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Chromatographic Properties of *s*-Triazines in the Presence of Soluble β-Cyclodextrin Polymer

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Abstract. The effect of a water-soluble β-cyclodextrin polymer on the lipophilicity and adsorption strength of 17 substituted *s*-triazine derivatives was studied by thin-layer chromatography. Beta-cyclodextrin polymer dissolved in the mobile phase modifies the chromatographic behaviour of *s*-triazine derivatives and, consequently, higher R_f and lower R_M values were observed. LiCl exerts an opposite influence, it decreases the R_f and increases the R_M values. The β-cyclodextrin polymer enhances the mobility of the *s*-triazine derivatives on silica gel and reduces their lipophilicity, thus promoting their penetration through the hydrophilic membranes of the target organism. The presence of LiCl decreases the stability of inclusion complexes. The first and second substituents on the *s*-triazine ring result in an increase of the inclusion complex stability but – due to steric hindrances – the third substituent decreases it.

Key words: β-cyclodextrin polymer, triazines, thin-layer chromatography.

1. Introduction

Cyclodextrins are able to form inclusion complexes with a fairly large number of organic compounds [1,2]. This phenomenon serves as a basis for the chromatographic separation of various compounds, e.g., amino acids [3], prostaglandins [4], alkaloids [5] and mandelic acid derivatives [6], and for the thin-layer chromatographic (TLC) separation of benzoic acid and phenol derivatives [7,8,9] etc. [1,10].

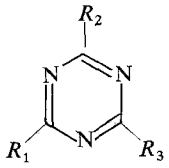
Chromatography is suitable, however, not only for the separation of different compounds but also for the determination of various physicochemical parameters, such as adsorption energy [11,12] and lipophilicity, often used in quantitative structure-activity relationship (QSAR) studies [13]. The aim of the present work was to study, by TLC, the effect of inclusion complex formation on the lipophilicity and adsorption energy of some bioactive compounds, because these two characteristics correlate generally with the biological activity.

The chromatographic behaviour of 17 symmetric triazine derivatives (see Table I) were studied. These compounds are applied widely in agricultural practice as preemergent herbicides.

2. Materials and Methods

Adsorptivity was determined on DC-Alufolien Kieselgel $60F_{254}$ (Merck) plates. For the lipophilicity measurements these plates were impregnated with 5% paraffin oil in *n*-hexane (reversed phase thin-layer chromatography, RPTLC). The triazine derivatives were dissolved

Table I. Structure of studied *s*-triazine derivatives



No	Common name	R_1	R_2	R_3
1	Simazine	Cl	$-NH-C_2H_5$	$-NH-C_2H_5$
2	Atrazine	Cl	$-NH-C_2H_5$	$-NH-CH-(CH_3)_2$
3	Propazine	Cl	$-NH-CH-(CH_3)_2$	$-NH-CH-(CH_3)_2$
4	Terbutylazine	Cl	$-NH-C_2H_5$	$-NH-C-(CH_3)_3$
5	Trietazine	Cl	$-NH-C_2H_5$	$-NH-C-(C_2H_5)_3$
6		Cl	$-NH-C-(CH_3)_3$	$-NH-C-(CH_3)_3$
7		Cl	Cl	$-NH-C-(CH_3)_3$
8		Cl	Cl	$-NH-C_2H_5$
9		Cl	H	H
10		Cl	H	$-NH-C_2H_5$
11	Atratone	OCH_3	$-NH-C_2H_5$	$-NH-CH-(CH_3)_2$
12	Prometone	OCH_3	$-NH-CH-(CH_3)_2$	$-NH-CH-(CH_3)_2$
13	Terbumetone	OCH_3	$-NH-C_2H_5$	$-NH-C-(CH_3)_3$
14	Etazine	OCH_3	$-NH-C_2H_5$	$-NH-CH-(CH_3)-C_2H_5$
15	Ametrine	SCH_3	$-NH-C_2H_5$	$-NH-CH-(CH_3)_2$
16	Prometrine	SCH_3	$-NH-CH-(CH_3)_2$	$-NH-CH-(CH_3)_2$
17	Terbutrine	SCH_3	$-NH-C_2H_5$	$-NH-C-(CH_3)_3$

