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Adsorption chromatography on cellulose

XI. Chiral separations with aqueous solutions of cyclodextrins as eluents

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ABSTRACT

The system cellulose–aqueous α-cyclodextrin was investigated for chiral separations of tryptophan, methyltryptophans and fluorotryptophans by thin-layer and paper chromatography. The chiral effects are essentially additive (for cellulose and α-cyclodextrin), hence some large R_F differences can be obtained for enantiomeric pairs. There is also a temperature effect, with an increase in ΔR_F values as the temperature is decreased.

INTRODUCTION

Thin-layer chromatographic (TLC) separations of enantiomers have been found to be of interest and two reviews [1,2] have summarized the extensive work published and its application to purity control. We have investigated the chiral properties of cellulose in paper and thin-layer chromatography [3–7] and our interest was centred on understanding adsorption on cellulose.

In this paper we report on chromatography using cellulose as adsorbent and aqueous solutions of cyclodextrins as eluents. Separations using a chiral adsorbent and a chiral eluent together have been reported by several workers.

Fujita *et al.* [8,9] seem to have been the first, using a tartrate ester linked to Sephadex as the stationary phase and 0.3 *M* disodium L-tartrate as eluent. Davankov *et al.* [10] considered theoretically the copper–amino acid type of chiral selector in the stationary and the mobile phase. They concluded that "Enantioselectivity of chiral chromatographic systems appears to be a complex function of the enantioselectivity effects of the selector–selectand adduct formation in both the mobile and stationary phases, as well as of the phase distribution of these adducts, unless the chiral selector resides entirely in one of these phases . . . The reciprocity relationship for mutual chiral selector–selectand recognition which are known to be valid for their association in solutions and for diastereomeric salt crystallization do not necessarily hold for chiral chromatographic systems".

The chiral selectivity of cyclodextrins has been summarized by Menges and Armstrong [11] and involves the size of the cavity of the cyclodextrin and hydrogen bonding between the hydroxyl groups of the cyclodextrin and the included molecule. We have to consider another parameter in our system: the cyclodextrin must not be strongly adsorbed on cellulose otherwise it cannot be used readily as constituent of the eluent.

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For our work only α-cyclodextrin, which moves to an R_F of 0.91 on paper and about 0.8 on cellulose thin layers, was suitable, β- and γ-cyclodextrins being fairly strongly adsorbed. The work reported here deals mainly with substituted tryptophans, which separate well with aqueous solvents on microcrystalline cellulose layers [3,6].

EXPERIMENTAL

Standard paper and thin-layer chromatographic techniques were used as reported in a previous paper [6]. The cyclodextrins used were obtained from Fluka (Buchs, Switzerland).

RESULTS

Reaction of iodine vapour with substituted tryptophans in the presence of α-cyclodextrin

In absence of α-cyclodextrin, iodine vapour produces pale brown spots on a white background irrespective of the type of cellulose used as the support. This reaction is about as sensitive as the reaction with ninhydrin. If left for prolonged periods in iodine vapour (*e.g.*, overnight), a black spot on a grey background is produced.

In the presence of α-cyclodextrin on cellulose layers, grey-black spots develop in a few seconds and these spots are very stable. Fig. 1 shows typical chromatograms after more than 60 days. We determined the sensitivity of this reaction for tryptophan and found that 5 ng can still be detected. This is about the lowest level generally detected by reagents in TLC.

Attempts to isolate the compound formed by mixing tryptophan in 1 *M* NaCl with α-cyclodextrin and adding iodine were unsuccessful. On Whatman 3MM paper the spots became intensely blue and not black and they disappeared within a few minutes. In the absence of NaCl or another salt sensitive coloration was not obtained. We suggest that it is a form of charge-transfer reaction involving the tryptophan, α-cyclodextrin, a salt and the cellulose support.

α-Cyclodextrin has been reported previously in conjunction with iodine vapour for the detection of lipids in TLC [12], but we could not find any reference to the reaction of tryptophan.

