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Note

Charge-transfer chromatographic study on inclusion complex formation between two hydroxypropyl-β-cyclodextrins and some chlorophenols

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Cyclodextrins (CDs) can form complexes with a wide variety of organic compounds[1,2], modifying their physico-chemical parameters, and are being increasingly applied in all types of chromatography[3–5]. In addition to solving separation problems, chromatographic techniques have been applied to determine the strength of various inclusion complexes[6]. Charge-transfer chromatography carried out on reversed-phase thin-layer chromatographic (TLC) plates has been used to study the inclusion complex formation of polymyxin[7], symmetric triazine[8], triphenylmethane[9], nitrostyrene[10] and barbituric acid derivatives[11]. Determination is based on the lipophilicity difference between the complexed and free forms of the guest compound[12]. A similar method was applied to determine the α-cyclodextrin complexes of 4-chloro- and 2,4,6-trichlorophenol[13].

The objectives of this investigation were to study the interaction of some chlorophenols with two hydroxypropyl-β-cyclodextrin derivatives and to elucidate the role of various chloro substitutions in the complex formation.

EXPERIMENTAL

The following chlorophenol derivatives were studied: 2-chloro-(I), 3-chloro-(II), 4-chloro-(III), 4-chloro-3-methyl-(IV), 2,4-dichloro-(V), 2,5-dichloro-(VI), 3,5-dichloro-(VII), 2,4,5-trichloro-(VIII) and 2,4,6-trichlorophenol (IX). Silufol UV254 TLC plates (Kavalier, Sklárny, Czechoslovakia) were impregnated with paraffin oil as described previously[7]. The chlorophenol derivatives were dissolved in acetone at a concentration of 2 mg/ml and 5 μl of each solution were spotted on the plates. As the aim was to study the complex formation between the chlorophenols and two hydroxypropyl-β-cyclodextrin (HPBCD) derivatives (average degree of substitution

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2.7 and 4.6) (henceforth called HPBCD 2,7 and HPBCD 4,6) and not to study the effect of HPBCDs on the separation of chlorophenols, the chlorophenols were spotted separately on the plates in each instance; in this manner the HPBCD: chlorophenol ratio was always identical for each chlorophenol derivative. This experimental design excluded the competition between the various chlorophenols for the cavities of HPBCD and their possible interaction with each other, which may influence the complex formation.

Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with β-cyclodextrins[14,15]. Methanol was incorporated in the eluent in the concentration range 0–30 vol.-% in steps of 5%. After development the plates were dried at 105°C and the chlorophenol spots and the HPBCD fronts were detected under UV light and with anthrone reagent, respectively. For each experiment five replicate determinations were carried out.

To separate the effect of methanol and HPBCD concentrations on the lipophilicity of chlorophenols, the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1C_1 + b_2C_2 \quad (1)$$

where

R_M = actual R_M value of a compound determined at given methanol and HPBCD concentrations;

R_{M0} = R_M value of a compound extrapolated to zero methanol and HPBCD concentrations;

b_1 = decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent;

b_2 = decrease in the R_M value caused by 1 mM change in the concentration of HPBCD in the eluent;

C_1, C_2 = methanol and HPBCD concentration, respectively.

Eqn. 1 was applied separately for each compound and for both HPBCD derivatives.

To elucidate the role of lipophilicity in the inclusion complex formation, linear correlations were calculated between the R_{MO} values and the b_2 values for each RPTLC system:

$$b_2 = a + bR_{MO} \quad (2)$$

To compare the complex-forming capacity of various cyclodextrin derivatives, the complex stability values of HPBCDs were linearly correlated with the complex stability values of a water-soluble β cyclodextrin polymer[16].

RESULTS AND DISCUSSION

The mean $R_F \times 100$ values of chlorophenols are given in Table I. The R_F values increase in each instance with increase in methanol concentration, *i.e.*, these compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using eqn. 1. An increase in HPBCD concentra-

tion also caused an increase in R_F values, proving the complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPBCDs with the chlorophenols reduces the lipophilicity of the latter.