in chloroform and from the 1 mg/cm^3 solutions 5 μl was spotted to the plates. The eluent systems are listed in Table II. To prove that the observed effect of the β-cyclodextrin polymer is not the result of an interaction between the β-cyclodextrin polymer and the plate surface, the investigations were repeated with glucose instead of β-cyclodextrin polymer.

Table II. Eluent systems for TLC and RPTLC studies of *s*-triazine derivatives

Eluent system	Layer	Water (cm^3)	Eluent composition			
			Methanol (cm^3)	Glucose (g)	β-cyclodextrin polymer (g)	$LiCl \cdot H_2O$ (g)
a		20	10			
b		20	10		2	
c	silica-	20	10			2.7
d	gel	20	10		2	2.7
e		20	10	2		
f			15			
g			15		2	
h	reversed		15			4.0
i	phase		15		2	4.0
j			15	2		

Because of the low solubility of β-cyclodextrin in the eluents, its water-soluble polymer [14] (5300 Dalton's molecular weight, on average) was used.

Methanol was used as an organic solvent component, because of its negligible tendency to form an inclusion complex with the β-cyclodextrin polymer. The influence of inorganic ions was studied by adding LiCl to the system [15]. To extract maximum information from the data, multivariate techniques such as principal component analysis (PCA) and Fujita Ban analysis [16] were applied.

3. Results and Discussion

The measured R_f and R_M values are summarized in Table III. Each value is the mean of five independent parallel determinations, where the coefficient of variation never exceeded 4%. The R_f and R_M values determined in eluent containing glucose do not deviate significantly from the corresponding values obtained in glucose-free eluent. This means that the modification of R_f and R_M values in the presence of the β-cyclodextrin polymer can probably be attributed to inclusion complexation and not to some nonspecific carbohydrate adsorption on the surfaces of TLC and RPTLC plates.

Table III. The R_f and R_M values of some *s*-triazine derivatives in different TLC and RPTLC systems

Compound number	R_f					R_M				
	System designation					System designation				
	a	*e*	*b*	*c*	*d*	*f*	*j*	*g*	*h*	*i*
1	0.21	0.24	0.71	0.80	0.76	− 0.03	− 0.06	− 0.40	0.37	0.24
2	0.69	0.71	0.64	0.39	0.56	0.09	0.06	− 0.31	0.58	0.55
3	0.41	0.39	0.05	0.21	0.31	0.31	0.30	− 0.08	0.88	0.90
4	0.43	0.45	0.68	0.20	0.34	0.36	0.37	− 0.20	0.79	0.57
5	0.46	0.43	0.57	0.19	0.34	0.47	0.50	0.06	0.96	0.95
6	0.08	0.13	0.45	0.03	0.09	0.76	0.83	0.18	1.30	1.14
7	0.41	0.46	0.79	0.20	0.38	0.37	0.39	− 0.13	0.87	0.62
8	0.73	0.71	0.81	0.61	0.77	− 0.09	− 0.07	− 0.39	0.28	0.29
9	0.81	0.79	0.78	0.79	0.86	− 0.48	− 0.50	− 0.55	− 0.26	− 0.31
10	0.84	0.81	0.73	0.74	0.74	− 0.47	− 0.50	− 0.40	− 0.18	− 0.36
11	0.71	0.71	0.80	0.49	0.55	0.08	0.03	− 0.39	0.42	0.28
12	0.64	0.63	0.66	0.39	0.47	0.23	0.25	− 0.22	0.61	0.48
13	0.55	0.60	0.80	0.38	0.62	0.31	0.33	− 0.32	0.61	0.39
14	0.58	0.57	0.72	0.41	0.51	0.24	0.26	− 0.19	0.61	0.49
15	0.49	0.43	0.63	0.27	0.59	0.29	0.30	− 0.20	0.70	0.61
16	0.34	0.32	0.41	0.14	0.34	0.51	0.52	− 0.02	0.99	0.86
17	0.25	0.23	0.70	0.14	0.25	0.60	0.58	− 0.08	0.92	0.66

