Inclusion Complexing by Water-Soluble β-Cyclodextrin Polymers

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Abstract. Cross-linked β-cyclodextrin with a molecular weight of less than 10 000 has good solubility in water, and it is a better inclusion complexing agent than the parent β-cyclodextrin. By including lipophilic guest molecules into the apolar cyclodextrin cavity, their apparent lipophilicity is reduced because the outer surface of the molecular 'wrapping' (the crosslinked β-CD) is highly hydrophilic. The relative stability of the inclusion complexes can be rapidly determined by reversed-phase thin-layer chromatography. The reversed-phase TLC behaviour of 25 triphenylmethane derivatives and analogues were studied in the presence of β-cyclodextrin polymers containing neutral and carboxyl groups. Increasing the molecular weight results in an increased complex-forming capacity. The carboxyl group modifies the accessibility of the CD cavity which in turn results in increased or decreased complex stability, depending on the guest molecule. The presence of organic solvents diminishes the stability of the CD complexes.

1. Introduction

Cross-linked β-cyclodextrin polymers of relatively low molecular weight (< 10 000) are considerably more soluble in water than unmodified β-cyclodextrin [1,4]. As a consequence of the substitution of some of the hydroxyl groups, a modification was expected in the inclusion complexing properties of the β-cyclodextrin 'capsule'. This modification must be reflected in the complex stability. For practical purposes, the relative stability characteristics are quite satisfactory. A rapid and simple method for obtaining such information is based on reversed phase thin-layer chromatography (RPTLC).

According to the polarity of the sorbent and mobile phase, chromatographic techniques can be divided in two groups:

(1) Polar sorbent–apolar mobile phase: this is adsorptive chromatography, (e.g., silica and alumina are the most typical sorbents, with water-insoluble organic solvents as mobile phases).

(2) Apolar sorbent–polar mobile phase: this is reversed-phase chromatography (e.g., silica or alumina impregnated with apolar oils as sorbents, water and organic solvents miscible with water as eluents).

The external surfaces of cyclodextrin molecules are hydrophilic, while the guest molecules are lipophilic, therefore complex formation decreases the lipophilicity of the molecules

included in the cavity of the cyclodextrins. This phenomenon can be utilized to estimate the complex stability: the differences between the lipophilicities of a compound in the presence, and in the absence, of complexing cyclodextrin or CD derivatives are related to the complex stability. The lipophilicity of polymixine in water-soluble β-cyclodextrin polymer (sCDP) has been studied by this method [5]. To extend the application of CDs in different fields of chromatography, various water-insoluble derivatives have been prepared and tested in gel [6, 7] and gas chromatography [8]. However, as a consequence of cross-linking, access to the sites of inclusion is partially hindered and furthermore, secondary cavities of different dimensions are formed within the polymer network. These dimensions can be partly commensurable with the dimensions of the cyclodextrin cavity. These facts may result in different inclusion complex formation energies of different CD polymers.

Table I.   Chemical structures of the studied compounds

No. of compound	R_1	R_2	R_3	R_4
1	-phenyl	-phenyl	-phenyl	–H
2	-phenyl	-phenyl	-phenyl	–OH
3	-phenyl	-phenyl	-phenyl	-2-imidazole
4	-phenyl	-phenyl	-phenyl	-1-triazole
5	-phenyl	-phenyl	-2-pyridine	–OH
6	-phenyl	-phenyl	-3-pyridine	–OH
7	-phenyl	-2,4-dichloro-phenyl	-5-pyrimidine	–OH
8	-phenyl	-phenyl	-4-chloro-phenyl	–OH
9	-phenyl	-4-chloro-phenyl	-2-pyridine	–OH
10	-phenyl	-phenyl	-2-chloro-phenyl	-2-imidazole
11	-phenyl	-phenyl	-2-chloro-phenyl	–OH
12	-phenyl	-4-methoxy-phenyl	-3-pyridine	–OH
13	-phenyl	-phenyl-SO_2-phenyl	-phenyl	–OH
14	-phenyl	-phenyl	-phenyl-SO_2-phenyl	–OH
15	-phenyl	-phenyl-SO_2-phenyl	-phenyl	-2-imidazole
16	-4-methoxy-phenyl	-4-methoxy-phenyl	-3-pyridine	–OH
17	$-C_2H_4-$	–H		
18	$-C_2H_4-$	–Cl		
19	$-C_2H_4-$	$-OCH_3$		
20	$-O-CH_2-$	–H		
21	$-O-CH_2-$	$-OCH_3$		
22	–H			
23	–Cl			
24	–OH			
25	$-CO-CH_3$			

