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Note

Reversed-phase thin-layer chromatography of barbiturates in the presence of soluble β-cyclodextrin polymer

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Cyclodextrins (CDs) form inclusion complexes with many different compounds, and are widely utilized for stabilization and solubilization, formulation of drugs, pesticides, flavouring substances, etc.[1,2]. β-Cyclodextrin (β-CD), although readily available, has a relatively low solubility; therefore its hydrophilic derivatives, methylated or cross-linked, have been employed for solubilization purposes[3–5]. The least expensive of such derivatives is the so-called water-soluble β-CD polymer (SCDP), prepared from the reaction of β-CD with epichlorohydrin in an alkaline medium. The reaction has to be well controlled in order to avoid the formation of insoluble true polymers (which can be utilized for other purposes).

SCDP is a highly water-soluble low-molecular-weight polymer, which consists of two to five β-CD moieties cross-linked with glyceryl bridges, and is highly substituted with very hydrophilic glyceryl ether groups. A 40 g/100 cm^3 aqueous solution of SCDP can easily be prepared, in which substances of very low solubility can be dissolved. Therefore, SCDP can be used for dissolving water-insoluble dyes in the photochemical industry[6], to enhance the bioavailability of orally administered drugs[7], to reduce the lipophilicity of components in reversed-phase thin-layer chromatography (RPTLC), etc.[8].

RPTLC is adequate to characterize the stability of the inclusion complex. Varying the structure of the guest molecule enables the study of structure–complex stability correlations. This method also provides direct information concerning the reduction of lipophilicity, which in turn is related to the complex stability. In previous papers, series of symmetric triazine[9] and triphenylmethane derivatives[10] were studied by means of this technique; the present paper deals with a series of barbiturate derivatives.

EXPERIMENTAL

The structures of the barbituric acid derivatives are shown in Table I.

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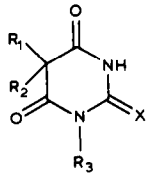
TABLE I

STRUCTURES OF THE BARBITURIC ACID DERIVATIVES



Compound No.	R_1	R_2	R_3	X
1	Methyl	1-Methylpentyl	H	O
2	Ethyl	1-methylbutyl	H	O
3	Ethyl	3-Methylbutyl	H	O
4	Ethyl	1-Methylpropyl	H	O
5	Ethyl	*n*-Pentyl	H	O
6	Butyl	1-Methylpropyl	H	O
7	Butyl	1-Methylbutyl	H	O
8	Butyl	3-Methylbutyl	H	O
9	Ethyl	*n*-Octyl	H	O
10	Ethyl	3-Dimethyloctyl	H	O
11	Allyl	Isopropyl	H	O
12	Allyl	1-Methylpropyl	H	O
13	Allyl	1-Methylbutyl	H	O
14	Methyl	1-Cyclohexenyl	Methyl	O
15	Allyl	2-Cyclopentenyl	H	O
16	Ethyl	1-Cyclohexenyl	H	O
17	Ethyl	Ethyl	H	O
18	Ethyl	1-Methylbutyl	H	S
19	Allyl	1-Methylbutyl	H	S
20	Ethyl	1,3-Dimethylbutyl	H	O
21	Ethyl	Phenyl	H	O

Polygram Sil G plates (Macherey-Nagel) were impregnated with paraffin oil as described in ref. 9. The barbituric acid derivatives were dissolved in methanol at a concentration of 4 mg/cm^3; 5 mm^3 of each solution was spotted on the plates. Ethanol was chosen as the organic solvent miscible with water because it forms only a very weak inclusion complex with β-CD[11,12]. The ethanol concentration in the eluent was varied from 3.3 to 50% (v/v) in steps of 3.3%.

The water-soluble β-CD polymer (weight-average molecular mass 4500; β-CD content 64%; intrinsic viscosity $5.7 \cdot 10^{-3}$ l g^{-1}) was prepared by cross-linking with epichlorohydrin[13], and dissolved in the ethanol–water eluent systems.

The determination of the lipophilicity of barbituric acid derivatives was carried out at each ethanol concentration without and with added SCDP at a concentration of 16.7 mg per cm^3 eluent. For each experiment, five replicate determinations were carried out.

After development the plates were dried at 105°C and the barbituric acid derivatives were detected by use of a mercurous nitrate reagent. The migration of SCDP was checked on separate plates with each solvent system: the SCDP front was detected by use of the anthrone reagent[14].

