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The Use of Principal Component Analysis for the Study of the Interaction of Anionic Surfactants with Hydroxypropyl-β-Cyclodextrin

TIBOR CSERHÁTI* and GERGELY CSIKTUSNÁDI KISS
Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O.Box 17, 1525 Budapest, Hungary

JOSEPH AUGUSTIN
Slovak Technical University, Faculty of Chemical Technology, Department of Biochemical Technology, Bratislava, Slovakia

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Abstract. The interaction of 11 sulfosuccinic acid ester anionic surfactants with hydroxypropyl-β-cyclodextrin (HPβCD) were determined with reversed-phase thin-layer chromatography and the relative strength of interaction was calculated. The relationship between the strength of interaction and the physicochemical parameters of anionic surfactants was elucidated with principal component analysis (PCA). HPβCD interacted with the anionic surfactants decreasing their hydrophobicity. The distribution of the points of the strength of interaction and physicochemical parameters on the two dimensional nonlinear map of PC loadings suggested that the strength of interaction between the anionic surfactants and HPβCD is of mixed steric character, with hydrophobic and electronic forces being involved in the interaction.

Key words: sulfosuccinic acid esters, hydroxypropyl-β-cyclodextrin, principal component analysis

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built up from 6–8 glucopyranose units. Due to their ring structures CDs have the capacity to form inclusion complexes with a wide variety of organic compounds and even with inorganic compounds [1, 2]. Both the biological efficiency and physicochemical characteristics of the guest molecule may deviate from those of the uncomplexed one resulting in improved application parameters and higher biological activity [3, 4]. Much effort has been devoted to the elucidation of the involvement of various binding forces in the host-guest interaction. It was assumed that dipole-dipole, van der Waals and hydrophobic interactions [5, 6], and hydrogen bond formation [7, 8] may influence the strength of host-guest interaction.

* Author for correspondence.

Anionic surfactants are extensively used in current agrochemical practice [9] to improve the application parameters of pesticide formulations [10]. Beside this beneficial effect they also display marked toxic activity [11]. Various bacteria can use anionic surfactants as carbon sources promoting in this way their degradation [12, 13]. As the practice of including both surfactants and CDs in pharmaceutical and agrochemical formulations continues to increase, studies of the interaction of CDs and their derivatives is of practical and theoretical importance. The interaction of nonionic [14–18] and anionic [19, 20] surfactants with various CDs and CD derivatives has been extensively studied using various chromatographic and other physicochemical methods.

Various chromatographic techniques can be successfully used for the determination of molecular interactions [21]. Their advantages are that they use a low quantity of compounds, and the interacting molecules need not be very pure because the impurities are separated during the chromatographic process.

The objectives of the study was the determination of the interaction of sulfosuccinic acid ester surfactants with hydroxy-propyl-β-cyclodextrin (HPβCD), the evaluation of the influence of salt on the strength of interaction, and the assessment of the relationship between molecular structure and complex forming capacity.

2. Experimental

Polygram UV_{254} (Macherey-Nagel, Dürren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane-paraffin oil 95 : 5 (v/v). The solutes were di-*n*-butyl- (compound **1**), di-iso-butyl- (**2**), di-*n*-pentyl- (**3**), di-*n*-hexyl- (**4**), dicyclohexyl- (**5**), di-(2-ethylhexyl)- (**6**), di-*n*-octyl- (**7**), di-iso-octyl- (**8**), 4-iso-decyl- (**9**) *n*-dodecyl- (**10**) and di-*n*-tridecylesters of sulfosuccinic acid (**11**). The esters were separately dissolved in methanol at a concentration of 3 mg/mL and 2 μL of the solutions were spotted on the plates. Water-methanol mixtures were used as eluents, the methanol concentration being 40 and 45 vol.%. HPβCD (CYCLOLAB Research and Development Company, Budapest, Hungary) was added to the eluents in the concentration range of 0–20 mg/mL. As the object was to study the complex formation between the solutes and HPβCD and not the study of the effect of HPβCD on the separation of solutes, they were separately spotted on the plates. In this way the competition between the anionic surfactants for the binding sites of HPβCD was excluded. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with β-cyclodextrins [22, 23]. In order to elucidate the effect of salt on the strength of the inclusion complexes the same experiments were carried out in ion-free eluents and in eluents containing 0.1 M NaCl end concentration. Developments were carried out in sandwich chambers (22 $\times$ 22 $\times$ 3 cm) at room temperature, with the distance of development at about 16 cm. After development the plates were dried at 105 °C and the spots of solutes were revealed by a pH indicator [24]. Each experiment was run in quadruplicate. The R_M value characterizing the molecular hydrophobicity in

