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Retention of 4-cyanophenyl herbicides on water-insoluble β-cyclodextrin support

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Abstract

The retention of eight cyanophenyl herbicides on water-insoluble β-cyclodextrin polymer beads (BCDP) was determined by thin-layer chromatography using BCDP as stationary phase. The effects of pH, salt concentration in the aqueous eluent systems and the various physico-chemical parameters of herbicides on the herbicide–BCDP interaction were calculated by using principal component analysis. The pH of the eluent exerted a higher influence than the salt concentration on the retention of herbicides. The hydrophobicity and steric characteristics of solutes had the highest impact on their retention behaviour. Calculations revealed that BCDP showed a mixed retention mechanism and hydrophobic and hydrophilic forces in addition to steric parameters are involved in the interaction.

1. Introduction

Owing to their capacity to form inclusion complexes, cyclodextrins (CDs) are used in the stabilization and formulation of drugs, flavours and fragrances and also in agrochemistry [1]. A CD derivative, the water-insoluble CD, has been proved to be a useful agent for removing organic impurities from solutions [2].

CDs have been extensively applied in thin-layer chromatography (TLC) to modify the retention of various solutes. CDs can be covalently bonded to the silica surface [3,4]. The use of silica-bonded CDs in chromatography has been reviewed previously [5–8]. Silica-bonded β-CD plates have been successfully applied to the separation of racemic and diastereomeric mixtures and structural isomers [9]. In other studies β-CD was used as an eluent additive for the separation of enantiomeric drugs and dansyl-amino acids [10,11]. Amino acid and alkaloid enantiomers have been well separated by reversed-phase TLC using maltosyl-β-CD as an eluent additive [12]. Polymerized β-CD derivatives bonded to silica [13] or to organic supports [14] have also been used in liquid chromatography for the successful separation of various bioorganic compounds. Enantiomer separation of various amino acid derivatives was achieved by adding β-CD to the eluent [15,16]. Hydroxypropyl-β-CD was also suitable for the RP-TLC separation of enantiomers of derivatized amino acids [17,18]. Nitro-substituted aromatic hydrocarbon isomers were separated using α- and β-CD additives [19]. The presence of α-CD in the eluent considerably improved the separation of substituted phenolic and naphtholic compounds [20]. Both α- and β-CDs improved

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the separation of cresols, nitrophenols, nitroanilines and nitronaphthalenes [21]. As the solubility of β-CD is low in common reversed-phase eluents, urea-solubilized β-CD solutions were used for the separation of polycyclic aromatic hydrocarbons and pesticides [22,23]. Hydroxypropyl-β-CDs appeared to be the most effective for increasing the TLC migration of laser dyes of the coumarin, rhodamine and bimane types [24].

The herbicidal character of 3,5-dihalo-4-hydroxy-benzonitriles has been known since 1963 [25]. They are primarily inhibitors of the photosynthetic electron transport chain [26] and uncouplers of oxidative photophosphorylation [27]. 3,5-Disubstituted 4-hydroxybenzonitriles containing a nitro group have a similar mode of action [28].

The objectives of this investigation were to study the possible application of water-insoluble β-CD polymer beads as TLC sorbents and to find the physico-chemical parameters of solutes that influence the retention. As it has been demonstrated that TLC can be successfully used to predict the retention of solutes in HPLC [29,30], the data can help in the prediction of the retention behaviour of solutes on a water-insoluble β-CD polymer-coated HPLC column [31,32].

2. Experimental

Water insoluble β-CD polymer (BCDP) was a pilot product of the Cyclolab Research and Development Laboratory (Budapest, Hungary). It was prepared by cross-linking β-CD monomers with epichlorohydrin and ethylene glycol bis(epoxypropyl) ether. It was ground and the 10–50-μm fraction was used in the experiments. As the common TLC sorbents contain the same fraction, we assumed that it can be successfully used for these preliminary investigations.