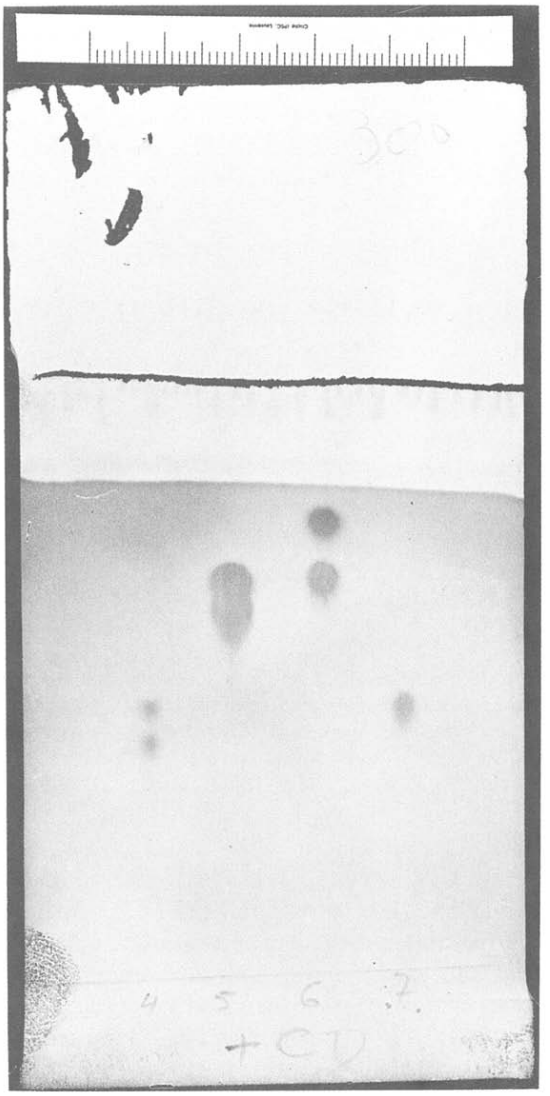

Fig. 1. Chromatogram of DL-methyltryptophans on native cellulose (Macherey–Nagel Cel-300) thin layers developed with aqueous 1 *M* NaCl containing 4% of α-cyclodextrin at room temperature (20–22°C). From left to right: 4-, 5-, 6- and 7-methyltryptophan. The thin layer was exposed to iodine vapour for a few seconds and then stored for over 60 days in the laboratory without a noticeable change in the intensity of the spots.

Effect of α-cyclodextrin concentration

We developed chromatograms with solutions of 1 *M* NaCl containing 1–10% of α-cyclodextrin. Table I shows the results obtained on microcrystalline cellulose (Merck 5577) and on "native" cellulose (Macherey–Nagel Cel-300). There is an increase in the separation from 1 to

TABLE I

EFFECT OF α-CYCLODEXTRIN CONCENTRATION ON THE R_F VALUES OF ENANTIOMERS OF SUBSTITUTED TRYPTOPHANS SEPARATED ON CELLULOSE THIN LAYERS WITH AQUEOUS α-CYCLODEXTRIN–1 *M* NaCl SOLUTIONS AT 22°C

