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Binding of environmental pollutants to the corn protein zein studied by high-performance liquid chromatography

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Abstract

The interaction of 16 ring-substituted phenols and anilines with the corn protein zein was studied by reversed-phase high-performance liquid chromatography by preparing silica- and alumina-based stationary phases coated with various concentrations of zein. The relationship between the strength of interaction and the physicochemical parameters of solutes was elucidated by principal component analysis followed by the nonlinear mapping technique. The binding of each phenol and aniline derivative to zein has been demonstrated. It was established that the electrostatical parameters of solutes exert the highest influence on the interaction and the involvement of hydrophobic binding forces is of secondary importance. The binding characteristics of phenol and aniline derivatives were different.

Keywords: Principal component analysis; Stationary phases, LC; Phenols; Anilines; Proteins; Zein

1. Introduction

During recent decades various protein-coated supports have been frequently used in high-performance liquid chromatography (HPLC). Proteins are generally covalently bonded to the support [1] which is mainly silica [2], or polyvinylimidazole-coated silica [3]. In the majority of cases human serum albumin has been applied for this purpose [4], however, ovoglycoprotein [5], ovomucoid [6], lysozyme [7] and other enzymes have also been applied [8,9]. The main field of application of protein-coated stationary phases has been the enhancement or the efficacy of chiral separation [10,11]. However, the preparation and application of silica containing on the surface a water-insoluble protein bonded by adsorptive forces has also been reported [12].

Principal component analysis (PCA) [13] is a multivariate mathematical–statistical method suitable for the assessment of the similarities and differences among the rows and columns of any data matrix without defining any rows or columns as being the dependent variable. Because of its simplicity PCA has found application in chromatography too. It has been used for the classification of hydrophobic interaction and hydrophobic interaction chromatographic media [14], for the study of the retention characteristics of porous graphitized carbon support [15], and for the investigation of the impact of molecular parameters of propargylamine derivatives on their retention in HPLC [16]. Although PCA decreases the dimensionality of the original data

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matrix the calculated matrices of principal component (PC) loadings and PC variables are generally multidimensional too. As the evaluation of data scattered in multidimensional space is difficult, the dimensions of the matrices of PC loadings and variables can be reduced further to two by nonlinear mapping technique (NLMAP) [17].

Zein is the main water-insoluble protein in corn (*Zea mays* L.) [18]. Because of its insolubility in water zein is probably in the solid state in the cells adsorbed on other water-insoluble cell components such as lignin, cellulose or hemicellulose.

The objectives of this study were the determination of the binding of ring-substituted phenol and aniline derivatives to zein by HPLC, and to assess the correlation between the strength of binding and the physicochemical parameters of solutes. The investigation of the binding of phenol and aniline derivatives to zein was motivated by the fact that zein is an important source of protein in many countries and phenols are priority pollutants in possible contact with zein. The elucidation of the mode of binding may facilitate not only the better understanding of the interactive forces between proteins and organic pollutants but also may promote the development of efficient environmental control procedures.

2. Experimental

Zein-coated silica and alumina were prepared by dissolving 0.5 and 1 g of zein in 200 ml 2-propanol–water (7:3, v/v) mixtures at 70 °C under continuous gentle stirring. After the dissolution of the protein, 20 g silica (particle size 5 μm, Macherey-Nagel, Dürren, Germany) or alumina (particle size 5 μm, Research and Development Laboratory of Hungarian Alumina Trust, Budapest, Hungary) were added and the mixture was stirred for 2 h at the same temperature then the solvents were removed under vacuum. The average pore diameter and specific surface area of supports were not provided by the producers. The zein-coated silica with 2.5 and zein-coated aluminas with 2.5 and 5% zein coatings were dried in a vacuum oven at 70 °C. As no loss of support or protein may occur during the preparation of the stationary phases, the mass ratio of support–protein was not determined; it was calculated from the initial mass ratio of the individual components. Columns of 250×4 mm I.D. were filled with a Shandon (Pittsburgh, PA, USA) analytical pump using bidistilled water as filling agent. The HPLC system consisted of a Liquopump Model 312 (Labor MIM, Budapest, Hungary) pump, a Cecil CE-212 variable-wavelength UV detector (Cecil Instruments, Cambridge, UK), a Valco injector (Valco, Houston, TX, USA) with a 20-μl sample loop and a Waters 740 integrator (Waters-Millipore, Milford, MA, USA). The flow-rate was 1 ml min^{-1} and the detection wavelength was 254 nm. In order to demonstrate the separation capacity of these protein-coated stationary phases, some concrete separations were carried out at lower flow-rates. Bidistilled water was used as eluent. Determinations were run at room temperature (22±2 °C). The chemical names of ring-substituted aniline and phenol derivatives are compiled in Table 1. The analytes were dissolved in bidistilled water at a concentration of 0.2 mg ml^{-1}. Each retention time was measured by three consecutive injections. The dead volume of the system was measured by injecting 1% aqueous $NaNO_3$ solution. The log k_w values were calculated by log $(t_R/t_0 - 1)$ where t_R is the retention time of the solute, t_0 is the retention time of