The simultaneous effects of methanol and HPBCD 2,7 concentrations on the R_M values of 2,4-dichloro- and 2,4,6-trichlorophenols are shown in Figs. 1 and 2. In both instances the lipophilicity decreases with increasing methanol and HPBCD concentration; the effect depends on the type of compound and on the composition of the eluent.

The presence of HPBCDs in the eluent did not affect the compactness and symmetry of the peaks, as shown in Figs. 3–5. This observation is in good agreement with previous work[17], where β-cyclodextrin in the eluent did not influence the peak shape and symmetry markedly.

The parameters of eqn. 1 are compiled in Table II. The equation fits the experi-

TABLE I

R_F × 100 VALUES OF CHLOROPHENOLS

Eluent composition			*Compound*								
Methanol (%, v/v)	*Compound*	*Cyclodextrin concentration (mg/ml)*	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
30	HPBCD 4,6	0	0.29	0.36	0.35	0.63	0.77	0.73	0.75	1.25	1.36
35		0	0.20	0.30	0.26	0.53	0.64	0.64	0.65	1.08	1.15
40		0	0.12	0.25	0.21	0.43	0.53	0.56	0.59	1.00	1.01
45		0	0.03	0.09	0.05	0.28	0.34	0.36	0.42	0.74	0.76
25		10	0.33	0.39	0.36	0.63	0.76	0.69	0.72	0.99	1.35
30		10	0.30	0.33	0.30	0.55	0.66	0.61	0.64	0.92	1.17
20		15	0.30	0.39	0.36	0.63	0.70	0.62	0.72	0.87	1.35
25		15	0.28	0.36	0.34	0.61	0.72	0.55	0.68	0.86	1.33
30		15	0.22	0.29	0.26	0.48	0.61	0.57	0.58	0.76	1.13
20		20	0.23	0.34	0.33	0.60	0.68	0.60	0.66	0.78	1.24
25		20	0.22	0.30	0.29	0.53	0.66	0.58	0.60	0.71	1.13
30		20	0.21	0.23	0.22	0.45	0.56	0.48	0.52	0.65	1.01
15		25	0.39	0.37	0.34	0.63	0.69	0.60	0.61	0.72	1.23
20		25	0.28	0.30	0.28	0.52	0.59	0.56	0.58	0.89	1.14
25		25	0.19	0.27	0.25	0.49	0.59	0.50	0.55	0.59	1.06
30		25	0.11	0.24	0.22	0.44	0.51	0.47	0.50	0.56	1.00
25	HPBCD 2,7	10	0.36	0.43	0.42	0.70	0.84	0.79	0.80	1.05	1.50
30		10	0.25	0.35	0.36	0.58	0.69	0.67	0.70	1.00	1.24
20		15	0.40	0.45	0.44	0.76	0.87	0.79	0.80	0.97	1.51
25		15	0.24	0.36	0.35	0.55	0.74	0.69	0.68	0.85	1.28
30		15	0.20	0.29	0.26	0.51	0.65	0.63	0.62	0.80	1.24
20		20	0.21	0.38	0.38	0.69	0.72	0.69	0.71	0.70	1.44
25		20	0.15	0.29	0.27	0.50	0.67	0.59	0.59	0.67	1.13
30		20	0.14	0.24	0.22	0.45	0.57	0.51	0.54	0.74	1.09
15		25	0.27	0.37	0.38	0.58	0.75	0.68	0.68	0.71	1.35
20		25	0.19	0.34	0.34	0.59	0.71	0.62	0.66	0.71	1.32
25		25	0.08	0.23	0.23	0.47	0.56	0.54	0.54	0.61	1.08
30		25	0.00	0.14	0.12	0.32	0.48	0.42	0.43	0.47	0.92

