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### Interaction of Hydroxypropyl- $\beta$ -Cyclodextrin with Peptides, Studied by Reversed-Phase Thin-Layer Chromatography

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## **Interaction of Hydroxypropyl- $\beta$ -Cyclodextrin with Peptides, Studied by Reversed-Phase Thin-Layer Chromatography**

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**Abstract:** The effect of various concentrations of hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) on the reversed-phase thin-layer chromatographic mobility of some low molecular mass peptides was measured in distilled water and in 0.05 M of LiCl, NaCl, KCl, RbCl, and CsCl solutions, and the relative strength of HP- $\beta$ -CD–peptide interaction has been calculated from the dependence of retention on the concentration of HP- $\beta$ -CD in the mobile phase. Lipophilicity of the peptides decreased regularly with increasing concentration of HP- $\beta$ -CD in the mobile phase, proving the interaction (probably the formation of inclusion complexes) between peptides and HP- $\beta$ -CD. Principal component analysis indicated that each salt influenced the interaction differently. Peptides formed clusters on the two-dimensional nonlinear map according to the character of the amino acid monomer and not according to the number of amino acid units, suggesting that only one terminal amino acid is included in the cavity of HP- $\beta$ -CD.

**Keywords:** Cyclodextrin, Peptides, TLC, Nonlinear map

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## INTRODUCTION

Cyclodextrins (CDs) offer a novel approach to protecting chemically unstable drugs from reacting with their environment because formation of drug-cyclodextrin inclusion complexes often has a marked stabilizing effect. Hydrophilic CDs are particularly useful for improving oral absorption of poorly water-soluble drugs, including steroids,<sup>[1–3]</sup> cardiac glycosides,<sup>[4]</sup> non-steroidal anti-inflammatory drugs,<sup>[5,6]</sup> barbiturates,<sup>[7]</sup> benzodiazepines,<sup>[8]</sup> etc. Hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) has been reported to be the most likely candidate of all chemically modified CDs for incorporation into formulations used by humans and animals. HP- $\beta$ -CD is the basic material in development of certain novel drug formulations with promising biological activities if the active agent is either too unstable or too insoluble in water. Several papers focused on correlation between the structure of the guest molecules and the complex stability,<sup>[9–12]</sup> and the altered properties of guest molecules, such as solubility, spectral changes, proton dissociation, surface activity, diffusion, electrochemical properties, and partition coefficient<sup>[13]</sup> have been investigated. Calorimetry,<sup>[14]</sup> spectrophotometry,<sup>[15]</sup> and liquid chromatography methods such as thin-layer chromatography (TLC)<sup>[16]</sup> and high performance liquid chromatography<sup>[17]</sup> have been successfully used for the assessment of the various aspects of the host–guest interactions. It is generally accepted that the structural features of the guest, such as geometric dimensions and structure-dependent properties, e.g. hydrophobicity, and polarizability, determine the stability of the complex.

Peptides and proteins play important roles in many biochemical reactions in living cells. They have vital functions in transportation of drug molecules, thereby regulating (prolonging) the effects of drug compounds in biological processes. Interaction of peptides and cyclodextrin in several respects, such as thermodynamics,<sup>[18]</sup> receptor-ligand binding,<sup>[19]</sup> and modification of response in human body,<sup>[20]</sup> have been recently examined. It has been demonstrated that CD complexation enhances the absorption of protein and peptide drugs (LHRH, and insulin).<sup>[21–23]</sup> CDs have a multifunctional role in peptide and protein delivery; they affect the permeability of the mucous membrane and stabilize the peptide-related compound against proteolysis.

