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α- AND β-CYCLODEXTRINS AS SELECTIVE AGENTS FOR THE SEPARATION OF ISOMERS BY REVERSED-PHASE HIGH-PERFORMANCE THIN-LAYER AND COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

Comparative studies on the application of cyclodextrins as sorbents for the separation of various isomers by two techniques, reversed-phase thin-layer chromatography and reversed-phase high-performance liquid chromatography were performed in parallel. The effects of α- or β-cyclodextrin concentration in the mobile phase solution on the k' and R_F values of o-, m- and p-cresols, -nitrophenols, -nitroanilines, and 1- and 2-methylnaphthalenes and -nitronaphthalenes were investigated.

With both techniques, substantial selectivity towards positional isomers (o-, m- and p-substituted benzenes and 1- and 2-substituted naphthalenes) was observed, which resulted from inclusion in β-cyclodextrin cavities.

Reversed-phase thin-layer chromatography was found to be a very useful, simple and fast procedure for preliminary studies of inclusion processes in the mobile phase solution and of their influence on chromatographic separation.

INTRODUCTION

α- and β-cyclodextrins (CD) are torus-shaped cyclic oligosaccharides, made up of six and seven α-1,4-linked D-glucopyranose units, respectively. The inside of the CD cavities is relatively hydrophobic; it is formed by a circular configuration of hydrogen atoms and glucoside oxygen atoms while all the hydroxyl groups are on the outside of the molecule. This structure gives rise to the remarkable ability of CD to form inclusion compounds with various molecules and ions[1]. The fit of the entire or at least part of the guest molecules in the CD (host) cavity determines the stability of the inclusion compounds and the selectivity of the complexation process.

This property of CD has been used to advantage in many separation tech-

niques, including classical liquid chromatography[2,3]. In high-performance liquid chromatography (HPLC) two approaches have recently been devised for separating various compounds through CD complexation: the use of chemically bonded α- or β-CD silica stationary phases[4-8]; and the use of CD as mobile phase components in reversed-phase systems[9-13].

Our previous studies taking the latter approach have dealt with the separation of *o*-, *m*- and *p*-nitrobenzoic acids[9], *cis*- and *trans-o*-, *m*- and *p*-nitrocinnamic acids[10], and the resolution of mandelic acid[11] and some of its derivatives into enantiomers[12,13]. CD, dissolved in the mobile phase, have also been used by Hinze and Armstrong[14] and Burkert *et al.*[15] in thin-layer chromatography (TLC) on a polyamide stationary phase for the resolution of *o*-, *m*- and *p*-substituted benzoic acids, phenols and naphthols.

The present paper reports further comparative studies on the application of CD as mobile phase components for the separation of various isomers, performed in parallel by two chromatographic techniques: reversed-phase HPLC and reversed-phase TLC. The model compounds were: *o*-, *m*- and *p*-cresols (C), -nitrophenols (NP), -nitroanilines (NA) and -chloromandelic acids (CMA); 1- and 2-methylnaphthalenes (MN) and -nitronaphthalenes (NN); and mandelic acid (MA).

The aim of this study was to establish a simple and fast procedure for preliminary optimization of separation conditions in reversed-phase systems containing CD in mobile phase solutions. In these experiments, we used precoated RP-18 $F_{254}s$ TLC plates, which have only recently become available. In contrast to those used previously[16,17], they are easily wettable by aqueous mobile phase solutions in prevailing concentrations of water used to dissolve CD for reversed-phase HPLC.

EXPERIMENTAL

α-CD was supplied by Janssen Chimical (Beerse, Belgium) and β-CD by Chinoin (Budapest, Hungary). All other materials were of analytical or reagent grade and were used without further purification.

Chromatographic experiments were performed with a HPLC unit constructed at the Institute of Physical Chemistry, Polish Academy of Sciences (Warsaw, Poland) and equipped with a UV detector (254 nm). For HPLC, use was made of stainless-steel columns (250 × 4.5 mm I.D.), packed with 10 μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.). For TLC, the TLC precoated plates RP-18 $F_{254}s$ of Merck (product no. 15683) were used. All were 5 cm in length with the exception of the plates for the resolution of cresols, which were *ca.* 17 cm long.