reversed-phase thin-layer chromatography was calculated for each solute in each eluent:

$$R_M = \log(1/R_f - 1) \tag{1}$$

When the coefficient of variation of the parallel determinations was higher than 5% the R_M value was omitted from the following calculations. To separate the effects of methanol and HPβCD on the hydrophobicity of the anionic surfactants the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 \tag{2}$$

where R_M is the R_M value for a surfactant determined at given methanol and HPβCD concentrations; R_{M0} is the R_M value extrapolated to zero methanol and HPβCD concentrations; b_1 is the decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent (related to the specific hydrophobic surface area of the surfactants [25]; b_2 is the decrease in the R_M value caused by a 1 mg/mL concentration change of HPβCD in the eluent (related to the relative strength of interaction); C_1 and C_2 are the concentrations of methanol and HPβCD, respectively. Equation (2) was applied separately for each surfactant.

The relationship between the relative strength of the surfactant – HPβCD interaction and the physicochemical parameters was elucidated by principal component analysis (PCA) [26]. The physico-chemical parameters included in the calculation as dependent variables were: π the Hansch–Fujita's substituent constant characterizing hydrophobicity; H-Ac and H-Do are the indicator variables for proton acceptor and proton donor properties, respectively; M-RE is the molar refractivity; F and R are electronic parameters characterizing the inductive and resonance effect, respectively; σ is Hammett's constant, characterizing the electron-withdrawing power of the substituent in the para and ortho+meta position ($\sigma_{\text{ortho+meta}}$, σ_{para}); Es is Taft's constant, characterizing steric effects of the substituent; B_1 and B_4 are Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment. The calculation of the physicochemical parameters of solutes was carried out by using the additivity rule. As the visual evaluation of the multidimensional matrices of PC loadings and variables is complicated, the dimensionality of the matrices was reduced to two by the non-linear mapping technique [27]. The iteration was carried out to the point where the difference between the last two iterations was less than 10^{-8}.

3. Results and Discussion

Compound **11** remained at the start point in each eluent system. This finding indicates that **11** is highly hydrophobic and its interaction with HPβCD cannot be determined under the chromatographic conditions employed. The simultaneous effect of methanol and HPβCD concentrations on the R_M values of compounds **3** and