Principal component analysis (PCA) is a versatile and easy-to-use multivariate mathematical-statistical method that has been developed to contribute to the extraction of maximal information from large data matrices containing numerous columns and rows.<sup>[24]</sup> PCA makes possible the elucidation of the relationship between the columns and rows of any data matrix without being one the dependent variable. PCA is a so-called projection method representing the original data in smaller dimensions. It calculates the correlations between the columns of the data matrix and classifies the variables according to the coefficients of correlations. PCA is a so-called projection method representing the original data in smaller dimensions. It calculates the correlations (similarities and dissimilarities) between the columns of the

data matrix and classifies the variables according to the coefficients of correlations taking into considerations simultaneously the magnitude and sign of the coefficients of correlation. PCA has been frequently used in many fields of up-to-date research. Thus, PCA has been employed in quantitative structure-activity relationship (QSAR) studies<sup>[25]</sup> for the exploration of molecular structure-property relationships,<sup>[26]</sup> evaluation of molecular lipophilicity,<sup>[27,28]</sup> theoretical organic chemistry,<sup>[29]</sup> quantitative structure-retention studies in chromatography,<sup>[30]</sup> elucidation of structure-biodegradation relationships,<sup>[31]</sup> clustering of amino acids,<sup>[32]</sup> assessment of solvent properties,<sup>[33]</sup> etc. As the resulting matrices of PC loadings and variables are generally multidimensional, they cannot be evaluated by visual methods except in the cases when the first two sets of PC loadings and components explain the overwhelming majority of variance. The nonlinear mapping technique (NLMAP) has been developed for reduction of the dimensionality of complicated multidimensional matrices.<sup>[34]</sup>

The objectives of the study were determination of the interaction of some peptides with HP- $\beta$ -CD by means of reversed-phase (RP) TLC, assessment of the effect of various salts on the relative strength of interaction, and elucidation of the impact of various molecular parameters of peptides and salts on the strength of interaction by using various multivariate mathematical-statistical methods.

## EXPERIMENTAL

### Materials and Methods

DC-aluminium oxide F<sub>254</sub> plates (Merck, Darmstadt, Germany) were impregnated by overnight predevelopment in *n*-hexane-paraffin oil (95 : 5, v/v). Homopeptides of alanine (Ala), glycine (Gly), lysine (Lys) and phenylalanine (Phe), (Ala<sub>2</sub>, Ala<sub>3</sub>, Ala<sub>4</sub>, Ala<sub>5</sub>, Gly<sub>2</sub>, Gly<sub>3</sub>, Gly<sub>4</sub>, Gly<sub>5</sub>, Lys<sub>2</sub>, Lys<sub>3</sub>, Lys<sub>4</sub>, Lys<sub>5</sub>, Phe<sub>2</sub>, Phe<sub>3</sub>, Phe<sub>4</sub>) were purchased from Sigma Chemical (St. Louis, MO); reduced and oxidized glutathion (GLT red and GLT ox) were obtained from REANAL Fine Chemicals (Budapest, Hungary). HP- $\beta$ -CD (average degree of substitution, 4.6) was a gift of Prof. Dr. József Szejtli (CYCLOLAB Research and Development Laboratory, Budapest, Hungary). The use of the highly water-soluble HP- $\beta$ -CD was necessitated by the low solubility of monomer  $\beta$ -CD in water and aqueous solutions. HP- $\beta$ -CD and peptides were used as received. Analytes were dissolved in water-2-propanol (3 : 1, v/v) at a concentration of 1 mg ml<sup>-1</sup>, and 5  $\mu$ L of solutions were separately spotted onto the plates using a microdispenser. This experimental design was motivated by the fact that the objective of the measurements was the determination of the relative strength of HP- $\beta$ -CD-peptide interaction and not the assessment of the impact of HP- $\beta$ -CD on the separation of peptides. Furthermore, this experimental design excluded the competition between

peptides for the binding sites of HP- $\beta$ -CD. Mobile phases consisted of distilled water and 0.05 M aqueous solutions of LiCl, NaCl, KCl, RbCl, and CsCl containing 0, 4, 7.5, 11, 14.5, 18, 21.5, and 25 mgml<sup>-1</sup> HP- $\beta$ -CD. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at ambient temperature, the distance of development being about 16 cm. After development, the plates were dried at 100°C, and the spots of analytes were revealed by use of ninhydrin reagent (0.3 g ninhydrin dissolved in 100 mL of *n*-butanol containing 3 mL acetic acid). In order to increase the sensitivity of detection, the plates were sprayed with 2 M aqueous acetic acid prior to the ninhydrin reaction. Each experiment was run in quadruplicate.