The spots were usually *ca.* 2 mm in diameter. Portions of 0.5–1 μl of 0.1–1% methanolic solutions of samples were applied 8 mm from the lower edge of the plates. Ascending TLC was performed in a glass jar. In all experiments with CD the plates were pretreated as follows: ascending development was carried out for 2 h with the same eluent that would be used for "normal" development. After the samples had been dried and spotted, the plates were developed in a second jar with the same eluent as in the pretreatment. Development times varied from 10 min to 30 min, depending on the percentage of ethanol and the concentration of CD in the eluents. The spots were located under UV light by quenching of fluorescence at 254 nm. The mobile phases (the same for HPLC and TLC experiments) consisted of aqueous solutions

containing various concentration of α- or β-CD, appropriate amounts of ethanol (20 vol. % and 50 vol. %), and in some cases suitable phosphate buffer components. HPLC and TLC chromatograms were carried out in parallel at room temperature (*ca.* 22°C).

RESULTS AND DISCUSSION

The k' and R_F values of the model compounds, determined on reversed phases by HPLC and TLC with mobile phase solutions containing various concentration of α- or β-CD are collected in Tables I–III.

TABLE I

k' AND R_F VALUES OF 1- AND 2-METHYLNAPHTHALENES (MN), 1- AND 2-NITRO-NAPHTHALENES (NN), *o*-, *m*- AND *p*-CHLOROMANDELIC ACIDS (CMA) AND MANDELIC ACID (MA) ON RP-18 HPLC COLUMNS AND TLC PLATES WITH MOBILE PHASES CONTAINING VARIOUS CONTENTRATIONS OF β-CD IN ETHANOL–PHOSPHATE BUFFER (pH 6.7) (50:50,v/v)

Compounds	$[\beta\text{-}CD]$ $(10^{-3}\ M)$							
	0.0		5		10		20	
	k'	R_F	k'	R_F	k'	R_F	k'	R_F
1-MN	29.8	0.03	>100	0.00	>100	0.00	>100	0.00
2-MN	31.5	0.02	33.6	0.04	33.1	0.03	22.7	0.04
1-NN	7.85	0.08	9.02	0.07	>100	0.00	>100	0.00
2-NN	9.98	0.08	11.35	0.06	10.67	0.07	8.5	0.08
o-CMA	7.48	0.08	7.88	0.06	8.3	0.09	>100	0.00
m-CMA	7.85	0.08	9.1	0.06	28.4	0.02	>100	0.00
p-CMA	0.34	0.19	0.28	0.16	0.32	0.24	0.30	0.29
MA	0.15	0.72	0.13	0.72	0.14	0.76	0.13	0.75

Examination of these data leads to the conclusion that similar effects due to CD complexation are observed by both techniques. The elution sequences are the same. The values of k' determined from HPLC and TLC data are approximately the same (or at least of the same order of magnitude). The results obtained appear to be consistent, considering the fact that we have sometimes observed similar discrepancies using CD solutions in columns packed with reversed-phase sorbents from different batches. Thus TLC on precoated reversed-phase plates can be very useful as a pilot procedure for studying CD complexation processes in the mobile phase solution and their influence on chromatographic separations.

As suggested earlier[10], β-CD imparts a substantial selectivity towards positional isomers on reversed-phase systems. The effects arising from α-CD comlexation, observed by both techniques, are slighter and not general for all disubstituted benzene derivatives.

TABLE II

k' AND R_F VALUES OF o-, m- AND p-CRESOLS (C), -NITROANILINES (NA) AND -NITRO-PHENOLS (NP) ON RP-18 HPLC COLUMNS AND TLC PLATES WITH MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS OF β-CD IN ETHANOL–WATER (20:80, v/v)