The higher R_f values observed in eluents containing β-cyclodextrin polymer indicate that the complex formation decreases the adsorption energy of symmetric triazine derivatives on the silica gel surface and so enchances their mobility. Increasing ion concentration exerts an opposite effect; the adsorption of triazines becomes stronger. This is an unexpected observation, since ions adsorbed on the free silanol groups of the silica surface ought to have decreased its adsorptivity. This effect was found in pure organic solvents. In eluents containing larger amounts of water, however the ions modify the solvate shell around the

triazine derivatives to such an extent that this adsorption strength-increasing effect outweighs the decrease in the triazine-adsorbing strength of silica caused by the ion environment.

The retention modifying effect of β-cyclodextrin polymer is reduced by the presence of inorganic ions. The R_m values show a similar behaviour pattern. The β-cyclodextrin polymer decreases the lipophilicity of the triazine derivatives thus facilitating their penetration through the lipophilic barriers (membranes) of the target organism. The ion concentration – due to its solvate shell-modifying effect – generally increases the lipophilicity of the triazine derivatives and decreases the stability of the inclusion complexes.

Since the R_f values measured in eluents containing glucose did not give any new information, they were not included in the calculations.

It can be established on the basis of the correlation matrix of PCA (Table IV) that, in most cases, the R_f and R_M values correlate well with each other. The negative regression coefficients of the linear correlations between R_f and R_M values point to the fact that higher lipophilicity follows a higher adsorption energy on the silica surface in an eluent containing a larger amount of water. In organic eluents the opposite correlation is found.

Table IV. Correlation matrix of principal component analysis. $r_{95\%} = 0.4821$, $r_{99\%} = 0.6055$, $r_{99.9\%} = 0.7246$

System designation	System designation						
	b	*c*	*d*	*f*	*g*	*h*	*i*
a	0.4298	0.6146	0.7223	− 0.7818	− 0.7256	− 0.7862	− 0.7280
b		0.4980	0.5580	− 0.4127	− 0.5949	− 0.5125	− 0.6070
c			0.9292	− 0.9323	− 0.8979	− 0.9353	− 0.9003
d				− 0.9104	− 0.9265	− 0.9223	− 0.8777
f					0.8907	0.9826	0.9350
g						0.9167	0.9077
h							0.9758

The first eigenvalue explains about 82% of the total variance (Table V), that is, one background factor (probably the chemical structure of triazines) governs the behaviour of the triazine derivatives in TLC, as well as in RPTLC systems.

The deciding role of that one background factor is established by studying the PCA loadings. We have to stress, however, that the adsorption strength of triazines in the presence

Table V. Parameters of principal component analysis

	Eigenvalue	Sum of variance explained (%)	System designation	Loadings	
1	6.56	81.98	*a*	− 0.80	0.11
2	0.71	90.85	*b*	− 0.61	− 0.79
3	0.43	96.28	*c*	− 0.94	0.09
4	0.15	98.16	*d*	− 0.96	0.03
5			*e*	0.96	− 0.23
6			*f*	0.96	0.03
7			*g*	0.98	− 0.12
8			*h*	0.97	0.02

of a β-cyclodextrin polymer also depends considerably on a second background factor which may be interpreted as the inclusion complex stability or some other physicochemical characteristics of the inclusion complexes.