Thin layer	Compound	α-Cycylodextrin (%)											
		0		1		2		4		6		10	
		R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F
Merck 5577 microcrystalline cellulose	Trp L-	0.41	0.07	0.40	0.09	0.41	0.10	0.48	0.12	0.47	0.13	0.59	0.12
	D-	0.48		0.49		0.51		0.60		0.60		0.67	
	4-Methyl-Trp L-	0.22	0.10	0.31	0.10	0.33	0.09	0.24	0.11	0.28	0.11	0.29	0.11
	D-	0.32		0.41		0.42		0.35		0.39		0.40	
	5-Methyl-Trp L-	0.25	0.08	0.24	0.09	0.32	0.10	0.41	0.13	0.47	0.11	0.52	0.11
	D-	0.33		0.33		0.42		0.54		0.58		0.63	
	6-Methyl-Trp L-	0.24	0.07	0.26	0.12	0.32	0.16	0.46	0.19	0.52	0.17	0.58	0.16
	D-	0.31		0.38		0.48		0.65		0.69		0.74	
	7-Methyl-Trp L-	0.26	0.07	0.26	0.08	0.28	0.07	0.28	0.08	0.32	0.08	0.34	0.07
	D-	0.33		0.34		0.35		0.36		0.40		0.41	
	4-Fluoro-Trp L-	0.30	0.08	0.19	0.09	0.21	0.10	0.34	0.11	0.42	0.10	0.44	0.12
	D-	0.38		0.28		0.31		0.45		0.52		0.56	
	5-Fluoro-Trp L-	0.35	0.06	0.33	0.08	0.38	0.08	0.42	0.09	0.46	0.08	0.56	0.05
	D-	0.41		0.41		0.46		0.51		0.54		0.61	
	6-Fluoro-Trp L-	0.32	0.09	0.35	0.11	0.40	0.14	0.43	0.17	0.49	0.16	0.57	0.15
	D-	0.41		0.46		0.54		0.60		0.65		0.72	
Macherey–Nagel Cel-300 native cellulose	Trp L-	0.56	0.02	0.53	0.03	0.58	0.04	0.60	0.07	0.60	0.06	0.62	0.07
	D-	0.58		0.56		0.62		0.67		0.66		0.69	
	4-Methyl-Trp L-	0.40	0.05	0.45	0.06	0.49	0.06	0.51	0.06	0.37	0.07	0.33	0.08
	D-	0.45		0.51		0.55		0.57		0.44		0.41	
	5-Methyl-Trp L-	0.43	0.04	0.42	0.05	0.50	0.05	0.58	0.06	0.60	0.06	0.61	0.07
	D-	0.47		0.47		0.55		0.64		0.66		0.68	
	6-Methyl-Trp L-	0.43	0.03	0.42	0.06	0.51	0.11	0.63	0.12	0.65	0.10	0.67	0.11
	D-	0.46		0.48		0.62		0.75		0.75		0.78	
	7-Methyl-Trp	0.50		0.46		0.43		0.47		0.44		0.47	
	4-Fluoro-Trp L-	0.48	0.04	0.34	0.07	0.37	0.07	0.38	0.06	0.50	0.07	0.47	0.09
	D-	0.52		0.41		0.44		0.44		0.57		0.56	
	5-Fluoro-Trp	0.51		0.49		0.53		0.58		0.57		0.57	
	6-Fluoro-Trp L-	0.48		0.47	0.05	0.53	0.06	0.60	0.11	0.60	0.10	0.60	0.13
	D-			0.52		0.59		0.71		0.70		0.67	

4% of α-cyclodextrin. The R_F difference between the two enantiomers does not increase measurably above 4% of cyclodextrin.

On "native" cellulose the separation of the enantiomers is slight, *e.g.*, an R_F difference of 0.02 between D- and L-tryptophan, whereas the difference on microcrystalline cellulose is 0.07. By comparing the behaviours on the two layers it is possible to obtain an estimate of the effect due to the α-cyclodextrin. It can be seen that the chiral effect due to the cellulose and that due to the α-cyclodextrin are essentially additive. On both layers an eluent containing 4% of α-cyclodextrin yields an increase in the R_F value with a difference of 0.05 for D,L-tryptophan.

The effect of different substituents and the position of the substituent is considerable in this system. Qualitatively it correlates with the data of Menges and Armstrong [11], who studied cyclodextrin-bonded phases in HPLC with 1% triethylammonium acetate at pH 5.1 as the eluent. There are several high R_F differences such as for 6-methyltryptophan ($\Delta R_F = 0.19$) and for 6-fluorotryptophan ($\Delta R_F = 0.17$), and there is a general increase for all the substituted tryptophans which were examined.