### Calculations

The  $R_M$  value characterizing the molecular hydrophobicity in RPTLC was calculated for each solute in each mobil phase using the equations:

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

where  $R_F$  is equal with the distance of chromatographic spot center from start divided by distance of solvent from start.

When the coefficient of variation of the parallel determinations was higher than 6%, the  $R_M$  value was omitted from the following calculations. This procedure was motivated by the fact that the standard error of traditional TLC measurements is generally lower than 6%. A higher standard error indicates inadequate experimental conditions and biased data.

In order to determine the effect of HP- $\beta$ -CD on the hydrophobicity of solutes, linear relationships were calculated between the  $R_M$  values and the concentration of HP- $\beta$ -CD in the mobile phases:

$$R_M = R_{M0} + bC \quad (2)$$

where  $R_M = R_M$  value for a solute determined at given HP- $\beta$ -CD concentration,  $R_{M0} = R_M$  value extrapolated to zero HP- $\beta$ -CD concentration (best estimate of the molecular lipophilicity),  $b$  = decrease in the  $R_M$  value caused by a 1 mgml<sup>-1</sup> increase in the HP- $\beta$ -CD concentration in the mobile phase (related to the relative strength of peptide-HP- $\beta$ -CD interaction), and  $C$  = concentration of HP- $\beta$ -CD in the mobile phase. Equation (2) was applied separately for each solute in each mobile phase system.

The similarities and differences among the effects of monovalent cations on the relative strength of HP- $\beta$ -CD-peptide interaction were evaluated by PCA. The relative strengths of interaction ( $b$  values of Equation (2) determined in distilled water and in the presence of salts were considered as variables, and the peptides were the observations. The limit of the variance explained was set to 95%. To facilitate the evaluation of the multidimensional matrices of principal component loadings and variables, their dimensionality

was reduced to two by the NLMAP technique. Iteration was carried out to the point where the difference between the last two iterations was lower than  $10^{-8}$ . In order to find the physicochemical parameters of peptides influencing their interaction with HP- $\beta$ -CD and those of monovalent cations modifying the strength of interaction, stepwise regression analysis (SRA) was applied. In the traditional multilinear regression analysis, the presence of independent variables that exert no significant influence on the dependent variable lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables having no significant impact on the strength and selectivity, increasing in this manner the information power of the calculation.

SRA was carried out twice. The first and second coordinates of the two-dimensional nonlinear map of principal component variables were separately the dependent variables. Independent variables were in both cases the hydrophobic ( $z_1$ ), sterical ( $z_2$ ), and electronical ( $z_3$ ) parameters of peptides and the pK values of the terminal carboxy and amino groups. The  $z$  parameters of peptides were calculated by using the additivity rule from the data.<sup>[35]</sup> The  $z$  parameters are the results of multivariate parametrization, including seven TLC, three NMR, and two theoretical variables. They characterize and summarize in one number the various aspects of the hydrophobicity, side chain bulk and electronic parameters of amino acids, without being identical with any concrete variables. In each instance, the number of accepted independent variables was not limited, and the acceptance limit was set to the 95% significance level.

Software for PCA and NLMAP was prepared by Dr. Barna Bordás (Plant Protection Institute of Hungarian Academy of Sciences, Budapest, Hungary), and software for stepwise regression analysis was purchased from CompuDrug Ltd. (Budapest, Hungary).

## RESULTS AND DISCUSSION

Homopeptides of Phe and oxidized GLT remained on the origin in each mobil phase system, indicating that they are highly hydrophobic; therefore, their interaction with HP- $\beta$ -CD cannot be investigated in aqueous mobile phases. Except for Ala<sub>5</sub>, alanine homopeptides formed diffuse spots in the presence of HP- $\beta$ -CD even at the lowest concentration. As the measurement of the exact spot position was not possible, the relative strength of interaction was not calculated.