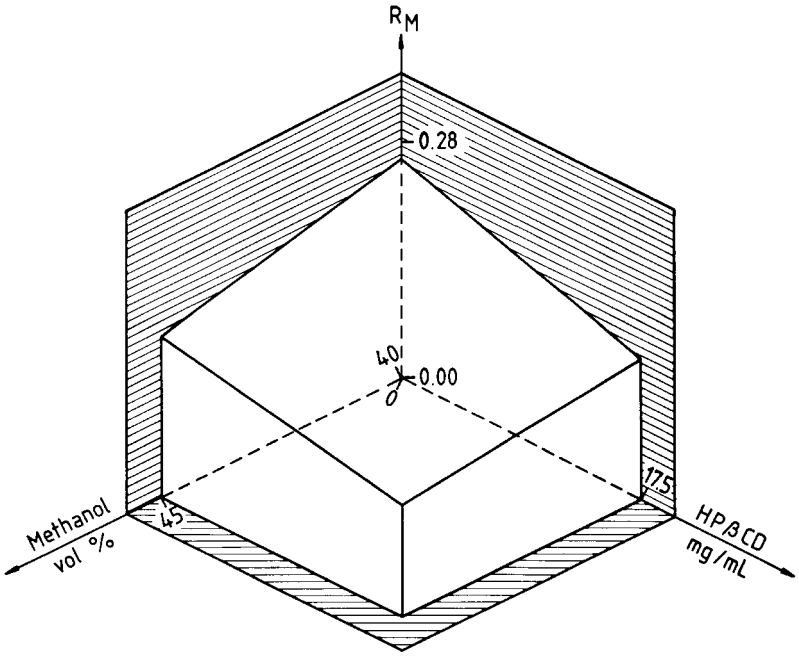

Figure 1. The effect of methanol and hydroxypropyl-β-cyclodextrin concentration on the R_M value of sulfosuccinic acid di-*n*-pentylester.

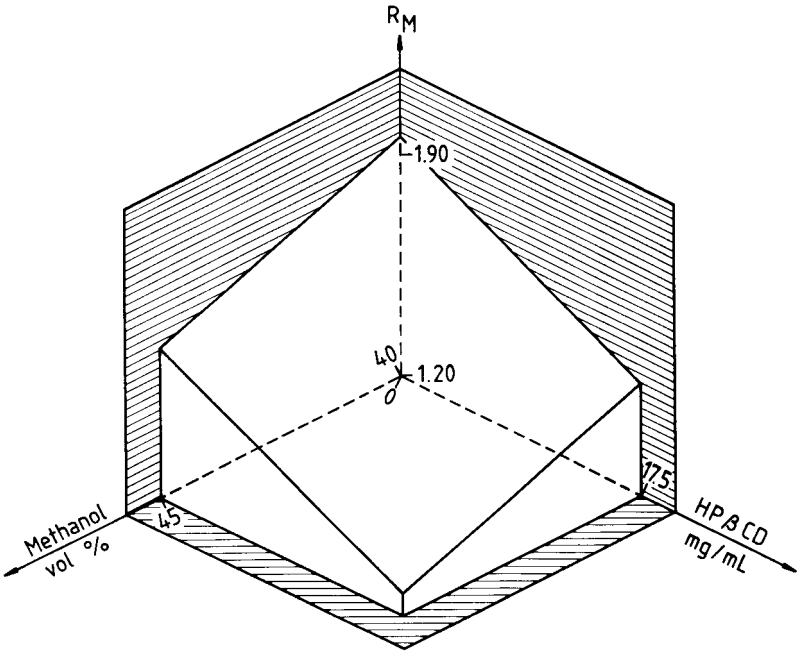

Figure 2. The effect of methanol and hydroxypropyl-β-cyclodextrin concentration on the R_M value of sulfosuccinic acid di-*n*-octylester.

Table I. Parameters of linear correlations between the R_M values of sulfosuccinic acid ester surfactants and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent. Salt-free eluents. Numbers refer to surfactant in Experimental. ($R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$)

Parameter	No of sulfosuccinic acid ester				
	1	2	3	4	5
R_{M0}	1.26	1.19	1.08	2.13	2.75
$-b_1 \times 10^2$	3.84	3.69	2.04	2.44	5.93
$s_{b1} \times 10^3$	5.87	6.12	2.01	3.79	18.19
$-b_2 \times 10^3$	–	–	3.72	8.03	17.18
$s_{b2} \times 10^3$	–	–	1.15	1.47	7.28
b_1'%	–	–	59.99	62.41	58.03
b_2'%	–	–	40.01	37.59	41.97
r^2	0.7408	0.7074	0.9312	0.8827	0.5929
$F_{\text{calc.}}$	42.86	36.26	54.16	56.42	9.47
Parameter	No of sulfosuccinic acid ester				
	6	7	8	9	10
R_{M0}	3.41	3.94	3.33	0.85	1.36
$-b_1 \times 10^2$	4.38	5.11	4.09	–	–
$s_{b1} \times 10^3$	5.11	9.94	7.08	–	–
$-b_2 \times 10^3$	19.73	21.93	20.17	30.47	34.09
$s_{b2} \times 10^3$	1.98	3.73	2.74	3.44	9.08
b_1'%	46.21	46.68	43.99	–	–
b_2'%	53.79	53.32	56.01	–	–
r^2	0.9202	0.8132	0.8540	0.8391	0.5020
$F_{\text{calc.}}$	86.46	30.47	43.88	78.21	14.11