TABLE II

100 · R_F VALUES OF BARBITURATES UNDER DIFFERENT RPTLC CONDITIONS

A = SCDP-free eluent; B = eluents with SCDP.

Compound	*Ethanol in eluent (%, v/v)*														
	3.33	*6.67*	*10*	*13.33*	*16.67*	*20*	*23.33*	*26.67*	*30*	*33.33*	*36.67*	*40*	*43.33*	*46.67*	*50*
1 A						4	9	12	12	20	22	28	46	53	64
B						12	15	17	22	24	31	39	49	53	67
2 A		4	9	7	12	14	24	29	30	38	42				
B		15	17	19	22	31	34	38	43	47	54				
3 A		6	8	7	11	15	24	29	30	38	43				
B		16	16	18	22	28	33	37	43	47	55				
4 A		15	18	19	25	29	40	47	45	52					
B		20	24	28	33	43	47	52	59	61					
5 A						12	19	24	26	33	38	43	62	68	76
B						24	27	30	35	40	49	57	66	71	79
6 A						4	6	9	9	16	20	26	44	52	62
B						10	11	13	16	20	28	34	48	54	69
7 A						4	6	9	9	16	20	25	44	51	63
B						8	11	12	16	19	27	34	47	54	68
8 A						3	6	8	10	15	19	25	43	51	62
B					12	14	16	18	22	29	35	48	55	68	
9 A							2	2	3	6	9	13	28	39	52
B							8	10	10	12	17	23	33	43	59
10 A						1	2	4	4	9	13	18	34	44	56
B						23	24	24	26	26	33	37	45	54	69

11	A	14	20	25	27	35	38	48	54	53	59				
	B	21	26	30	34	41	51	54	59	62	66				
12	A	8	14	15	17	22	25	35	40	41	47				
	B	16	19	22	25	29	36	40	43	49	53				
13	A						10	16	21	23	29	35	40	56	65	72
	B						25	26	29	33	38	45	3	64	68	73
14	A	5	7	11	12	16	21	30	35	36	43					
	B	12	16	16	19	23	31	36	38	44	49					
15	A	7	13	14	16	19	24	34	38	40	46					
	B	18	22	25	28	34	47	50	52	56	60					
16	A	6	11	15	18	22	26	38	44	44	49					
	B	19	23	25	31	38	49	54	57	60	65					
17	A	20	26	33	35	40	41	50	52	53	57					
	B	25	31	35	39	44	49	56	59	61	64					
18	A						6	10	13	14	19	23	28	43	53	61
	B						19	22	22	24	28	33	40	49	54	68
19	A						4	7	9	10	15	18	23	36	45	56
	B						18	17	18	23	23	27	33	43	48	61
20	A						9	13	17	19	25	31	35	51	62	72
	B						26	31	37	37	39	48	51	62	63	75
21	A	12	15	21	23	27	32	43	44	47	52					
	B	20	24	26	33	41	54	64	63	67	69					

TABLE III

PARAMETERS OF THE LINEAR CORRELATION BETWEEN THE R_M VALUES OF SOME BARBITURIC ACID DERIVATIVES AND THE ETHANOL CONCENTRATION OF ELUENT, ΔR_{M0} VALUES RELATED TO THE COMPLEX STABILITY AND THE DEPENDENCE OF COMPLEX STABILITY ON THE ETHANOL CONCENTRATION OF THE ELUENT, Δb

$b = 10$, $r_{99.9\%} = 0.8721$.