Effect of concentration of NaCl in the eluent

Table II shows the R_F values obtained on microcrystalline and "native" cellulose when the NaCl concentration was varied from 0 to 1 *M*, keeping the concentration of cyclodextrin at 4% throughout. There is a small but measurable salt

TABLE II

EFFECT OF CONCENTRATION OF NaCl ON R_F VALUES OF ENANTIOMERS OF SUBSTITUTED TRYPTOPHAN SEPARATED ON CELLULOSE THIN LAYERS WITH AQUEOUS 4% α-CYCLODEXTRIN–NaCl SOLUTIONS AT 22°C

Thin layer	Compound	NaCl concentration (M)							
		0		0.1		0.5		1	
		R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F
Merck 5577 microcrystalline cellulose	Trp L-	0.50	0.04	0.53	0.10	0.48	0.10	0.48	0.12
	D-	0.54		0.63		0.58		0.60	
	4-Methyl-Trp L-	0.27	0.09	0.45	0.09	0.38	0.10	0.24	0.11
	D-	0.36		0.54		0.48		0.35	
	5-Methyl-Trp L-	0.40	0.09	0.45	0.11	0.39	0.10	0.41	0.13
	D-	0.49		0.56		0.49		0.54	
	6-Methyl-Trp L-	0.43	0.16	0.48	0.17	0.44	0.18	0.46	0.19
	D-	0.59		0.65		0.62		0.65	
	7-Methyl-Trp L-	0.30	0.07	0.37	0.07	0.30	0.08	0.28	0.08
	D-	0.37		0.44		0.38		0.36	
	4-Fluoro-Trp L-	0.37	0.08	0.32	0.09	0.25	0.09	0.34	0.11
	D-	0.45		0.41		0.34		0.45	
	5-Fluoro-Trp L-	0.43	0.06	0.49	0.06	0.42	0.09	0.42	0.09
	D-	0.49		0.55		0.51		0.51	
	6-Fluoro-Trp L-	0.40	0.16	0.50	0.13	0.46	0.13	0.43	0.17
	D-	0.56		0.63		0.59		0.60	
Macherey–Nagel Cel-300 native cellulose	Trp L-	0.63	0.04	0.64	0.05	0.64	0.04	0.60	0.07
	D-	0.67		0.69		0.68		0.67	
	4-Methyl-Trp L-	0.45	0.05	0.57	0.05	0.55	0.05	0.51	0.06
	D-	0.50		0.62		0.60		0.57	
	5-Methyl-Trp L-	0.61	0.05	0.63	0.06	0.61	0.04	0.58	0.06
	D-	0.66		0.69		0.65		0.64	
	6-Methyl-Trp L-	0.65	0.08	0.65	0.09	0.66	0.08	0.63	0.12
	D-	0.73		0.74		0.74		0.75	
	7-Methyl-Trp	0.53		0.54		0.51		0.47	
	4-Fluoro-Trp L-	0.57	0.04	0.45	0.07	0.41	0.06	0.38	0.06
	D-	0.61		0.52		0.47		0.44	
	5-Fluoro-Trp	0.63		0.65		0.61		0.58	
	6-Fluoro-Trp L-	0.64	0.08	0.65	0.08	0.61	0.09	0.60	0.11
	D-	0.72		0.73		0.70		0.71	

effect both for the R_F values in general and for the ΔR_F values for a chiral pair. The optimum results were obtained with 1 M NaCl.

Effect of temperature

In previous work [3] on the separation of enantiomers in the absence of α-cyclodextrin in the range −10 to 63°C, the R_F values increased with increase in temperature but the chiral effect remained almost constant, diminishing only slightly at the highest temperature (63°C) and in the presence of high salt concentrations (*e.g.*, in 4.7 M LiCl).