TLC plates were prepared on 20 × 20 cm glass plates: 5 g of BCDP and 25 mg of poly(vinyl alcohol) were suspended in the intended eluent, spread on the glass surface and dried at room temperature. Owing to the considerable swelling of the β-CD polymer, the preparation of the TLC plates has to be carried out in the eluent. The use of poly(vinyl alcohol) was motivated by the low mechanical stability of the BCDP layer; it considerably increased the adhesion of polymer particles to the glass surface.

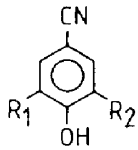
The structures of solutes are shown in Table 1. They were dissolved in methanol at a concentration of 5 mg/ml and 5-μl volumes of solution were spotted on the plates. The eluents were water–methanol (3:7, v/v) (eluent I), 0.1 *M* HCl–methanol (3:7, v/v) (II), 0.1 *M* NaOH–methanol (3:7, v/v) (III), 1 *M* $MgCl_2$–methanol (3:7, v/v) (IV) and 1 *M* glycine–methanol (3:7, v/v) (V). The choice of the various eluent additives was motivated by the supposition that the extent of dissociation of polar groups in the solutes may influence the capacity to interact with cyclodextrins [33–35], and the salts [36,37] and bulky additives may inhibit inclusion complex formation.

Development was carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, the distance of development being about 16 cm. After development, the spots were detected by their UV and visible spectra. Each determination was run in quadruplicate.

The R_M value characterizing the molecular lipophilicity in RP-TLC was separately calculated for each solute in each eluent system:

$$R_M = \log (1/R_F - 1) \quad (1)$$

Table 1
Structures of 4-cyanophenol derivatives



No.	R_1	R_2
1	H	H
2	H	Br
3	H	NO_2
4	NO_2	NO_2
5	Br	Br
6	I	NO_2
7	Cl	NO_2
8	Br	NO_2

To find the similarities and dissimilarities between the effects of the various eluent additives on the retention capacity of BCDP beads and to find the physico-chemical parameters of solutes governing their retention, principal component analysis (PCA) was applied [38]. PCA differs markedly from linear free energy calculations (LFE), which have also been applied to evaluate retention data [39,40]. LFE methods can calculate only the relationship between one chromatographic parameter (dependent variable) and one or more physico-chemical characteristics of the solute molecule. PCA with two-dimensional non-linear mapping [41] can evaluate the relationship between a large number of chromatographic and physico-chemical parameters without defining one as a dependent variable [42]. PCA was used twice:

(A) eluent systems I–V, the measured lipophilicity and adsorption capacity values of 4-cyanophenyl derivatives determined on impregnated and unimpregnated silica [43] being the variables; and

(B) Eluent systems I–V and the various calculated physico-chemical parameters of herbicides being the variables.

The physico-chemical parameters included in the calculation were the following:

π = Hansch–Fujita substituent constant characterizing hydrophobicity [44,45];

H-Ac and *H-Do* = indicator variables for proton acceptor and proton donor properties, respectively [46];

M-RE = molar refractivity [47];

F and *R* = electronic parameters characterizing the inductive and resonance effects, respectively [48];

σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent [49];

Es = Taft's constant, characterizing steric effects of the substituent [50];

B_1 and B_4 = Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment [51,52].

The limit of variance explained was set to 95% in both instances. The two-dimensional non-linear map of principal component loadings and variables and the varimax rotation of PC loadings in two axes was also calculated. The iteration was carried out to the point where the difference between the last two iterations was less than 10^{-8}. Eluent systems and solutes form clusters on the map when their retention characteristic are similar and they are well separated when their retention behaviour is highly different. To compare the information contents of varimax rotation and the non-linear mapping technique, linear correlations were calculated between the corresponding coordinates.

