables the enantioseparation of the mandelic acid derivatives, which are not separated in the partition system. Conversely, the partition systems separate the enantiomers of binaphthol (**2**), which is not the case with the adsorption-type systems [17]. Thus, changing the retention mechanism from the LSC mode to the LLC mode influences chiral selectivity.

The results in Table IV show that the capacity factors depend on the concentration of the chiral additive in the stationary phase, as reflected by the K_i^{L-L} values listed in Table II. Selectivity of the liquid–liquid system is also dependent on the concentration of chiral agent. For solutes **4** and **5** the enantioselectivity increases only slightly, but for binaphthol the increase in selectivity, and therefore the improvement in resolution, is rather significant (see Fig. 4a and b). The observed dependence of chiral selectivity on the concentration of the chiral additive allows the users to control selectivity, within some limits, by simply modifying the concentration of the chiral agent in the system. This flexibility is not observed in chiral LSC systems.

Table V shows the efficiency of the chromatographic column for an LSC and LLC system without the chiral component and for liquid–liquid systems with different amounts of TM-β-CD in the stationary phase. All chromatographic determinations

TABLE V

COLUMN EFFICIENCY (NO. OF THEORETICAL PLATES) FOR LSC AND LLC SYSTEMS WITH DIFFERENT CONCENTRATIONS OF TM-β-CD IN THE STATIONARY PHASE

Substance	LSC	LLC		
		0 mg/ml	65 mg/ml	130 mg/ml
2. 2,2'-Dihydroxy-1,1'-binaphthol	4600	4600	–	4200 3350
3. Morsuximide	4120	3640	3550	3600
4. Methylphenobarbital	9860	8900	5430 5350	2690 2560
5. Glutethimide	6180	4990	4700 3640	4220 3600
6. Methylmandelate	9300	8410	6240	3300
7. Mephenytoin	8850	7800	4590	1000

were carried out on the same column. The efficiency of the column is about the same for LSC and LLC systems operated without chiral component.

The use of the chiral additive always caused a decrease of column efficiency and peak symmetry. It suggests that the processes of complexation reaction between the enantiomers and TM-β-CD in the stationary liquid phase contributing to the mass-transfer effect are relatively slow. The decrease of efficiency depends very strongly on the solute to be separated and also on the nature of the chiral additive. This question is discussed in more detail elsewhere [13].

It is probable that the drastic decrease in column efficiency, due to the slow kinetics of complexation in the chiral liquid stationary phase for methyl mandelate, makes resolution of its enantiomers impossible. Resolution was successful in an LSC system exhibiting relatively a low decrease in column efficiency [13].

CONCLUSIONS

The results of the present work show convincingly that (1) the ternary liquid–liquid phase systems may be supplemented by chiral modifiers: the systems tested retain the properties of the partition system and show, in addition, chiral properties; (2) the enantioselectivity, which is determined by the type and amount of modifier used, is similar to the enantioselectivity found in previously described solvent-generated adsorption systems [11–13,16,17] but this technique prove to be more flexible in selectivity, *i.e.*, by modifying the amount of the chiral agent used one may exert substantial control over the selectivity of the system.

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