It can be clearly seen from the two-dimensional nonlinear mapping of PCA loadings (Figure 1, number of iterations: 24, max. error: 0.0137) that the TLC and RPTLC systems separate well from each other and they form definite clusters. The systems containing β-cyclodextrin polymers deviate markedly from the others, pointing to a divergent phenomenon, the inclusion complex formation. The systems containing both β-cyclodextrin polymers and LiCl do not deviate from the others; again proving the antagonistic effect of inorganic ions on inclusion complex formation.

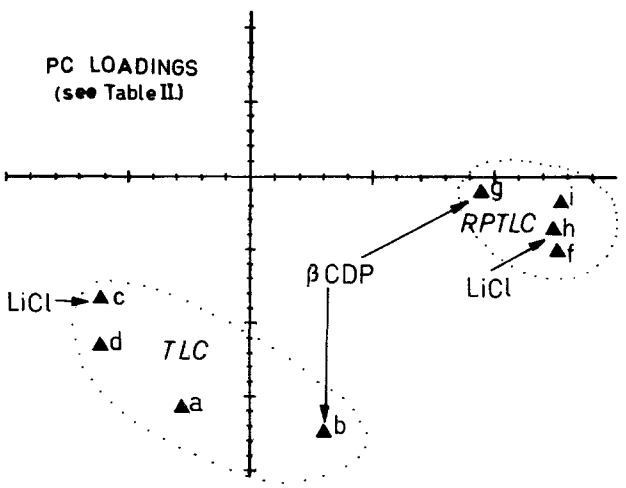


Fig. 1. Two-dimensional nonlinear mapping of PCA loadings (for signs see Table II).

The pattern of the two-dimensional nonlinear mapping of PCA variables is more complicated than that of PCA loadings (Figure 2, number of iterations: 34, max. error: 0.0161). Only compounds 9 and 10 containing dissociable hydrogen separate well from the others. The compounds with one or two chloro-substituents (compounds 1–10) do not form clusters.

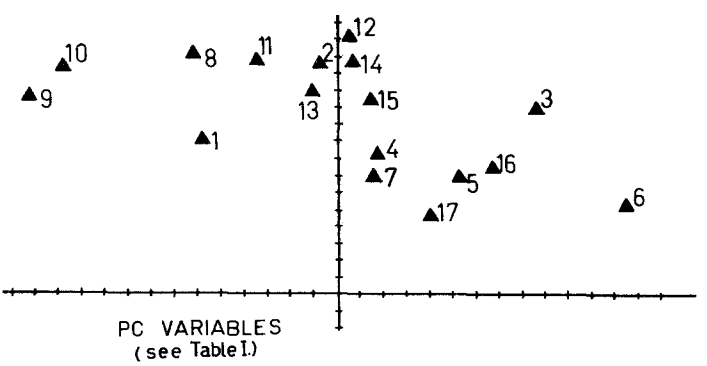


Fig. 2. Two-dimensional nonlinear mapping of PCA variables (for signs see Table I).

A fairly good correlation was found between the compounds with OCH_3 substituents (11–14) but, surprisingly, compound 2 belongs to this cluster. The triazines with SCH_3 groups form a loose cluster which also includies compounds 4, 5 and 7.

We supposed that the change of R_f or R_M values caused by the β-cyclodextrin polymer is related to the stability of the inclusion complexes. To determine the effect of various substituents on the inclusion complex formation, our data were subjected to Fujita Ban analysis. The F values of the data measured under adsorptive conditions did not show significant correlations (for β-cyclodextrin: $F = 1.56$; $r = 0.880$; for LiCl: $F = 1.57$: $r = 0.881$). It is not unexpected, considering that three factors simultaneously influence the measured R_f-values: the modification of the adsorption energy of the sorbent, the change of adsorption energy of the triazine derivatives caused by the transformed solvate shell around the molecule, and the stability of the inclusion complex. It is hardly to be expected that the outcome of these effects could be described by a simple Fujita Ban analysis.

The data on lipophilicity show highly significant correlations mainly in the case of β-cyclodextrin polymer (Table VI).