Basic structure for

compounds 1–16:

$$R_1 - \underset{R_4}{\overset{R_3}{\underset{|}{\overset{|}{C}}}} - R_2$$

compounds 17–21:

$$R_2 - \bigcirc - \overset{OH}{\underset{|}{C}} - \bigcirc - R_1$$
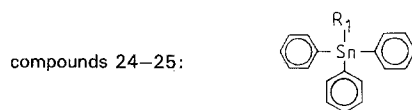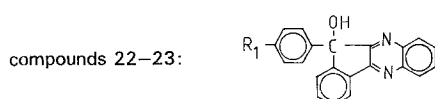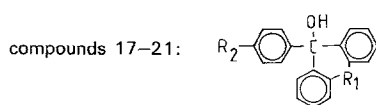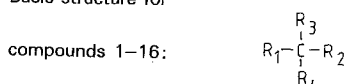
compounds 22–23:

compounds 24–25:

Table II. RPTLC systems to study the complexing ability of soluble β-cyclodextrin polymers

No. of System	Support	Eluent composition			
		water : ethanol ratio	Polymer mg/cm³ eluent		
			sCDPi-4300	sCDP-5300	sCDP-4500
I	Polygram Sil G	8 : 7	–	–	–
II	(Macherey–Nagel)	8 : 7	100	–	–
III		8 : 7	–	100	–
IV		8 : 7	–	–	100
V	DC Alufolien Aluminium-	8 : 7	–	–	–
VI	oxid 60 F$_{254}$ (Merck)	8 : 7	100	–	–
VII		8 : 7	–	100	–
VIII	DC Alufolien	2 : 1	–	–	–
IX	Cellulose F$_{254}$ (Merck)	2 : 1	100	–	–
X		2 : 1	–	100	–
XI	Polygram Sil G	8 : 7[a]	–	–	–
XII	(Macherey–Nagel)	8 : 7[b]	–	–	–
XIII		8 : 7[a]	100	–	–
XIV		8 : 7[b]	100	–	–
XV		2 : 1	–	–	–
XVI		2 : 1	100	–	–
XVII		2 : 1	–	100	–
XVIII		2 : 1	–	–	100

[a] 0.1 M NaOH instead of water.   [b] 0.1 M Acetic acid instead of water.

The objectives of our work were to compare the complexing ability of three different water soluble β-cyclodextrin polymers, to study the effect of molecular weight, and the presence of ionic groups built into the structure of the polymer, on the inclusion complex-forming properties.

The compounds investigated are commercially available or experimental fungicides. Besides being of theoretical interest their succesful cyclodextrin complexation may result in improved biological activity and in more effective pesticide formulations.

2. Materials and Methods

The polymers to be compared were prepared from β-cyclodextrin with epichlorohydrin, according to published methods [3, 9, 10]:

(1) sCDPi-4300: a water-soluble ionic (carboxymethylated) β-cyclodextrin polymer, $\overline{M}_w = 4300$ D, β-CD content: 57% COO^-/β-CD = 1.5; Ca^{2+}/β-CD = 0.45; Mg^{2+}/β-CD = 0.24; Na^+/β-CD = 0.33; $[\eta] = 4.7 \times 10^{-3}$ l/g.

(2) sCDP-5300: water soluble (non-ionic) β-CD polymer, $\overline{M}_w = 5300$ D, β-CD content: 60%; $[\eta] = 8.7 \times 10^{-3}$ l/g.

(3) sCDP-4500: as above, $\overline{M}_w = 4500$; β-CD content: 64%, $[\eta] = 5.7 \times 10^{-3}$ l/g.