In most cases, the retention of peptides decreased in the presence of HP- $\beta$ -CD with the effect being higher at higher concentrations of HP- $\beta$ -CD. The less hydrophobic HP- $\beta$ -CD decreased the lipophilicity of the more hydrophobic peptides, indicating complex (probably inclusion complex)

formation. This finding further suggests that the biological properties (adsorption, uptake, self-life, etc.) of peptide-HP- $\beta$ -CD complexes may be different from those of uncomplexed peptides resulting in modified effectiveness.

The parameters of Equation (2) are compiled in Tables 1–3. In the majority of cases, Equation (2) fit the experimental data well, the significance level being in each instance over 95% (compare calculated  $r$  values with those tabulated). The ratio of variance explained was high in each instance, indicating the good reproducibility of the method. The parameters of Equation (2) are markedly different for different mobile phases and peptides, suggesting that the relative strength of HP- $\beta$ -CD-peptide interactions depends both on the chemical structure of the peptide and the presence of salts. The differences between

**Table 1.** Parameters of linear relationships between the  $R_M$  values of peptides and the concentration (C) of hydroxypropyl- $\beta$ -cyclodextrin in the mobile phase ( $\text{mg mL}^{-1}$ )

Solute	$n^a$	$R_{M0}$	$-b \cdot 10^2$	$s_b \cdot 10^{3b}$	$r_{\text{calc.}}^c$	$r_{95\%}^d$
Mobile phase, distilled water						
Ala <sub>5</sub> <sup>e</sup>	5	0.40	1.98	4.58	0.928	0.8783
Gly <sub>2</sub>	5	0.26	0.58	0.87	0.9681	0.8783
Gly <sub>3</sub>	5	0.36	0.88	1.06	0.9787	0.8783
Gly <sub>4</sub>	7	0.40	1.00	2.41	0.8779	0.7545
Gly <sub>5</sub>	5	0.65	1.29	2.99	0.9277	0.8783
Lys <sub>2</sub>	6	0.22	0.82	1.07	0.9681	0.8114
Lys <sub>3</sub>	5	0.28	0.45	4.45	0.9855	0.8783
Lys <sub>4</sub>	5	0.46	0.64	1.52	0.9254	0.8783
Lys <sub>5</sub>	7	0.56	0.46	1.33	0.8379	0.7545
GLT red	7	1.47	0.67	1.23	0.9255	0.7545
Mobile phase, 0.05 M aqueous LiCl solution						
Ala <sub>5</sub> <sup>e</sup>	8	0.42	2.48	5.49	0.8792	0.7067
Gly <sub>2</sub>	6	0.42	1.88	3.78	0.9277	0.8114
Gly <sub>3</sub>	8	0.31	0.89	2.46	0.8266	0.7067
Gly <sub>4</sub>	8	0.35	1.14	2.00	0.9185	0.7067
Gly <sub>5</sub>	7	0.47	0.94	3.36	0.7827	0.7545
Lys <sub>2</sub>	7	0.12	0.72	2.70	0.7666	0.7545
Lys <sub>3</sub>	7	0.19	0.83	1.97	0.8322	0.7545
Lys <sub>4</sub>	7	0.48	0.75	1.63	0.8984	0.7545
Lys <sub>5</sub>	Not significant					
GLT red	6	1.49	1.60	1.64	0.9795	0.8114

<sup>a</sup>Number of data points.

<sup>b</sup>Standard deviation of  $b$ .

<sup>c</sup>Calculated coefficient of correlation.

<sup>d</sup>Tabulated  $r$  value indicating the significance level of the fitness of Equation (2) to the experimental data.

<sup>e</sup>Symbols refer to peptides in the Experimental section ( $R_M = R_{M0} + bC$ ).