Table 1
Chemical names and log k_w values of ring-substituted phenol and aniline derivatives on zein-coated stationary phases

No.	Name	Log k_wI	Log k_wII	Log k_wIII
1	Phenol	−0.395	−0.316	0.172
2	4-Hydroxyphenol	−0.788	−0.475	−0.070
3	3-Hydroxyphenol	−0.620	−0.224	0.156
4	3-Methoxyphenol	−0.351	−0.204	0.291
5	2-Methylphenol	−0.252	−0.105	0.462
6	3-Methylphenol	−0.253	−0.111	0.443
7	4-Methyphenol	−0.220	−0.112	0.441
8	4-Cyanophenol	−0.391	−0.329	0.578
9	3-Fluorophenol	−0.292	−0.005	0.480
10	2-Aminophenol	0.261	0.501	0.602
11	3-Aminophenol	0.097	−0.562	−0.254
12	4-Aminophenol	0.750	−0.699	−0.458
13	Aniline	0.316	−0.609	−0.218
14	2-Nitroaniline	0.015	0.047	0.630
15	2-Methoxyaniline	0.419	−0.535	−0.080
16	4-Methoxyaniline	0.771	−0.648	−0.274

I, silica support coated with 2.5% (w/w) zein; II, alumina support coated with 2.5% (w/w) zein; III, alumina support coated with 5% (w/w) zein.

the unretained $NaNO_3$. The standard deviations were also calculated. It was supposed that a higher log k_w value indicates higher affinity of the environmental pollutants to zein, therefore, it can be used as a quantitative indicator of the strength of the solute–protein interaction [19].

We are well aware that the binding characteristics of zein may be different in free and adsorbed states even depending on the type of the adsorbent. Because of the insolubility of zein in water it is probably in the adsorbed state also in its natural environment making reasonable the assumption that the differences in the log k_w values really reflect the differences in the binding strengths of pollutants to zein.

To find the correlation between the strength of interaction and the physicochemical parameters of ring-substituted phenol and aniline derivatives, PCA was applied. The three sets of log k_w values measured on zein-coated (2.5%) silica and zein-coated alumina supports (2.5 and 5%), and the physicochemical parameters of solutes were the variables (altogether 13 variables) and the 16 solutes the observations. The physicochemical parameters included in the calculation were: π which is Hansch–Fujita's substituent constant characterizing hydrophobicity; H-Ac and H-Do which are indicator variables for proton acceptor and proton donor properties, respectively; M-RE, the molar refractivity; F and R which are electronic parameters characterizing the inductive and resonance effect, respectively; σ which is Hammett's constant, characterizing the electron-withdrawing power of the substituent; Es which is Taft's constant, characterizing steric effects of the substituent; B_1 and B_4 which are Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment [20]. The parameters were calculated according to the additivity rule from the fragmental constants. These molecular characteristics have been frequently employed in chromatography, e.g.: π value for the characterization of hydrophobic interaction and hydrophobic interaction chromatography media [14]; M-RE value for the assessment of the relationship between solute structure and retention on polybutadiene-coated alumina stationary phase [21]; Es constant for the study of selectivity in gas–liquid chromatography (GLC) [22]; various electronic and steric parameters for quantitative structure–retention relationship (QSRR) studies in GLC [23,24] and in HPLC [25], etc. The ratio of variance explained by PCA was set to 95% of the total variance. Two-dimensional NLMAPs of the data matrices of PC loadings and components have also been calculated. Iteration was carried out to the point where the difference between the last two iterations was lower than 10^{-8}.

Software for PCA and NLMAP were prepared by Dr. Barna Bordás (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary).