The solubility in water of these cyclodextrin polymers is practically infinite. Solutions containing 10 g/100 ml were used as eluents. (Solubility of β-cyclodextrin at room temperature is 1.8 g/100 ml, but the solubility of its complexes is seldom more than 0.1 g/100 ml.)

Table III. $100 \cdot R_M$ values in various RPTLC systems (for symbols see Tables I and II). s = spot on start.

No. of compound	Number of RPTLC system													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XV	XVI	XVII	XVIII
1	181	113	102	114	146	103	92				s	147	121	136
2	81	56	52	63	46	35	23				149	108	83	93
3	65	36	21	28	−10	−21	−20				138	96	118	133
4	50	37	37	45	3	9	12				102	105	103	103
5	47	21	6	19	16	5	−4				86	57	41	47
6	10	−6	−18	−10	−25	−33	−43				53	39	30	37
7	35	20	5	16	2	−7	−18				98	71	37	58
8	127	87	80	81	84	70	56	104	31	5	179	135	110	116
9	79	52	43	49	55	39	30	74	18	−2	138	91	74	82
10	61	41	22	38	19	19	16				141	106	118	127
11	118	85	74	82	85	69	59	149	77	54	165	135	103	115
12	4	−10	−6	−13	−32	−42	−60				49	33	16	29
13	82	61	57	64	18	22	14				135	133	129	128
14	81	60	58	66	17	22	16				133	132	119	124
15	40	20	24	35	−18	−14	−15				117	102	124	117
16	−2	−21	−38	−31	−37	−52	−46				41	21	1	17
17	118	64	55	61	72	50	36				169	93	76	77
18	148	101	90	96	111	83	73	151	39	13	s	131	110	114
19	110	60	48	54	70	42	29				165	86	68	68
20	81	41	27	34	36	19	8				124	71	50	51
21	80	36	24	29	30	13	2				127	68	48	48
22	26	15	−8	10	−12	−16	−22				64	65	52	56
23	71	46	35	43	26	21	11				117	104	87	91
24	143	31	113	125	s	141	112				s	86	s	s
25	142	32	117	135	s	131	130				s	82	s	s

The chemical structure of the compounds to be complexed is shown in Table I. The characteristics of the RPTLC systems are compiled in Table II. As the sCDPi-4300 and the majority of the studied compounds contain dissociable polar groups, it was necessary to study the effect of pH value on the complex formation (see systems XI–XIV in Table II).

The chromatographic plates were impregnated with 5% paraffin oil in n-hexane overnight. After evaporating the n-hexane at room temperature, 5 μl of solutions of 2 mg compound/cm³ ethanol was spotted onto the plates. The spots were detected by UV adsorption. Each R_f value is the mean of five independent parallel determinations.

3. Results and Discussion

The R_M values measured in nonbuffered systems are listed in Table III. The low adsorptive capacity of cellulose also becomes manifest after impregnation with paraffin oil therefore we had to use a weaker eluent. However, under these conditions, the spots were deformed and the R_f values could not be accurately determined. These data are omitted from Table III. Corresponding to our expectations, each polymer reduced the lipophilicity of the guest compounds on each support proving the formation of the more hydrophilic inclusion complexes. The lipophilicity-reducing effect of sCDP-5300 is higher than that of sCDPi-4300, that is, the complex formation energy of the former is higher than that of the latter. The lipophilicity-reducing effect of sCDP-4500 was between those of ionic sCDPi-4300 and

nonionic sCDP-5300. The effect is not uniform for all compounds. The polymers reduce markedly the lipophilicity of compounds 1, 24 and 25 but only weakly compounds 3, 4, 12, 13, 14 and 15. The tin derivatives show a considerable affinity to the ionic sCDPi-4300, the phenyl-SO_2-phenyl group counteracts the complex formation. The cause of these phenomena is not clearly understood; it is assumed that not only steric, but also polar (adsorptive) properties may influence the interaction energy.

The few results on the cellulose support indicate that the effect of polymers is higher at a higher dielectric constant (lower ethanol ratio). The presence of organic solvents always diminishes the stability of cyclodextrin complexes.