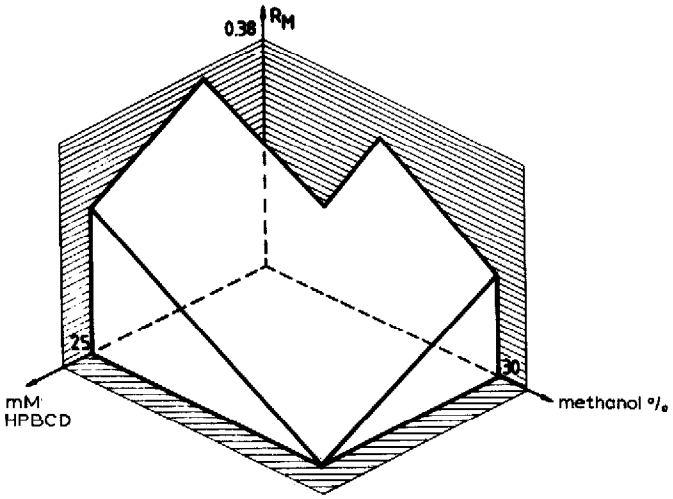

Fig. 1. Effect of methanol and HPBCD 4,6 concentrations on the R_M value of 2,4-dichlorophenol.

mental data well, the significance levels in each instance being over 99.9%; the ratios of variance explained were about 75–90% (see r^2 values). The complex stability (b^2 values) increases with increasing number of substituents. The monohalogenated derivatives form the weakest and the trichlorinated derivatives the strongest complexes. Methyl substitution has a similar influence to chloro substitution on the complex

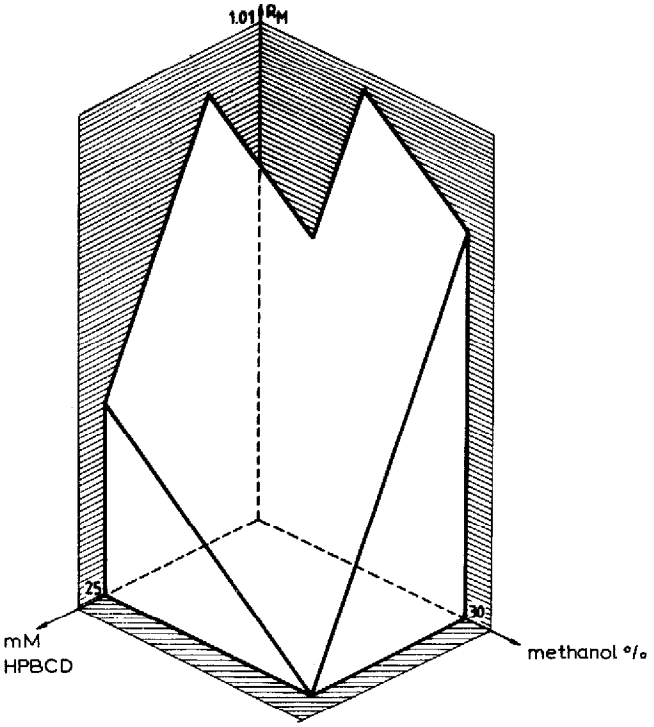

Fig. 2. Effect of methanol and HPBCD 4,6 concentrations on the R_M value of 2,4,6-trichlorophenol.

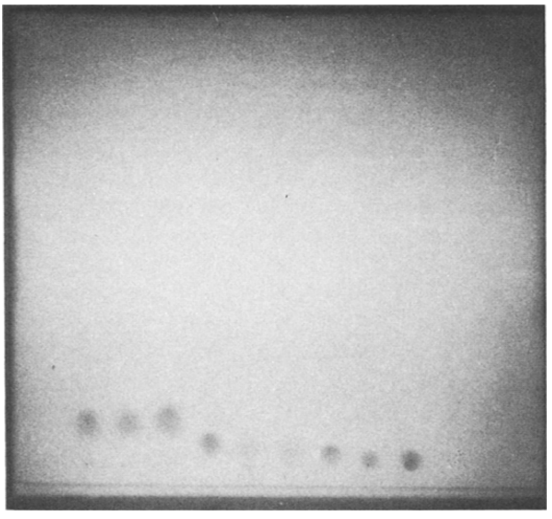

Fig. 3. UV detection of chlorophenols with water–methanol (4:1) as eluent. Chlorophenols I–IX appear consecutively from left to right.