In eluents containing α-cyclodextrin there is a large temperature effect, as shown in Table III. With an increase in temperature from 8 to 60°C the ΔR_F values can change as much as fivefold (*e.g.*, for 6-methyltryptophan on Macherey–Nagel Cel-300 cellulose from 0.15 at 8°C to 0.03

TABLE III

EFFECT OF THE TEMPERATURE OF DEVELOPMENT ON THE R_F VALUES OF ENANTIOMERS OF SUBSTITUTED TRYPTOPHANS SEPARATED ON CELLULOSE THIN LAYERS WITH AQUEOUS 4% α-CYCLODEXTRIN–1 *M* NaCl SOLUTION

Thin layer	Compound	8°C		22°C		40°C		60°C	
		R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F	R_F	ΔR_F
Merck 5577 microcrystalline cellulose	Trp L-	0.35	0.16	0.48	0.12	0.56	0.08	0.62	0.08
	D-	0.51		0.60		0.64		0.70	
	4-Methyl-Trp L-	0.28	0.11	0.24	0.11	0.44	0.11	0.56	0.06
	D-	0.39		0.35		0.55		0.62	
	5-Methyl-Trp L-	0.29	0.12	0.41	0.13	0.46	0.08	0.55	0.07
	D-	0.41		0.54		0.54		0.62	
	6-Methyl-Trp L-	0.31	0.21	0.46	0.19	0.50	0.13	0.59	0.10
	D-	0.52		0.65		0.63		0.69	
	7-Methyl-Trp L-	0.20	0.08	0.28	0.08	0.39	0.06	0.51	0.04
	D-	0.28		0.36		0.45		0.55	
	4-Fluoro-Trp L-	0.14	0.10	0.34	0.11	0.32	0.10	0.44	0.08
	D-	0.24		0.45		0.42		0.52	
	5-Fluoro-Trp L-	0.31	0.08	0.42	0.09	0.50	0.06	0.59	0.05
	D-	0.39		0.51		0.56		0.64	
	6-Fluoro-Trp L-	0.35	0.17	0.43	0.17	0.53	0.11	0.60	0.08
	D-	0.52		0.60		0.64		0.68	
Macherey–Nagel Cel-300 native cellulose	Trp L-	0.48	0.09	0.60	0.07	0.69	0.04	0.73	0.02
	D-	0.57		0.67		0.73		0.75	
	4-Methyl-Trp L-	0.42	0.07	0.51	0.06	0.59	0.06	0.68	0.03
	D-	0.49		0.57		0.65		0.71	
	5-Methyl-Trp L-	0.46	0.07	0.58	0.06	0.64	0.05	0.69	0.04
	D-	0.53		0.64		0.69		0.71	
	6-Methyl-Trp L-	0.49	0.15	0.63	0.12	0.68	0.08	0.73	0.03
	D-	0.64		0.75		0.76		0.76	
	7-Methyl-Trp L-	0.39	0.03	0.47		0.57		0.67	
	D-	0.42							
	4-Fluoro-Trp L-	0.28	0.08	0.38	0.06	0.50	0.07	0.61	0.03
	D-	0.36		0.44		0.57		0.64	
	5-Fluoro-Trp L-	0.48	0.03	0.58		0.66		0.71	
	D-	0.51							
	6-Fluoro-Trp L-	0.50	0.12	0.60	0.11	0.67	0.05	0.70	0.04
	D-	0.62		0.71		0.72		0.74	

at 60°C). Further, high ΔR_F values are possible at low temperatures ($\Delta R_F = 0.21$ for 6-methyltryptophan at 8°C on Merck 5577 layers). The effect of temperature on the enantiomer separation varies considerably with the substituent and its position and there is always a decrease with increase in temperature.

The best results shown in Table III are as good as the best separations obtained by Günther [2] on chiral plates.

Paper chromatography

The chiral separations obtained on cellulose paper are usually of the same order as those obtained in TLC with "native" cellulose and as paper has a lower plate number not many baseline separations can be obtained [5]. It was therefore considered of interest to try eluents containing α-cyclodextrin as high ΔR_F values were recorded.