The lipophilicity values (expressed as R_M) measured in buffered systems are compiled in Table IV. The R_M values of all compounds are lower in both alkaline and acidic environments than in an ion-free system. The probable reason for this observation is that the ions in eluents are readily absorbed on the silanol groups of a silica surface which is not covered by paraffin oil, thus reducing the overall retention capacity of the layer. A further possibility is that the pH value also influences the dissociation of polar substituents resulting in modified lipophilicity. The strongest fall in lipophilicity was found at acidic pH-values for tin compounds (Nos. 24 and 25) as well as for compounds containing the imidazole group (Nos. 3, 10 and 15).

Table IV. Effect of pH on the complexing capacity of sCDPi-4300 ($100 \times R_M$ values were measured in buffered system)

No. of compound	Number of RPTLC system			
	XI (alkaline)	XII (acidic)	XIII (alkaline)	XIV (acidic)
1	156	164	118	117
2	73	73	66	64
3	44	14	57	24
4	35	46	46	43
5	35	36	25	24
6	6	0	-3	-5
7	31	31	29	18
8	97	120	95	91
9	70	70	57	57
10	45	15	57	25
11	94	105	95	92
12	3	-3	-12	-11
13	61	66	70	67
14	58	68	70	68
15	22	9	37	19
16	-2	-11	-19	-25
17	96	103	76	66
18	127	137	105	104
19	88	103	71	60
20	62	68	52	43
21	64	70	48	42
22	17	18	16	14
23	51	55	50	44
24	127	57	104	28
25	129	54	107	25

The ionic soluble polymer (sCDPi-4300) formed the most stable complexes with compounds 1, 24 and 25. Except in these compounds, the lipophilicities in the presence of sCDPi-4300 are similar in both neutral and acidic environments, and a slight decrease was observed for compounds 3 and 10. Under alkaline conditions, there was a considerable enhancement of the lipophilicity of some compounds (Nos. 3, 4, 10, 14 and 15) in the presence of sCDPi-4300.

The lipophilicity orders of compounds determined in the absence and presence of soluble CD polymers correlate well with each other (Table V); that is, the polymers exert a similar effect on the compounds investigated. The intercept values represent the average effect of cyclodextrin polymers on the lipophilicity of all compounds. The intercepts ('a') values again prove that the effect of sCDP-5300 on lipophilicity is about twice that of sCDPi-4300 (see the 'a' values for Equations 1–2 and 19–20 in Table V). This effect is about twice as high in an eluent of lower ethanol concentration (lower ethanol concentration results in a higher complex stability, i.e., a stronger reduction of lipophilicity). It means that the complex-forming capacity of all β-cyclodextrin polymers decreases with an increasing ethanol concentration. The lower complexing ability of sCDP-4500, as compared with sCDP-5300, indicates that the molecular weight is a factor that must not be neglected. A higher degree of polymerization results in more stable inclusion complexes (Figure 1). However, the molecular weight differences do not totally explain the differences between the complexing capacities of polymers (e.g., sCDPi-4300 forms the more stable complexes with compounds 24 and 25). The present experimental data allows us to suppose that the bulky carboxyl groups – independent of the fact that they are easily polarizable – can sterically modify the accessibility of the β-cyclodextrin cavity. This is manifested directly in an increase or decrease of the complex stability.

Table V. Linear correlations between the R_M values of compounds determined in various RPTLC systems. $y = a + b \cdot x r_{99.9\%} = 0.6524$ (for 20 degree of freedom). (Roman numerals indicate R_M values measured in a given RPTLC system.)