Compound No.	*Without SCDP*			*With SCDP*			ΔR_{M0}	Δb
	R_{M0}	b	r	R_{M0}	b	r		
1	234.7	−5.12	0.9855	166.7	−3.75	0.9890	68.0	−1.37
2	154.8	−4.03	0.9813	96.2	−2.79	0.9931	58.6	−1.25
3	150.3	−3.86	0.9872	98.1	−2.83	0.9935	52.2	−1.03
4	99.3	−3.19	0.9854	80.3	−3.13	0.9942	20.0	−0.06
5	170.3	−4.28	0.9874	130.0	−3.61	0.9909	40.3	−0.68
6	248.2	−5.32	0.9925	196.1	−4.36	0.9841	52.0	−0.96
7	253.9	−5.44	0.9930	201.6	−4.47	0.9890	52.1	−0.98
8	253.1	−5.40	0.9935	176.2	−3.93	0.9821	76.9	−1.47
9	352.2	−7.04	0.9949	222.0	−4.46	0.9688	130.1	−2.58
10	317.9	−6.53	0.9961	121.4	−2.71	0.9260	196.5	−3.81
11	82.4	−3.13	0.9873	65.1	−2.95	0.9928	17.3	−0.17
12	109.3	−3.24	0.9855	80.6	−2.66	0.9880	28.7	−0.59
13	179.6	−4.33	0.9910	123.8	−3.30	0.9851	55.7	−1.03
14	134.1	−3.82	0.9916	95.7	−2.83	0.9928	38.4	−0.99
15	114.5	−3.34	0.9848	74.3	−2.91	0.9859	40.3	−0.43
16	118.5	−3.77	0.9846	74.7	−3.20	0.9895	43.8	−0.57
17	60.1	−2.33	0.9796	51.5	−2.44	0.9924	8.5	0.11
18	210.8	−4.59	0.9804	133.7	−3.03	0.9862	77.1	−1.55
19	231.2	−4.70	0.9923	136.6	−2.83	0.9525	94.6	−1.87
20	192.1	−4.46	0.9891	105.7	−2.86	0.9779	86.5	−1.61
21	93.4	−3.06	0.9904	71.4	−3.34	0.9621	22.0	0.28

To increase the accuracy of our investigations, the R_M values were extrapolated to zero ethanol concentration separately for eluents with and without SCDP

$$R_M = R_{M0} + bC \tag{1}$$

where R_M = the actual R_M value of a compound determined at the given ethanol concentration, R_{M0} = the R_M value of a compound extrapolated to zero ethanol concentration, b = decrease in the R_M value caused by a 1% increase in the ethanol concentration in the eluent and C = ethanol concentration (%, v/v) in the eluent. The differences between the R_{M0} values, ΔR_{M0}, calculated for water and for water with added SCDP were considered to be related to the stability of the inclusion complex.

RESULTS AND DISCUSSION

The retention data are listed in Table II and the results calculated by eqn. 1 are compiled in Table III.

Due to its highly hydrophilic character, the SCDP front coincided with the

eluent front in each eluent system, which means that the retention data for the barbiturates do not need correction for the different mobilities of SCDP in the different eluent systems.

In all cases, the R_M values were correlated linearly with the ethanol concentration at a significance level of 99.9%. These lipophilicity values are in good agreement with data in refs. 15–17.

The empirical complex stability values, ΔR_{M0}, indicate that the complex stability increases with increasing chain length of the alkyl substituents both at R_1 and R_2 (see compound pairs 2, 4; 2, 7; 4, 6; 5, 9; 6, 7 and 11, 12). This means that the barbituric acid derivatives containing longer alkyl chains fit the cyclodextrin cavity better than do the other derivatives. Branching of the alkyl chain (compounds 2, 3 and 5) exerts a similar effect, increasing the complex stability: the bulkier molecules fill the cyclodextrin cavity more completely. A cyclohexyl ring increased the complex stability more than did a benzene ring (compounds 16 and 21). The substitution of oxygen by sulphur considerably enhanced the complex stability (compounds 2, 18 and 13, 19).

A good linear correlation was found between the ΔR_{M0} values expressing complex stability and the ethanol concentration of the eluent

$$\Delta R_{M0} = 11.67 + 44.85 \cdot \Delta b$$
$$n = 21, \qquad r_{calc.} = 0.9797$$

where $\Delta b = b_1 - b_2$ and b_1, b_2 = slope of eqn. 1 for SCDP-free eluents and for eluents containing SCDP respectively. This finding shows that the higher the complex stability the more rapidly it deteriorates with increasing organic solvent concentration, and emphasizes the necessity in the preparation of inclusion complexes to use an organic solvent concentration as low as possible.

The R_{M0} and b values determined in SCDP-free eluents showed a very good agreement:

$$R_{M0} = -109.4 - 66.59b$$
$$n = 21, \qquad r_{calc.} = 0.9906$$

This demonstrates again that not only the lipophilicity value extrapolated to zero organic phase concentration but also the slope, b, of eqn. 1 characterize the lipophilicity of a molecule.

Our complex stability data showed good agreement with the values published in refs. 18 and 19, the coefficients of the linear regressions being 0.7949 ($n = 8$) and 0.8737 ($n = 7$) respectively. This indicates that the RPTLC method is suitable to characterize the complex stability. It does not necessitate complicated instrumentation and is very easy to carry out.

The conclusions of the present work are in perfect accord with those drawn from studies of the correlation between the structure and inclusion-forming capacity of barbituric acid derivatives[20].

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