**Table 2.** Parameters of the linear relationships between the  $R_M$  values of peptides and the concentration (C) of hydroxypropyl- $\beta$ -cyclodextrin in the mobile phase (mg mL<sup>-1</sup>)

Solute	$n^a$	$R_{M0}$	$-b \cdot 10^2$	$s_b \cdot 10^{3b}$	$r_{\text{calc.}}^c$	$r_{95\%}^d$
Mobile phase, 0.05 M aqueous NaCl solution						
Ala <sub>5</sub> <sup>e</sup>	8	0.37	2.22	2.64	0.9600	0.7067
Gly <sub>2</sub>	6	0.13	0.69	1.32	0.9324	0.8114
Gly <sub>3</sub>	5	0.25	0.86	0.89	0.9843	0.8783
Gly <sub>4</sub>	7	0.29	0.77	0.98	0.9613	0.7545
Gly <sub>5</sub>	8	0.53	1.36	3.69	0.8320	0.7067
Lys <sub>2</sub>	Not significant					
Lys <sub>3</sub>	Not significant					
Lys <sub>4</sub>	Not significant					
Lys <sub>5</sub>	7	0.39	0.26	0.73	0.8497	0.7545
GLT red	6	1.18	0.50	1.20	0.9009	0.8114
Mobile phase, 0.05 M aqueous KCl solution						
Ala <sub>5</sub> <sup>e</sup>	7	0.43	2.39	4.54	0.9077	0.7545
Gly <sub>2</sub>	6	0.12	0.78	1.45	0.9379	0.8114
Gly <sub>3</sub>	5	0.31	1.54	1.91	0.9775	0.8783
Gly <sub>4</sub>	5	0.30	1.17	1.60	0.9730	0.8783
Gly <sub>5</sub>	8	0.44	1.24	1.82	0.9409	0.7067
Lys <sub>2</sub>	6	0.28	1.71	1.37	0.9875	0.8114
Lys <sub>3</sub>	6	0.19	0.82	1.55	0.9351	0.8114
Lys <sub>4</sub>	6	0.37	0.45	0.78	0.9445	0.8114
Lys <sub>5</sub>	5	0.41	0.68	1.00	0.9694	0.8783
GLT red	5	1.57	2.17	0.61	0.8982	0.8783

<sup>a</sup>Number of data points.<sup>b</sup>Standard deviation of b.<sup>c</sup>Calculated coefficient of correlation.<sup>d</sup>Tabulated r value indicating the significance level of the fitness of Equation (2) to the experimental data.<sup>e</sup>Symbols refer to peptides in the Experimental section ( $R_M = R_{M0} + bC$ ).

the effects of salts suggest that not only the concentration of salts but also the type of cations exert a considerable influence on the interaction.

The results of PCA are compiled in Table 4. Three principal components explained the overwhelming majority of the variation present in the original six variables. Unfortunately, PCA does not define these background (theoretical) variables as concrete physicochemical or physical entities, it only indicates their mathematical possibility. Each variable has a high loading in the first principal component. This finding suggests that the relative strength of the peptide-HP- $\beta$ -CD interaction is similar but not identical in the various mobile phases.

The two-dimensional nonlinear map of principal component loadings is shown in Figure 1. The scales of the maps are dimensionless numbers indicat-



**Table 3.** Parameters of linear relationships between the  $R_M$  values of peptides and the concentration (C) of hydroxypropyl- $\beta$ -cyclodextrin in the mobile phase ( $\text{mg mL}^{-1}$ )

Solute	$n^a$	$R_{M0}$	$-b \cdot 10^2$	$s_b \cdot 10^{3b}$	$r_{\text{calc.}}^c$	$r_{95\%}^d$
Mobile phase, 0.05 M aqueous RbCl solution						
Ala <sub>5</sub> <sup>e</sup>	8	0.34	2.08	4.80	0.8707	0.7067
Gly <sub>2</sub>	8	0.25	1.49	4.69	0.7923	0.7067
Gly <sub>3</sub>	8	0.28	1.45	4.49	0.7958	0.7067
Gly <sub>4</sub>	8	0.30	1.37	2.88	0.8822	0.7067
Gly <sub>5</sub>	8	0.47	1.44	3.35	0.8681	0.7067
Lys <sub>2</sub>	7	0.06	0.75	2.33	0.8195	0.7545
Lys <sub>3</sub>	8	0.22	1.01	2.83	0.8257	0.7067
Lys <sub>4</sub>	7	0.31	0.69	1.14	0.9391	0.7545
Lys <sub>5</sub>	Not significant					
GLT red	8	1.34	1.06	2.91	0.8304	0.7067
Mobile phase, 0.05 M aqueous CsCl solution						
Ala <sub>5</sub> <sup>e</sup>	6	0.43	2.49	2.97	0.9726	0.8114
Gly <sub>2</sub>	6	0.33	1.91	2.39	0.9700	0.8114
Gly <sub>3</sub>	7	0.34	1.71	3.61	0.9045	0.7545
Gly <sub>4</sub>	7	0.42	2.01	4.88	0.8790	0.7545
Gly <sub>5</sub>	8	0.49	1.31	4.35	0.7763	0.7067
Lys <sub>2</sub>	Not significant					
Lys <sub>3</sub>	6	0.19	0.90	2.01	0.9131	0.8114
Lys <sub>4</sub>	8	0.30	0.58	1.62	0.8262	0.7067
Lys <sub>5</sub>	8	0.46	0.48	1.34	0.8236	0.7067
GLT red	7	1.31	0.77	2.02	0.8621	0.7545