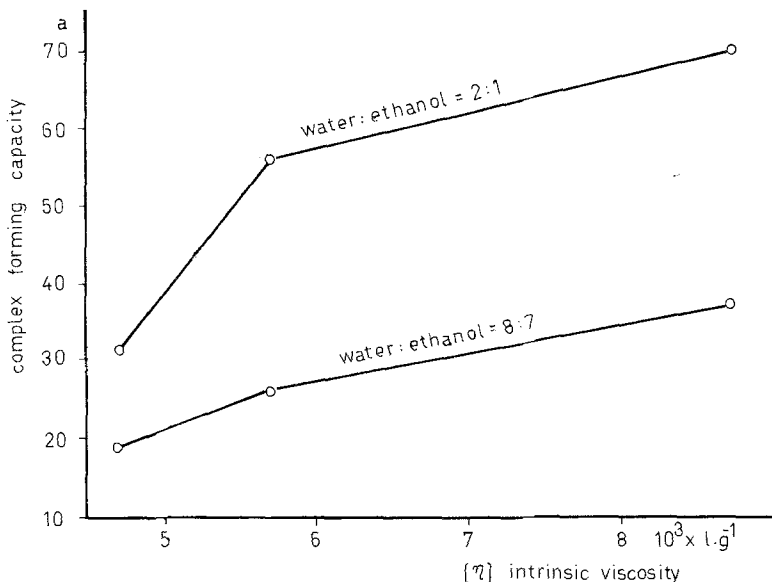| No. of equation	y	x	a	b	s_b	r	$t_{calculated}$	$t_{tabulated}$	n
1	I	II	17.88	1.23	0.06	0.9714	3.66	2.81 (99%)	25
2	I	III	37.45	1.22	0.16	0.8538	1.41		25
3	I	IV	26.32	1.08	0.09	0.9321	0.89		25
4	V	VI	8.43	1.17	0.05	0.9783	3.15	2.83 (99%)	23
5	V	VII	17.46	1.21	0.08	0.9609	2.77	2.08 (90%)	23
6	I	V	45.01	0.94	0.05	0.9661	1.15		23
7	I-II	I	0,65	0.38	0.06	0.7840			25
8	I-III	I	13.15	0.40	0.08	0.7446			25
9	V-VI	V	−0.26	0.27	0.03	0.8913			23
10	V-VII	V	11.16	0.25	0.05	0.7274			23
11	I	XI	8.18	1.11	0.03	0.9924			25
12	I	XII	24.73	0.93	0.06	0.8831			25
13	XI	XII	16.14	0.81	0.10	0.8673			25
14	II	XIII	−1.60	0.79	0.09	0.8713			25
15	II	XIV	2.56	0.93	0.02	0.9921			25
16	XI	XIII	2.34	1.07	0.07	0.9521			25
17	XII	XIV	6.16	1.19	0.08	0.9514			25
18	XIII	XIV	19.15	0.86	0.12	0.8206			25
19	XV	XVI	31.55	0.99	0.16	0.8100	0.06		21
20	XV	XVII	69.90	0.74	0.18	0.6943	1.44		21
21	XV	XVIII	56.10	0.76	0.19	0.6829	1.26		21

Fig. 1. Effect of the intrinsic viscosity of soluble CD polymers ([η], proportional to the molecular weight, or degree of polymerization) on the 'a' value (see in Table V) which is a measure of the complex forming capacity.

The effects of polymers are about two times higher on a silica than on an aluminium oxide support (Equations 4 and 5) because the support influences the effect of polymers, even after impregnation. The aluminium oxide support results in a lower retention than the silica, and this difference also remains after impregnation.

In some cases, the slope (b') values deviate significantly from the theoretical b = 1 value. This finding again emphasizes that the effect of polymers on the compounds is not uniform. To find the explanation, the original lipophilicity of the compounds has been correlated to the lipophilicity differences resulting from the presence of polymer (Equations 7–10). In all cases, highly significant linear correlations were obtained. The effect of polymers is greater on the more lipophilic compounds, i.e., the lipophilicity is a very important factor in determining the complex stability. This observation is further support for the theory in which the apolar–apolar interaction between host and guest is the most important factor in inclusion complex formation.

The lipophilicity values in the presence of sCDPi-4300 do not generally depend on pH (low intercept values of Equation 14 and 15), that is, the effect of the dissociation of carboxyl groups of sCDPi-4300 on the complex forming is nearly always negligible. The stability of the inclusion complexes is similar in both neutral and acidic environments ('a' values of Equations 1 and 18) and somewhat weaker under alkaline conditions ('a' value of Equation 17) but it never reaches that level which is characteristic for the nonionic sCDP-5300 polymer (Equation 2). This means that the dissociation of carboxyl groups is not responsible for the lower complexing ability of sCDPi-4300.

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