3. Results and discussion

Some chromatograms of pollutants are shown in Fig. 1. Phenol and aniline derivatives are well separated on zein-coated stationary phases, and the peaks are symmetric even at high elution times. Fig. 1A clearly shows that structural isomers can also be separated indicating that the sterical differen-

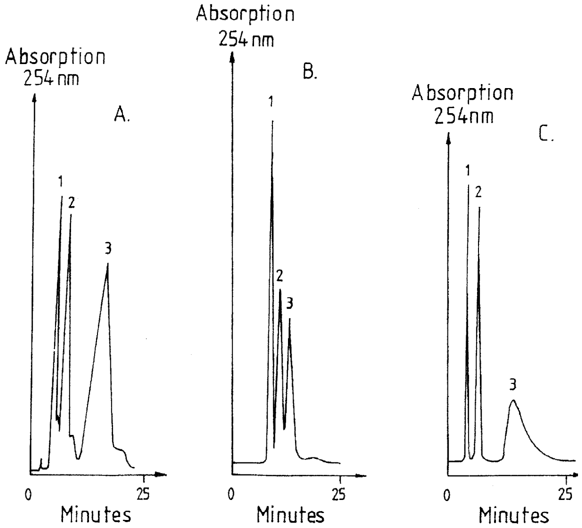

Fig. 1. Separation of phenol and aniline derivatives on zein-coated stationary phases. (A) Zein-coated silica. 1, 3-Aminophenol; 2, 2-aminophenol; 3, 4-aminophenol. Flow-rate, 0.5 ml/min. (B) Zein-coated alumina (2.5%, w/w). 1, 3-Aminophenol; 2, 3-methoxyphenol; 3, 3-fluorophenol. Flow-rate, 0.2 ml/min. (C) Zein-coated alumina (2.5%, w/w). 1, 4-Methoxyaniline; 2, 4-nitroaniline; 3, 4-hydroxyaniline. Flow-rate, 0.4 ml/min. Detection wavelength 254 nm and mobile phase bidistilled water in each instance.

tiating capacity of zein is higher than that of the traditional reversed-phase stationary phases. Solutes containing various substituents at the same position can be separated too (Fig. 1B and C) suggesting that besides the measurement of the strength of zein–pollutant interaction, these stationary phases may be chromatographically useful.

The mean log k'_{w} values of ring substituted aniline and phenol derivatives are compiled in Table 1. The relative standard deviation was in each instance lower than 1.7% showing the good stability of the stationary phases and good reproducibility of the HPLC system. The data in Table 1 indicate that each pollutant eluted with retention times higher than the dead volume on each stationary phase suggesting that they are bonded more or less strongly to the protein. It can be further established that the solutes have been more strongly retained on stationary phases with higher concentration of coating protein, however, the retention order was different on silica and alumina supports. This finding can be tentatively explained by the supposition that the protein does not cover entirely the active adsorption centers of the original supports and they are available for the relatively small pollutant molecules. The polar interactions between the hydrophobic substructures of phenol and aniline derivatives and the adsorption centers on the surface of stationary phases may result in modified retention behaviour. This result draws attention to the fact that the binding characteristics of zein may depend on the type of adsorbent and this effect has to be taken into consideration.

The results of principal component analysis are compiled in Table 2. Six background (hypothetical) variables explained the overwhelming majority of total variance present in the original 13 variables with a loss of 5.61% of information. Unfortunately, PCA does not define these hypothetical variables as concrete physical or physicochemical entities; it only indicates their mathematical possibility. The high loading of both retention characteristics and physicochemical parameters in the first PC component

Table 2
Similarities and differences among the binding of ring substituted phenol and aniline derivatives to the corn protein zein and their physicochemical parameters. Results of principal component analysis. For symbols see Section 2

No. of principal component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	3.93	30.26	30.26
2	3.68	28.33	58.59
3	2.09	16.06	74.65
4	1.26	9.63	94.39
5	0.67	5.17	89.50
6	0.64	4.89	94.39

No. and symbol of variables	No. of principal components					
	1	2	3	4	5	6
Log k_{w}I	−0.54	0.14	−0.56	−0.31	0.40	0.14
Log k_{w}II	0.61	0.30	0.43	−0.28	0.41	−0.32
Log k_{w}III	0.85	0.29	0.28	0.00	0.29	−0.09
π	0.78	0.38	−0.31	0.08	−0.24	−0.09
H-Ac	−0.63	0.50	0.29	0.41	0.21	0.06
H-Do	−0.60	−0.01	0.67	−0.20	−0.10	−0.14
M-RE	−0.22	0.81	−0.45	0.11	0.21	0.12
F	0.28	0.32	0.56	0.62	0.01	0.26
R	0.82	−0.19	−0.38	0.22	0.07	0.16
σ	0.36	−0.29	0.43	−0.48	0.05	0.60
Es	−0.41	−0.78	0.11	0.29	0.24	−0.03
B_1	−0.14	0.88	0.17	−0.33	−0.19	0.02
B_4	−0.23	0.92	0.03	0.02	−0.07	0.11

indicates that these parameters considerably influence the retention behaviour of solutes.