<sup>a</sup>Number of data points.<sup>b</sup>Standard deviation of b.<sup>c</sup>Calculated coefficient of correlation.<sup>d</sup>Tabulated r value indicating the significance level of the fitness of Equation (2) to the experimental data.<sup>e</sup>Symbols refer to peptides in the Experimental section ( $R_M = R_{M0} + bC$ ).

ing only the distribution of points on the two-dimensional plane. The results entirely support the previous qualitative conclusions. Mobile phases are widely distributed on the map suggesting again that salts exert a different influence on the formation of peptide-HP- $\beta$ -CD complexes, and that not only the concentration but also the physicochemical parameters of cations may have a marked impact on the complex formation.

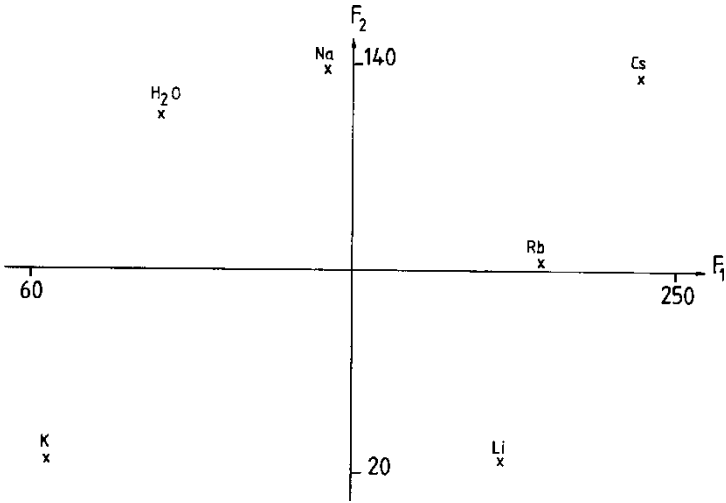
The two-dimensional nonlinear map of peptides is shown in Figure 2. Interestingly, peptides form clusters according to the character of the amino acid building units and not according to the length of the peptide chain. This finding can be tentatively explained by the supposition that only the terminal amino acid unit of the peptides enters the cavity of HP- $\beta$ -CD molecule, and the contribution of the other amino acids to the formation of complexes is negligible.

**Table 4.** Similarities and differences among the effects of mobile phase on the relative strength of HP-β-CD–peptide interaction. Results of principal component analysis