3. Results and discussion

The R_F values of 4-cyanophenol derivatives are compiled in Table 2. Solutes show higher retention in acidic and lower retention in alkaline eluents; the effect of $MgCl_2$ and glycine is negligible. This finding indicates that the degree of dissociation of the polar head group has a considerable impact on the retention even on this support, and a change in pH may result in a change in retention order. These results entirely support previous conclusions [33–35] that the strength of inclusion complex formation may depend on the degree of dissociation of the guest molecule. We assume that the bulky substituted benzene ring enters the cavity of the β-CD polymer, forming inclusion complexes the stability of which depends on the steric correspondence of the interacting molecules. The polar groups probably point out of the cavity and can bind to the hydrophilic surface substructures on the outer part of the cavities (electrostatic interactions). We are well aware that the cross-linking of the CD may modify its inclusion complex-forming capacity [53]. However, the CD cavities remaining on the polymer surface and the irregular cavities formed during the polymerization process [54] may influence the retention of various solutes on β-CD polymer beads resulting in uncommon retention characteristics of the above support.

The results of principal component analyses A

Table 2
Retentions (R_F) of 4-cyanophenol derivatives on β-CD polymer beads

Compound	Eluent system									
	I		II		III		IV		V	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	0.38	0.04	0.45	0.03	0.28	0.03	0.36	0.07	0.39	0.01
2	0.39	0.03	0.46	0.05	0.20	0.01	0.37	0.03	0.42	0.05
3	0.42	0.05	0.57	0.04	0.19	0.02	0.42	0.05	0.49	0.06
4	0.43	0.06	0.52	0.03	0.21	0.04	0.41	0.02	0.52	0.04
5	0.41	0.02	0.52	0.04	0.20	0.05	0.37	0.01	0.39	0.02
6	0.42	0.03	0.63	0.07	0.22	0.01	0.39	0.04	0.49	0.03
7	0.40	0.02	0.51	0.08	0.19	0.02	0.35	0.06	0.47	0.02
8	0.40	0.03	0.58	0.06	0.21	0.05	0.38	0.04	0.52	0.07

and B are compiled in Tables 3 and 4, respectively. In both instances the first principal component explains most of the variance, that is, the effect of various eluent additives can be explained by one background variable. Unfortunately, principal component analysis does not specify this background variable as a concrete physico-chemical entity but only indicates its mathematical possibility.

The measured physico-chemical parameters of the herbicides and also eluent systems I and V have a high loading in the first principal component, indicating that both the hydrophobicity and adsorption capacity of herbicides influence their binding to the BCDP surface. The relatively high loadings of acidic, basic and salt-containing eluents in the second, third and fourth principal components emphasizes the importance of the degree of dissociation of polar substructures in the retention and the consider-

Table 3
Similarities and dissimilarities between the effects of eluent additives on the retention characteristics of water-insoluble β-polymer beads and their measured physico-chemical parameters: results of principal component analysis A

No. of principal component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	4.60	65.72	65.72
2	0.98	13.97	79.69
3	0.74	10.62	90.31
4	0.41	5.82	96.13

Principal component loadings

Parameter	No. of principal component			
	1	2	3	4
Water	0.93	0.24	−0.04	0.06
0.1 *M* HCl	0.78	0.24	0.18	−0.54
0.1 *M* NaOH	−0.62	0.33	0.69	0.08
1 *M* $MgCl_2$	0.63	0.71	−0.11	0.26
1 *M* Glycine	0.96	−0.10	0.11	0.05
Lipophilicity	0.92	−0.27	−0.04	0.08
Adsorption capacity	0.75	−0.40	0.45	0.17

Table 4
Similarities and dissimilarities between the effects of eluent additives on the retention characteristics of water-insoluble β-CD polymer beads and their calculated physico-chemical parameters: results of principal component analysis B

No. of principal component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	8.79	62.81	62.81
2	3.17	22.67	85.48
3	0.97	6.91	92.39
4	0.70	5.01	97.40