7 are shown in Figures 1 and 2. The R_M values decrease with increasing methanol concentration, i.e., these compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Equation 2. An increase in HPβCD concentration also caused a decrease in R_M values, indicating some type of interaction (possibly inclusion complex formation). We have to emphasize that the data do not prove unambigously the existence of inclusion complex formation between the anionic surfactants and HPβCD. As the effect of HPβCD on the change of R_M value is much higher than that of methanol in the same concentration range, a specific interaction between these molecules was assumed. As the strongest interaction between CDs and other compounds is the inclusion complex formation, it is possible that this phenomenon occurs between the

Table II. Parameters of linear correlations between the R_M values of sulfosuccinic acid ester surfactants and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent. Salt-containing eluents. Numbers refer to surfactant in Experimental. ($R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$)

Parameter	No of sulfosuccinic acid ester				
	1	2	3	4	5
R_{M0}	0.48	0.71	1.16	1.77	1.30
$-b_1 \times 10^2$	1.24	1.84	1.64	2.13	2.24
$s_{b1} \times 10^3$	2.27	2.39	2.93	2.79	2.56
$-b_2 \times 10^3$	7.33	6.90	14.33	13.67	18.53
$s_{b2} \times 10^3$	0.88	0.93	1.14	1.08	0.99
b_1'%	39.66	50.87	30.76	37.68	31.93
b_2'%	60.34	49.13	69.24	62.32	68.07
r^2	0.8692	0.8844	0.9272	0.9358	0.9660
$F_{\text{calc.}}$	49.85	57.38	95.47	109.37	212.82
Parameter	No of sulfosuccinic acid ester				
	6	7	8	9	10
R_{M0}	3.99	3.28	2.70	1.54	1.63
$-b_1 \times 10^2$	5.30	3.17	2.04	1.98	–
$s_{b1} \times 10^3$	10.29	8.81	8.91	5.38	–
$-b_2 \times 10^3$	21.96	20.69	20.41	22.97	68.67
$s_{b2} \times 10^3$	4.13	3.47	3.69	2.08	6.09
b_1'%	49.21	37.62	29.23	25.01	–
b_2'%	50.78	62.38	70.77	74.99	–
r^2	0.7826	0.7724	0.7108	0.9000	0.8883
$F_{\text{calc.}}$	23.40	18.67	15.97	67.53	127.30

anionic surfactants and HPβCD. Interaction of the more hydrophilic HPβCD with the surfactants reduces the lipophilicity of the latter. This finding suggests that the biochemical and biophysical properties (surfactant activity, penetration capacity, leakage, uptake, decomposition rate, etc.) of surfactant-HPβCD complexes may be different from those of uncomplexed surfactants resulting in modified efficiency. The parameters of Equation (2) calculated in salt-free and in salt-containing eluents are compiled in Tables I and II, respectively.

Blank sites in Tables I and II indicate that these independent variables did not influence significantly the R_M value of the surfactant. Equation (2) fits the experimental data well, the significance levels in each instance being over 99% (see calculated F values); the ratios of variance explained varied between 50–96%

Table III. Similarities and dissimilarities between the physico-chemical parameters of sulfosuccinic acid esters and their capacity to interact with hydroxypropyl-β-cyclodextrin. Results of principal component analysis

No of component	Eigenvalue	Variance explained (%)	Sum of variance explained (%)
1	5.52	46.04	46.04
2	4.26	35.47	81.51
3	0.94	7.85	89.36
4	0.73	6.09	95.45

Parameters	Principal component loadings No of principal components			
	I	II	III	IV
π	−0.89	0.34	−0.08	0.23
H-Ac and H-Do[a]	0.49	0.84	0.14	−0.10
M-RE	−0.81	0.54	0.08	0.21
F	0.69	0.61	−0.07	−0.23
R	0.60	−0.41	0.29	0.61
$\sigma_{(ortho+meta)}$	0.87	0.44	0.15	0.10
$\sigma_{(para)}$	0.96	0.22	0.00	0.08
Es	0.65	−0.56	−0.41	0.25
B_1	−0.56	−0.50	0.63	−0.12
B_4	−0.76	0.42	−0.40	0.20
b_2 (water)	−0.12	0.91	0.26	0.26
b_2 (0.1 M NaCl)	0.08	0.88	−0.02	−0.02

[a] H-Ac and H-Do values were identical for each sulfosuccinic acid ester.