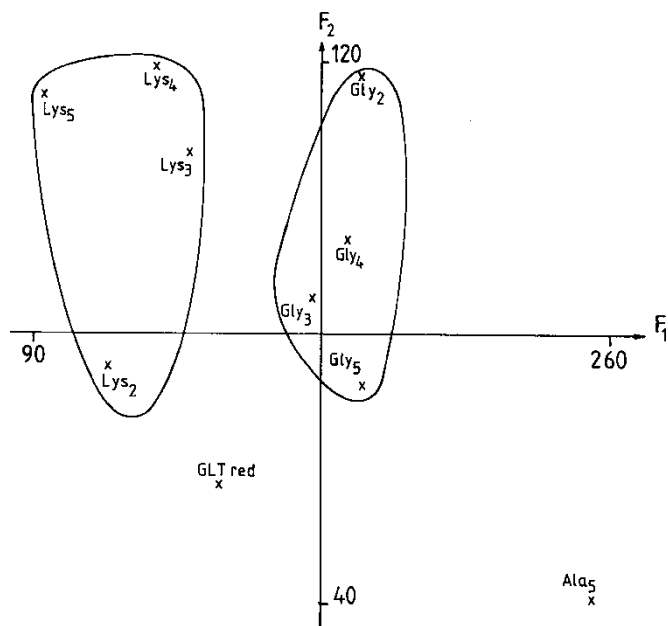
No. of principal component	Eigenvalue	Variance explained (%)	Total variance (%)
1	4.45	74.19	74.19
2	0.78	13.06	87.25
3	0.47	7.92	95.17

Principal component loadings  
No. of principal component

Parameter	1	2	3
Water	0.88	0.19	−0.37
LiCl	0.87	−0.04	0.44
NaCl	0.93	−0.02	−0.31
KCl	0.69	0.69	0.15
RbCl	0.93	−0.18	0.16
CsCl	0.84	−0.49	−0.02



**Figure 1.** Similarity and dissimilarity between the effect of mobile phases on the relative strength of HP-β-CD–peptide interaction. Two-dimensional nonlinear map of principal component loadings. Number of iterations, 59; maximum error,  $1.12 \cdot 10^{-2}$ . For symbols see the experimental section. The scales of the map are dimensionless numbers indicating only the distribution of points on the two-dimensional plane.



**Figure 2.** Similarity and dissimilarity between peptides. Two-dimensional nonlinear map of principal component variables. Number of iterations, 183; maximum error,  $9.81 \cdot 10^{-3}$ . Symbols refer to peptides in the experimental section. The scales of the map are dimensionless numbers indicating only the distribution of points on the two-dimensional plane.

The parameters of significant relationships between the relative strength of peptide-HP- $\beta$ -CD interaction and the physicochemical parameters of peptides are compiled in Table 5. SRA found significant relationships between the dependent and independent variables, the significance level being in both cases over 99% (compare calculated and tabulated F values). The independent variables explain 89% and 60% of the total variance (see  $r^2$  values). The hydrophobic ( $z_1$ ) and electronic ( $z_3$ ) parameters of peptides significantly influenced the complex formation. This result demonstrates the importance of the hydrophobic binding forces between the guest molecule and the apolar inner surface of the CD cavity, and indicates that the hydrophilic substructures of peptides may interact with the polar surface groups on the outer sphere of HP- $\beta$ -CD molecule.

## CONCLUSION

It can be concluded from the data that peptides can form complexes (probably inclusion complexes) with HP- $\beta$ -CD. The strength of complex formation con-

**Table 5.** Parameters of significant relationships between the relative strength of peptide–HP- $\beta$ -CD interaction and the physicochemical parameters of peptides. Results of stepwise regression analysis. Equation I,  $NLM1 = a + b_1 \cdot z_1 + b_2 \cdot z_3^a$ ; Equation II,  $NLM2 = a + b_1 \cdot z^a$

Parameter	Equation I	Equation II
n	10	10
a	142	75.8
$b_1$	6.07	−4.06
$s_{b1}$	2.15	1.16
$b_2$	12.7	—
$s_{b2}$	2.04	—
$b_1$ %	31.28	—
$b_2$ %	68.72	—
$r^2$	89.06	60.62
$F_{calc.}$	28.50	12.31
$F_{99,9\%}$	21.69	—
$F_{99\%}$	—	11.26

<sup>a</sup>NLM1 and NLM2 are the first and second coordinates of the two-dimensional nonlinear map of principal component variables. For other symbols see the Experimental section and Table 1.

siderably depends on the structure of the peptide molecule and on the presence of salts in the mobile phase. It is probable that the complex formation of peptides with HP- $\beta$ -CD modifies the various pharmacokinetic parameters and consequently, the biological activity of peptides in living organisms.

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