stability. The normalized slopes (b') show that a change in HPBCD concentration has a similar effect to a change in methanol concentration on the retention of chlorophenols.

The R_F values of the front of the eluent additives are given in Table III. Each additive front is well ahead of the chlorophenol spots, *i.e.*, the differences observed between the retention behaviours of chlorophenols in various eluent systems are really caused by the presence of HPBCD.

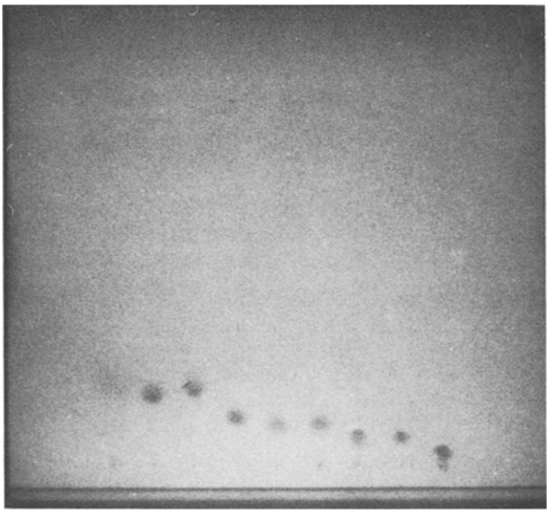

Fig. 4. UV detection of chlorophenols with water–methanol (4:1) + 25 mM HPBCD 2,7 as eluent. Chlorophenols as in Fig. 3.

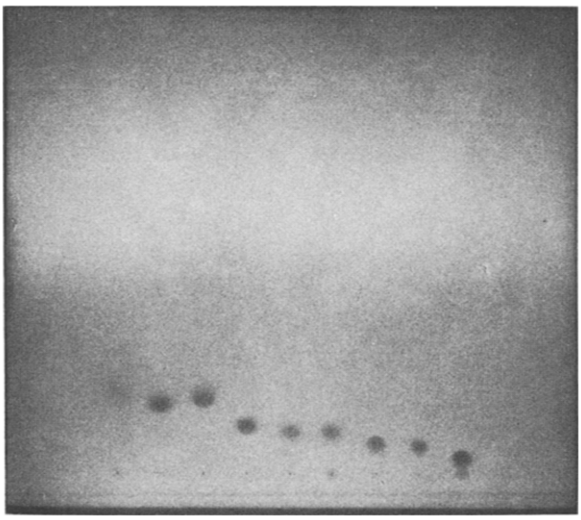

Fig. 5. UV detection of chlorophenols with water–methanol (4:1) + 25 m*M* HPBCD 4,6 as eluent. Chlorophenols as in Fig. 3.

TABLE II

CORRELATIONS BETWEEN R_M VALUES OF CHLOROPHENOLS AND CONCENTRATIONS OF METHANOL AND HPBCD IN THE ELUENT

Eqn. 1: $n = 16$; $F_{99.9\%} = 12.31$; $t_{99.9\%} = 4.22$.