Compound	$[\beta$-CD$](10^{-3} M)$							
	0.0		5		10		20	
	k'	R_F	k'	R_F	k'	R_F	k'	R_F
o-C	17.0	0.05	13.07	0.06	11.05	0.08	8.87	0.09
m-C	16.16	0.06	11.71	0.07	9.64	0.08	7.48	0.11
p-C	16.42	0.06	10.37	0.07	7.97	0.11	5.68	0.13
o-NP	15.5	0.04	13.4	0.05	10.7	0.05	8.5	0.08
m-NP	12.7	0.06	9.8	0.07	8.2	0.13	6.9	0.16
p-NP	7.9	0.07	6.3	0.11	4.4	0.17	3.1	0.21
o-NA	14.8	0.04	11.07	0.05	9.13	0.06	7.27	0.08
m-NA	7.72	0.07	5.87	0.09	4.93	0.08	4.00	0.14
p-NA	5.82	0.09	2.65	0.19	1.78	0.22	1.12	0.38

The R_F values listed in Tables I and II show that in some cases the separation of positional isomers by TLC is easily obtained (e.g., o-, m- and p-nitroanilines), whereas in other cases particular conditions are required (e.g., o-, m- and p-cresols, known to be difficult to separate by LC). However, improved separation by TLC may be obtained by multiple development or by increasing the migration distance. Under these conditions, clear-cut separation of the three isomers was obtained. A comparison of HPLC and TLC separations with β-CD solutions is shown in Fig. 1.

TABLE III

k' AND R_F VALUES OF o-, m-, AND p-CRESOLS (C), -NITROANILINES (NA) AND -NITRO-PHENOLS (NP) ON RP-18 HPLC COLUMN AND TLC PLATES WITH MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS OF α-CD IN ETHANOL–WATER (20:80, v/v)

Compound	$[\alpha$-CD$] (10^{-3} M)$							
	0.0		5		10		20	
	k'	R_F	k'	R_F	k'	R_F	k'	R_F
o-C	17.0	0.05	15.40	0.05	14.84	0.05	13.22	0.05
m-C	16.16	0.06	14.55	0.06	13.96	0.06	12.66	0.06
p-C	16.42	0.06	14.73	0.06	14.02	0.06	12.56	0.06
o-NP	15.5	0.04	13.11	0.04	11.72	0.04	10.84	0.05
m-NP	12.7	0.06	12.14	0.07	9.84	0.07	7.98	0.09
p-NP	7.9	0.07	6.52	0.08	4.21	0.09	3.40	0.13
o-NA	14.80	0.04	13.10	0.04	11.60	0.04	9.12	0.06
m-NA	7.72	0.07	6.74	0.08	5.76	0.09	4.60	0.11
p-MA	5.82	0.09	4.58	0.12	3.22	0.19	2.24	0.25