The two-dimensional nonlinear map of the three sets of log k_w values and the physicochemical parameters are shown in Fig. 2. Zein-coated silica and zein-coated alumina stationary phases are far away from each other indicating again the influence of the adsorption centers of the original supports not covered by the protein. The hydrogen donor and hydrogen acceptor capacity of solutes and their log k_w values on the zein-coated silica form a separate cluster (cluster A) demonstrating the importance of electrostatic interaction in the protein–pollutant binding.

The molecular lipophilicity, the resonance effect and the log k_w values measured on zein-coated alumina stationary phases form a distinct cluster (cluster B). The fact that the lipophilicity also exerts a marked impact on the binding indicates the involvement of more than one type of interactive forces in the binding. It can be assumed that the apolar ring structure of solutes can bind to the hydrophobic side chain of amino acids (Van der Waals or stacking interactions) and the polar head groups can interact with the hydrophilic peptide bonds or with the polar side chains of basic or acidic amino acids.

Phenol and aminophenol derivatives formed clear-cut clusters on the two-dimensional NLMAP proving again the relevance of electrostatic (polar) interactions in the protein–pollutant interactions (Fig. 3).

It can be concluded from the data that the binding of ring-substituted aniline and phenol derivatives to zein can be successfully studied by reversed-phase HPLC. The strength of interaction mainly depends on the electrostatical parameters of the solutes, and the involvement of hydrophobic forces is of secondary importance.

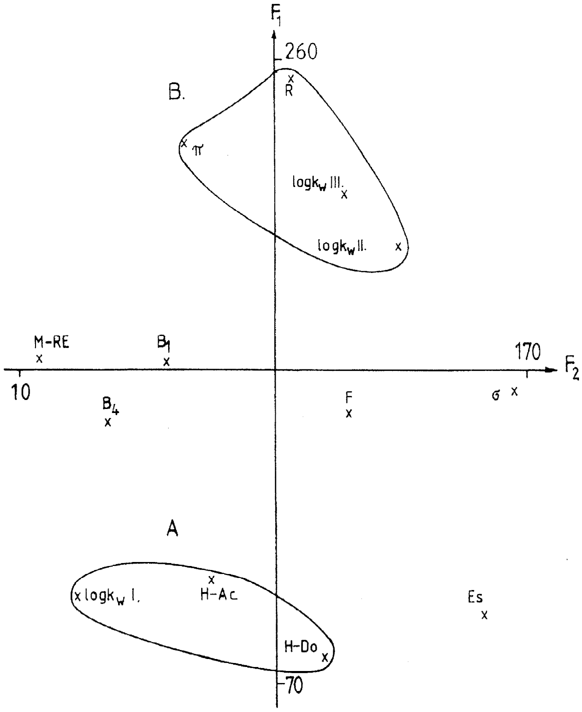

Fig. 2. Similarities and dissimilarities between the retention characteristics and physicochemical parameters of ring-substituted phenol and aniline derivatives. Two-dimensional nonlinear map of PC loadings. Number of iterations, 266; maximum error, 5.40× 10^{-2}. For symbols see Section 2.

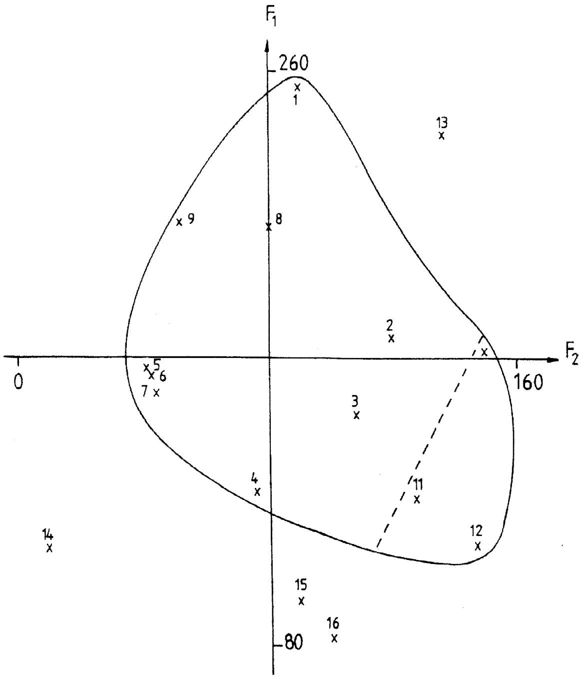

Fig. 3. Similarities and dissimilarities between the ring-substituted phenol and aniline derivatives. Two-dimensional nonlinear map of PC variables. Number of iterations, 166; maximum error, 4.94× 10^{-2}. Numbers refer to solutes in Table 1.

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