Principal component loadings

Parameter	No. of principal component			
	1	2	3	4
Water	−0.88	0.23	0.32	−0.01
0.1 *M* HCl	−0.75	−0.05	0.42	0.40
0.1 *M* NaOH	0.58	0.27	−0.26	0.71
1 *M* $MgCl_2$	−0.46	0.62	0.59	0.00
1 *M* Glycine	−0.95	0.09	0.04	−0.03
π	−0.05	0.98	−0.17	−0.06
H-Ac	0.80	−0.55	0.22	0.00
M-RE	0.83	0.50	−0.02	−0.19
F	0.97	0.09	0.20	0.04
R	0.35	−0.91	0.19	0.00
σ	0.97	0.08	0.23	0.03
Es	−0.97	−0.17	−0.14	0.04
B_1	0.88	0.46	0.12	0.00
B_4	0.96	0.20	0.16	−0.02

able influence of salt on the BCPD–herbicide interaction (Table 3). The distribution of variables on the two-dimensional non-linear map of PC loadings entirely supports our previous conclusions (Fig. 1: both the pH of eluent and the salt concentration exert marked effects on the retention of the solutes. Herbicides form distinct clusters according to the number of substituents on the two-dimensional non-linear map of PC variables (Fig. 2). This finding suggests that not only the lipophilicity and adsorption capacity but also the dimensions of the solute may influence the retention.

The various calculated physico-chemical parameters for 4-cyanophenyl herbicides and their retention behaviour on the BCDP layer are strongly related (Table 4). The steric characteristics (*Es*, B_1 and B_4 values) have a high loading in the first PC, proving again the importance of molecular dimensions in the herbicide–BCDP interaction.

The retention characteristics of eluent systems form a loose cluster with the lipophilicity and the steric effect of substituents on the two-dimensional non-linear map of PC loadings (Fig. 3). This finding suggests that more than one type of binding forces are involved in the herbicide–BCDP interaction. We assume that the ring structure of the herbicide enters the CD cavity on the surface of the BCDP (governed by the dimensions of the solute), and the binding forces between the cavity walls and the surface of guest molecules are of hydrophobic character (role of solute hydrophobicity). The dissociable hydroxyl group points towards the outer sphere of the BCDP and binds to it by hydrophilic forces. The strength of interaction depends in part on the degree of dissociation of the hydroxy group.

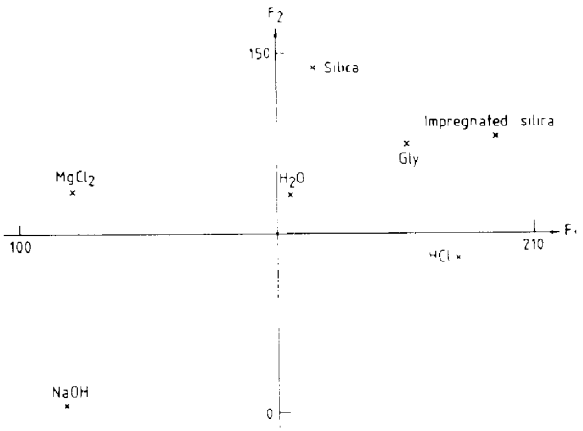


Fig. 1. Similarities and dissimilarities between the retention characteristics of various supports and eluent systems. Two-dimensional non-linear map of PC loadings; No. of iterations, 162; maximum error, $2.81 \cdot 10^{-2}$. H_2O = BCDP support, water–methanol (3:7, v/v); HCl = BCDP support, 0.1 *M* HCl–methanol (3:7, v/v); NaOH = BCDP support, 0.1 *M* NaOH–methanol (3:7, v/v); $MgCl_2$ = BCDP support, 1 *M* $MgCl_2$–methanol (3:7, v/v); Gly = 1 *M* glycine–methanol (3:7, v/v); Silica = adsorption capacity of solutes determined on unimpregnated silica; Impregnated silica = lipophilicity of solutes determined on impregnated silica.