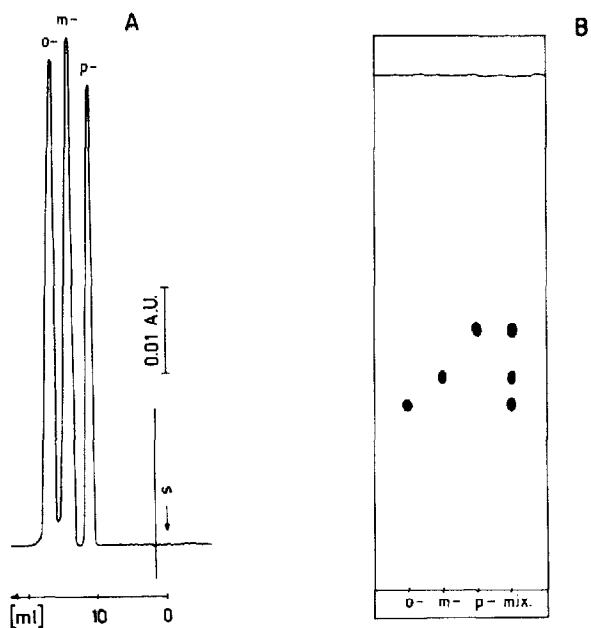Fig. 1. Separation of o-, m- and p-cresols by (A) HPLC and (B) TLC with a mobile phase containing 0.02 M β-CD in ethanol–water (20:80, v/v). (A) HPLC column (250 × 4.5 mm I.D. RP-18), flow-rate 1.8 ml/min; (B) TLC (RP-18) chromatogram after six developments of a plate, 17 × 5 cm.
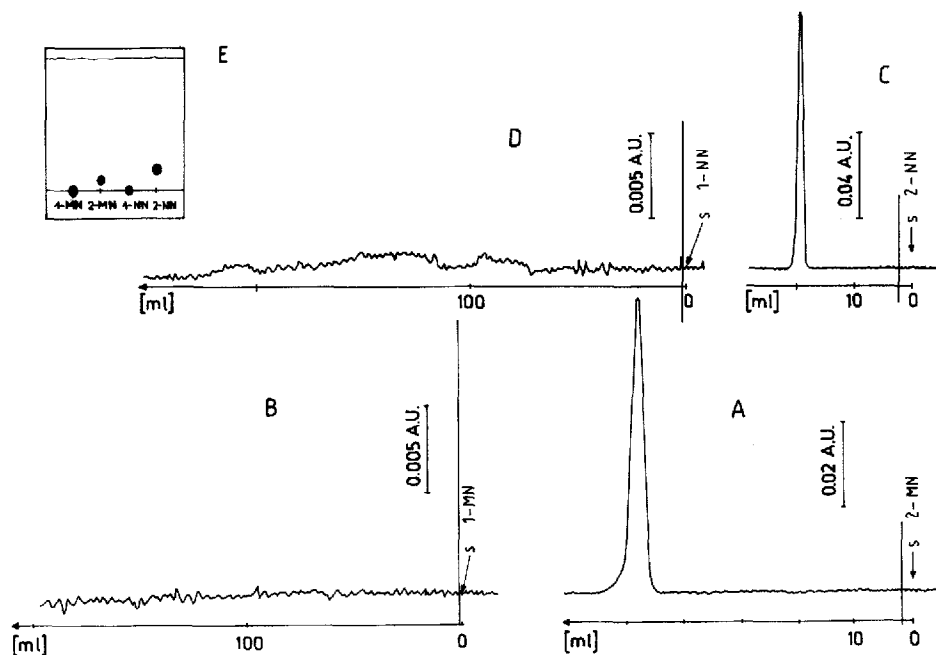

Fig. 2. Chromatograms of 1- and 2-methylnaphthalenes (MN) and 1- and 2-nitronaphthalenes (NN) by HPLC and TLC with a mobile phase containing 0.02 M β-CD in ethanol–phosphate buffer (pH 6.7) (50:50, v/v). (A) 2-MN; flow-rate, 1.8 ml/min; (B) 1-MM; flow-rate, 2.4 ml/min (no peak eluted); (C) 2-NN; flow-rate, 1.8 ml/min; (D) 1-NN; flow-rate, 2.4 ml/min (no peak eluted); (E) TLC of 1-MN, 2-MN, 1-NN, and 2-NN: chromatogram after two developments of a plate 5 × 4 cm.

It was observed that both 1-MN and 1-NN are irreversibly adsorbed on the RP-18 phase from β-CD solutions. Fig. 2 demonstrates the behaviour of 1- and 2-MN and 1- and 2-NN in aqueous ethanol solution (50 vol. %), containing β-CD, observed by reversed-phase HPLC and TLC techniques.

The separation on reversed-phase HPLC columns is too specific for the determination of two isomers in a mixture: only one peak is eluted (that of naphthalene, substituted on position 2) whereas 1-NN and 1-MN remain adsorbed on the column. In such a case, only TLC may be used for evaluating the composition of mixtures of 1- and 2-MN or 1- and 2-MN. Two spots are detected by TLC, the first corresponding to 1-substituted naphthalenes at the start (initial position) and the second corresponding to 2-substituted naphthalenes. It should be noted that the resolution of two isomeric monosubstituted naphthalenes is very poor without β-CD (see Table I). The unusual irreversible adsorption of (1-MN · β-CD) and (1-NN · β-CD) complexes on the RP-18 phase is under further investigation.

The attempts to perform a direct comparison of the results described above with those of Hinze and Armstrong[14] and Burkert et al.[15] seem to be unreasonable because they concern two different sorbents (RP-18 and polyamide phases) and perhaps different mechanisms.

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