(see r^2 values). The majority of surfactants interact with HPβCD (b_2 values differ significantly from zero) indicating that in formulations containing both surfactants and HPβCD their possible interaction has to be taken into consideration. The parameters of Equation (2) show high variations between the surfactants proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity to form inclusion complexes with HPβCD (b_2) differ considerably. This result suggests also that the inclusion complex formation may influence differently the efficiency of the individual surfactants. The relative strength of the surfactant – HPβCD interaction increases with increasing length of the alkyl substituent both in salt-free and salt-containing eluent systems. This phenomena can be explained by the supposition that the alkyl chains enter the hydrophobic cavity of HPβCD enhancing the stability of the complex. The path coefficients (b_i'% values) indicate that the change of methanol and HPβCD concentrations has a similar effect on the retention of anionic surfactants.

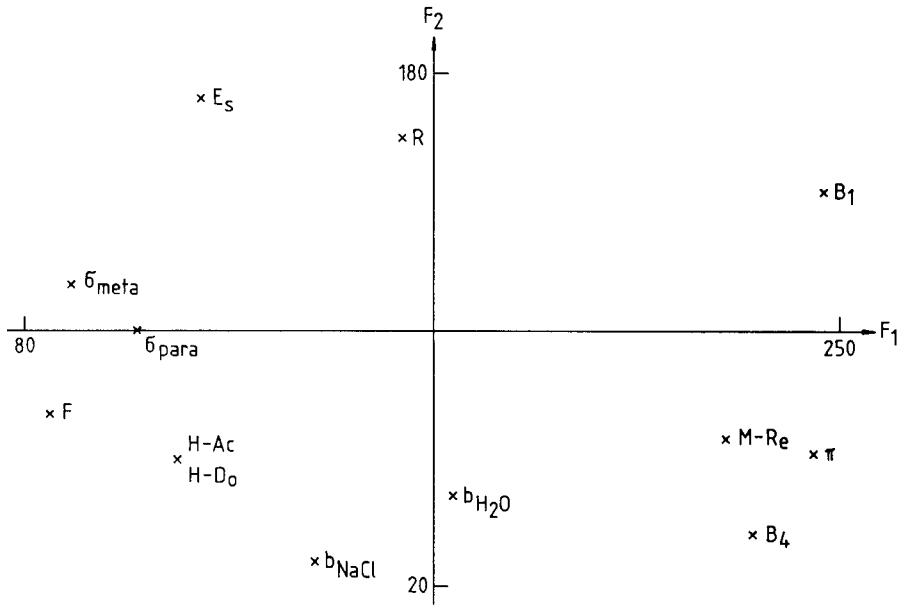

Figure 3. Relationship between the various physicochemical parameters of sulfosuccinic acid esters and their capacity to interact with hydroxypropyl-β-cyclodextrin. Two-dimensional nonlinear map of principal component loadings (number of iterations: 140, maximum error: 1.53×10^{-2}). For symbols see Experimental Section.

The results of principal component analysis are compiled in Table III. Four principal components explain the overwhelming majority of variance indicating that the 12 original variables can be substituted by 4 background (abstract) variables with only 4.55% loss of information. Unfortunatly, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility. The complex forming capacities of surfactants – together with many physicochemical parameters – have high loadings in the second PC indicating the marked influence of these parameters on the complex forming capacity of surfactants. The distribution of variables on the two-dimensional nonlinear map of PC loadings supports our previous conclusions (Figure 3). The relative strengths of interaction determined in salt-free and salt-containing eluent systems are similar proving that salt concentration exerts a negligible influence on the strength of the surfactant -HPβCD interaction. The role of salts in the inclusion complex formation has been vigorously discussed. Salts can increase or decrease the stability of inclusion complexes or cannot exert any effect [28]. The influence of salts was tentatively explained by the modification of the activity of the guest molecule [29], by the formation of ternary complexes [30] and by the changing of the microenvironment of the CD cavity [31].