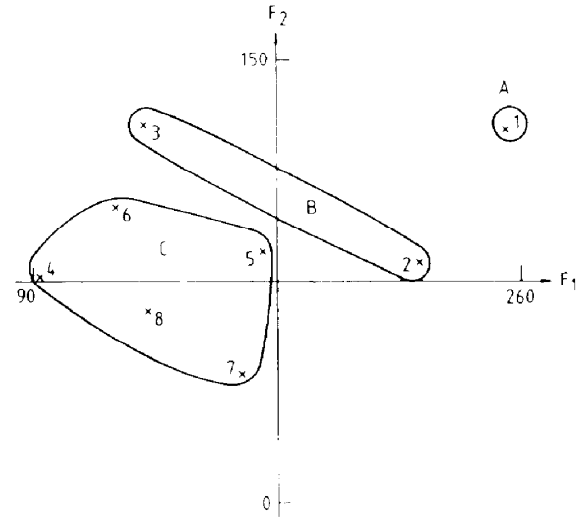


Fig. 2. Distribution of 4-cyanophenyl herbicides according to their retention behaviour. Two-dimensional non-linear map of PC variables. No. of iterations, 111; maximum error, $1.80 \cdot 10^{-2}$. Numbers refer to 4-cyanophenyl derivatives in Table 1. A = Unsubstituted 4-cyanophenol; B = monosubstituted 4-cyanophenols; C = disubstituted 4-cyanophenols.

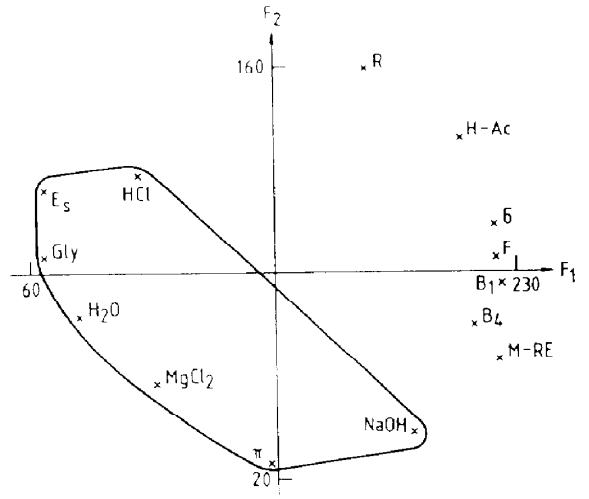


Fig. 3. Relationship between the retention behaviour and physico-chemical parameters of 4-cyanophenol derivatives. Two-dimensional non-linear map of PC loadings; No. of iterations, 79; maximum error, $1.62 \cdot 10^{-2}$. For symbols, see Fig. 1 and Experimental.

Good linear correlations were found between the coordinates of varimax rotation and the two-dimensional non-linear map:

$$\mathrm{Nlmap}_1 = -1.68 + (1.17 \cdot 10^{-2} \pm 6.69 \cdot 10^{-4}) \cdot \mathrm{varimax}_1$$

$$n = 14; \quad r_{\mathrm{calc.}} = 0.9810; \quad r_{99.9\%} = 0.7800$$

$$\mathrm{Nlmap}_2 = 1.04 - (1.10 \cdot 10^{-2} \pm 2.33 \cdot 10^{-3} \cdot \mathrm{varimax}_2$$

$$n = 14; \quad r_{\mathrm{calc.}} = 0.8064; \quad r_{99.9\%} = 0.7800$$

These data indicate that the information contents of the results of varimax rotation and two-dimensional non-linear mapping are similar but not identical. We must emphasize that this conclusion is possibly valid only for this data matrix and it is not the result of theoretical considerations, and therefore its use for other data matrices is probably subject to considerable error.

As the use of BCDP as a TLC support is relatively new [55], we do not have sufficient retention data to compare its advantages or

disadvantages with those of polymer-coated silica.

It can be concluded from our data that BCDP can be successfully used as a TLC support for the study of the interaction of various organic xenobiotics with BCDP, which facilitates the application of BCDP in environmental protection.

Acknowledgement

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