Table VI. Parameters of Fujita–Ban analysis (effect of substituents on the lipophilicity of inclusion complexes of some *s*-triazine derivatives). $t_{95\%} = 2.57$, $t_{99\%} = 4.03$, $t_{99.9\%} = 6.86$, $F_{99.9\%} = 9.58$. Activity of unsubstituted compound (Table I, No 9): − 0.070

	Activity contribution	s	t
R_1 = $-OCH_3$	−0.069	0.013	5.31
$-SCH_3$	−0.116	0.013	8.92
R_2 = $-NH-C_2H_5$	−0.437	0.021	20.81
$-NH-CH-(CH_3)_2$	−0.438	0.025	17.52
$-NH-C-(CH_3)_3$	−0.455	0.028	16.25
Cl	−0.372	0.021	17.71
R_3 = $-NH-C_2H_5$	0.140	0.023	6.09
$-NH-CH-(CH_3)_2$	0.112	0.029	3.86
$-NH-C-(CH_3)_3$	−0.055	0.028	1.96
$-NH-C-(C_2H_5)_3$	0.098	0.031	3.16
$-NH-CH-(CH_3)C_2H_5$	0.147	0.033	4.45

$r = 0.999$, $s = 0.016$, $F = 192.36$.

The substitution at R_1 (for symbols, see Table I) increases the stability of the inclusion complex; the strongly lipophilic and voluminous $-SCH_3$ group exerts the highest effect. In all cases the substitution at R_2 had the greatest stabilizing effect on the inclusion complexes. The more bulky the substituent, the higher is its stabilizing effect because the substituents probably make the compounds sterically more favourable for inclusion complex formation.

At first it seems to be surprising that the same substituents at R_3 decrease the inclusion complex stability (except the *t*-butylamino group; but its activity contribution does not deviate significantly from zero). The probable explanation is that, with a third substituent, the guest molecule becomes too bulky to fit comfortably in the cavity of the β-cyclodextrin polymer. The differences of calculated and measured R_M values never exceeded the accuracy limit ($0.03\ R_M$) of the method.

Table VII. Parameters of Fujita–Ban analysis (effect of substituents on the increase of lipophilicity caused by LiCl). For tabulated statistical values, see Table VI. Activity of unsubstituted compound (Table I, No. 9): 0.220

	Activity contribution	*s*	*t*
R_1 = $-OCH_3$	−0.166	0.024	6.92
$-SCH_3$	−0.102	0.024	4.25
R_2 = $-NH-C_2H_5$	0.083	0.039	2.13
$-NH-CH-(CH_3)_2$	0.146	0.046	3.17
$-NH-C-(CH_3)_3$	0.174	0.051	3.41
Cl	0.107	0.039	2.74
R_3 = $-NH-C_2H_5$	0.070	0.042	1.67
$-NH-CH-(CH_3)_2$	0.200	0.053	3.77
$-NH-C-(CH_3)_3$	0.146	0.051	2.86
$-NH-C-(C_2H_5)_3$	0.187	0.058	3.22
$-NH-CH-(CH_3)C_2H_5$	0.233	0.061	3.82

$r = 0.985$, $s = 0.030$, $F = 14{,}53$.

The substitutions at R_1 decrease, and at R_2 and R_3 increase the effect of LiCl (Table VII). The fact that the signs of the activity contributions for R_2 and R_3 are identical for LiCl and opposite for the β-cyclodextrin polymer proves the different nature of LiCl and β-cyclodextrin polymer effects. The inclusion complex formation depends chiefly on the steric features of the guest molecule. This would explain the opposite signs of activity contributions of the same substituents at R_2 and R_3 positions. The effect of LiCl depends rather on the lipophilicity of the substituents, therefore the effects are similar with R_2 and R_3. In the case of LiCl, too, the deviation of calculated and measured R_M values did not exceed the accuracy limit of the method.

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