The complex forming capacities do not form a well defined cluster with any one physicochemical parameter indicating that more than one molecular character-

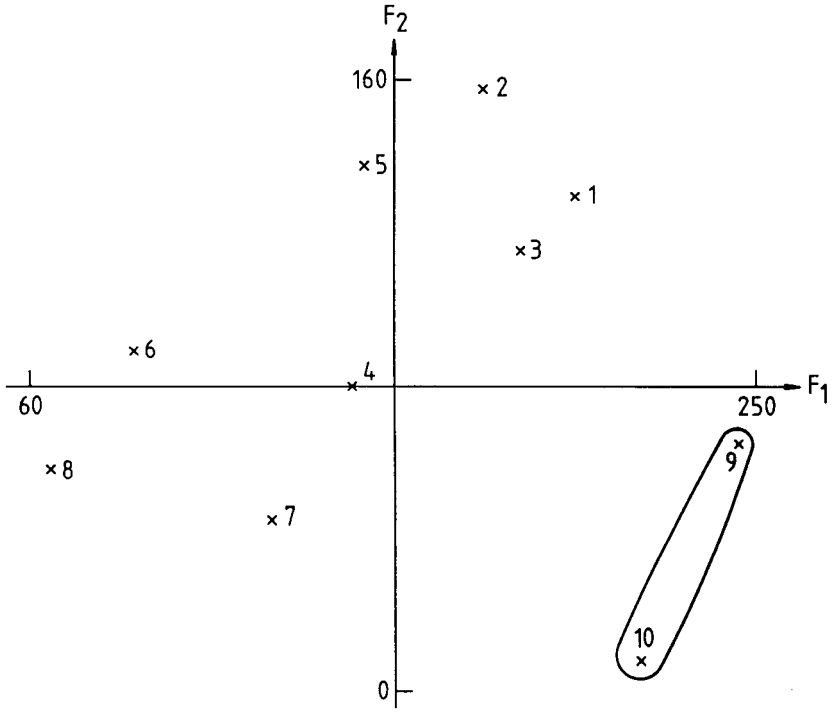

Figure 4. Similarity and dissimilarity of sulfosuccinic acid esters. Two-dimensional nonlinear map of principal component variables (number of iterations: 71, maximum error: 1.90×10^{-2}). Numbers refer to sulfosuccinic acid esters in Experimental Section.

istic influence the interaction. Electronic, steric, and hydrophobic parameters are relatively near to the points representing the relative strengths of interaction. This result suggests that more than one interactive force is involved in the formation of inclusion complexes.

The data indicate that the hydrophobic alkyl chains enter the cavity of HPβCD. As the longer alkyl chains are also more hydrophobic the impact of these steric and hydrophobic parameters on the strength of interaction cannot be adequately separated. The involvement of electronic parameters can be explained by the assumption that the polar substructures of surfactants pointing out of the HPβCD cavity can bind to the hydrophilic substructures on the surface of the HPβCD molecule resulting in enhanced stability of the host–guest complex. The distribution of the surfactants on the two-dimensional nonlinear map of PC variables supports entirely our previous conclusions (Figure 4). Mono-substituted derivatives form a distinct cluster emphasizing the importance of the highly polar free carboxyl group in the interaction.

It can be concluded from the data that sulfosuccinic acid ester surfactants readily form complexes with HPβCD. The stability of the complex increases considerably with increasing length of the alkyl substituents. Principal component analysis indi-

cated that not only steric and hydrophobic but also electronic forces may contribute to the formation of surfactant – HPβCD complexes. The advantages of PCA for the study of the relationship between the relative strengths of the surfactant-HPβCD interaction and a large set of physicochemical parameters are obvious. Traditional correlation methods are able only to calculate one to one relationships whereas PCA makes possible the evaluation of the similarities and dissimilarities between the columns and rows of any data matrix in one calculation step.

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