Parameter	*Cyclodextrin*	*Compound*								
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
R_{M0}	HPBCD 4,6	0.74	0.76	0.73	1.13	1.27	1.12	1.18	1.73	2.16
$-b_1 \cdot 10^3$		15.2	13.2	13.4	17.4	18.2	14.4	15.1	19.3	28.4
$-b_2 \cdot 10^3$		6.23	6.32	5.89	8.41	9.73	10.4	10.7	25.4	15.6
$r^2(\%)$		82.21	84.80	84.30	90.13	77.75	75.13	83.96	83.81	83.99
$s \cdot 10^2$		4.11	3.19	3.38	3.29	5.46	4.76	3.78	7.98	6.97
F		30.04	36.26	34.91	59.36	22.72	19.63	34.02	35.66	34.09
t_1		7.37	8.34	8.04	10.68	6.71	6.08	8.06	4.89	8.23
t_2		3.83	5.01	4.41	6.46	4.50	5.51	7.14	8.05	5.66
b_1'		1.28	1.34	1.31	1.38	1.30	1.25	1.33	0.81	1.36
b_2'		0.67	0.81	0.72	0.84	0.88	1.13	1.18	1.33	0.93
R_{M0}	HPBCD 2,7	0.90	0.93	0.97	1.36	1.52	1.41	1.39	1.81	2.50
$-b_1 \cdot 10^3$		19.1	17.7	19.4	23.3	24.6	21.8	20.5	20.6	37.2
$-b_2 \cdot 10^3$		12.6	9.72	9.73	12.8	12.5	13.4	13.4	28.6	18.2
$r^2(\%)$		84.59	89.80	90.79	87.68	89.65	88.96	90.06	87.84	88.98
$s \cdot 10^2$		4.60	3.34	3.51	4.91	4.73	4.29	3.84	7.68	7.46
F		35.68	57.24	64.09	46.25	56.32	52.38	58.89	46.95	52.50
t_1		8.38	10.67	11.17	9.59	10.49	10.22	10.78	5.39	10.07
t_2		6.91	7.35	7.01	6.59	6.69	7.90	8.82	9.41	6.18
b_1'		1.36	1.40	1.40	1.39	1.39	1.40	1.40	0.78	1.38
b_2'		1.12	0.97	0.88	0.95	0.89	1.08	1.15	1.35	0.85

TABLE III

R_F VALUES OF HPBCD 2,7 AND HPBCD 4,6 FRONTS IN VARIOUS ELUENT SYSTEMS

Eluent composition		R_F *value*	
Methanol (%, v/v)	*HPBCD 2,7 or HPBCD 4,6 (mM)*	*HPBCD 2,7*	*HPBCD 4,6*
25	10	0.74	0.42
30	10	0.82	0.46
20	15	0.80	0.47
25	15	0.81	0.46
30	15	0.85	0.52
20	20	0.84	0.52
25	20	0.89	0.53
30	20	0.89	0.56
15	25	0.87	0.52
20	25	0.89	0.60
25	25	0.88	0.56
30	25	0.92	0.56

The correlation coefficients of eqn. 2 were 0.7171 and 0.6365 for HPBCD 4,6 and 2,7, respectively, which suggests that the lipophilicity of chlorophenol derivatives does not explain adequately the strength of interaction and the steric parameters are probably more important in the inclusion complex formation.

Significant linear correlations were found between the complex stability values of various cyclodextrin derivatives:

$$b_{\text{HPBCD 2,7}} = -0.88 + 0.66 b_{\text{SCDP}};\ r = 0.8803$$

$$b_{\text{HPBCD 4,6}} = -6.22 + 0.73 b_{\text{SCDP}};\ r = 0.9197$$

where SCDP is a water-soluble β-cyclodextrin polymer.

The results demonstrate that the various substituents on the β-cyclodextrin ring modify the complex-forming capacity, but the order of complex stabilities remains the same, that is, the interaction in each instance is governed by the insertion of guest molecules in the cyclodextrin cavity.

CONCLUSIONS

Chlorophenol derivatives form inclusion complexes with hydroxypropyl-β-cyclodextrins and the complex stability increases with increase in the number of chloro substituents whereas the position of substitution has smaller effect on the complex stability. Charge-transfer chromatography proved to be a suitable method for studying such interactions.

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