Table IV gives the R_F values on Whatman

TABLE IV

R_F VALUES OF SUBSTITUTED TRYPTOPHANS DEVELOPED ON WHATMAN NO. 3MM PAPER WITH AQUEOUS 1 *M* NaCl CONTAINING 4% OF α-CYCLODEXTRIN

Time, 7 h; distance moved, 25 cm; temperature, 20–22°C; R_F value of α-cyclodextrin front, 0.91.

Compound	R_F	ΔR_F
L-Trp	0.54	0.07
D-Trp	0.63	
DL-4-Methyl-Trp	0.38 0.44	0.06
DL-5-Methyl-Trp	0.53 0.61	0.08
DL-6-Methyl-Trp	0.60 0.69	0.09
DL-7-Methyl-Trp	0.46 (single spot)	0
DL-4-Fluoro-Trp	0.46 0.53	0.07
DL-5-Fluoro-Trp	0.51 (single spot)	0
DL-6-Fluoro-Trp	0.52 0.64	0.12

3MM paper; ΔR_F values identical with those on Macherey–Nagel Cel-300 thin layers were obtained. A number of enantiomeric pairs (as indicated in Table IV) yield baseline separations with a development of 20–25 cm in 5–7 h. The reaction with iodine vapour is different to that on thin layers, as already discussed.

Effect of Cu^{2+} ions on chiral separations with α-cyclodextrin

In a previous paper [7] we reported that the chiral discrimination of tryptophan and substituted tryptophans on cellulose is diminished when an excess of Cu^{2+} ions ($CuSO_4$ as eluent) is present in the eluent. Table V shows that the same occurs when α-cyclodextrin is used for chiral separations. It seems that complexation of the NH_2 and COOH groups interferes with the chiral interactions not only with cellulose but also with α-cyclodextrin.

CONCLUSIONS

Davankov *et al.* [10] pointed out that systems with a chiral support and a chiral eluent are complex owing to the numerous equilibria that are possible in solution and on the support. Our results with the cellulose–aqueous α-cyclodextrin system show a general but only qualitative additivity of the chiral separation effects, that is, the ΔR_F values are always larger on microcrystalline cellulose, which has a strong chiral discrimination, than on native cellulose.

However, in addition to this general trend there is a considerable change in R_F values in the presence of α-cyclodextrin and there is a strong temperature dependence for the chiral separations. This permits a wide choice of conditions for separations between the enantiomers and also between numerous substituted tryptophans which do not separate without α-cyclodextrin. The reaction of iodine vapour with thin-layer

TABLE V

R_F VALUES OF SUBSTITUTED TRYPTOPHANS ON CELLULOSE THIN LAYERS DEVELOPED WITH AQUEOUS α-CYCLODEXTRIN SOLUTION CONTAINING $CuSO_4$

Thin layer	Compound	R_F	
		4% α-cyclodextrin + 0.05 *M* $CuSO_4$	0.05 *M* $CuSO_4$
Merck 5577 microcrystalline cellulose	L-Trp	0.60	0.70
	D-Trp	0.63	0.74
	DL-4-Methyl-Trp	0.47	0.48
	DL-5-Methyl-Trp	0.60 0.64	0.50 0.53
	DL-6-Methyl-Trp	0.68	0.52
	DL-7-Methyl-Trp	0.53	0.54
	DL-4-Fluoro-Trp	0.57	0.55
	DL-5-Fluoro-Trp	0.61	0.57
	DL-6-Fluoro-Trp	0.63	0.56
Macherey–Nagel Cel-300 native cellulose	L-Trp	0.61	0.56
	D-Trp	0.63	0.58
	DL-4-Methyl-Trp	0.45	0.43
	DL-5-Methyl-Trp	0.62	0.50
	DL-6-Methyl-Trp	0.66	0.48
	DL-7-Methyl-Trp	0.52	0.51
	DL-4-Fluoro-Trp	0.54	0.51
	DL-5-Fluoro-Trp	0.57	0.52
	DL-6-Fluoro-Trp	0.60	0.51

plates developed with an aqueous NaCl–α-cyclodextrin mixture yields a colour reaction which seems to be as good as the best yet reported.

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