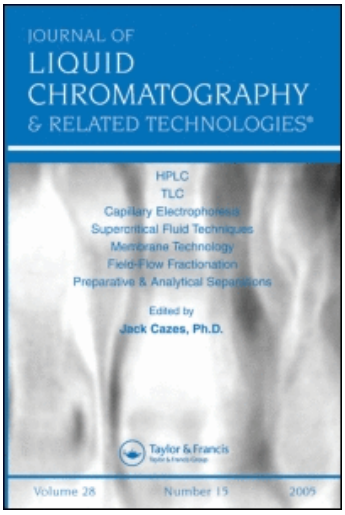

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USE OF A MODIFIED NONLINEAR MAPPING METHOD IN QUANTITATIVE STRUCTURE RETENTION RELATIONSHIP STUDY

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ABSTRACT

The interaction of 20 nonsteroidal anti-inflammatory drugs with a model protein was studied by reversed-phase high-performance liquid chromatography and the relationship between the strength of interaction and the physicochemical parameters of drugs was elucidated by principal component analysis followed by modified nonlinear mapping and cluster analysis. It was established that the polarity and and sterical parameters of drugs exert the highest influence on the interaction. Cluster analysis and nonlinear mapping using the absolute values of principal component loadings explain more precisely the interaction than the traditional nonlinear mapping and cluster analysis do.

INTRODUCTION

Last decades various multivariate mathematical statistical methods have found growing acceptance and application in many fields of chromatography such as gas-liquid,[1] thin-layer,[2] micellar electrokinetic,[3] and high performance liquid chromatography (HPLC).[4] Their applicaton facilitates the evaluation of multidimensional retention data matrices, helps the elucidation of the relationship between retention characteristics and physicochemical parameters of solutes, promotes the classification of chromatographic systems, etc. Due to the different mode of calculation each multivariate method highlights only one or two aspects of the relationships mentioned above.

Thus, stepwise regression analysis selects the independent variables exerting a significant influence on one dependent variable from any set of independent variables;[5] canonical correlation analysis calculates the relationship between two sets of variables, one of them containing the dependent variables,[6] factor analysis,[7] whereas hierarchical cluster analysis,[8] and principal component analysis (PCA)[9] calculate the similarities and dissimilarities between the rows and columns of any data matrix without being any row or column the dependent variable. Due to its versatility, PCA has been frequently used in chromatography. It has been employed for the characterisation of hydrophobic interaction and hydrophobic interaction chromatographic media,[10] for the elucidation of the retention mechanism of porous graphitized carbon support,[11] and for the assessment of the influence of molecular parameters on HPLC retention of propargylamine derivatives.[12]

Although PCA reduces the dimensionality of the original data matrix the resulting matrices of PC loadings and PC variables are sometimes even multidimensional. As the capacity of the human brain to evaluate data distributed in multidimensional space is limited, the dimensions of the matrices of PC loadings and variables can be reduced either to two by nonlinear mapping technique[13] or to one by cluster analysis.[14] Both traditional cluster analysis and nonlinear mapping takes into consideration the positive or negative signs of the correlations by constructing the corresponding dendograms and maps.

Necessarily, the variables with strong negative correlation are far from each other on the map. The situation is the same when two variables are not intercorrelated: they also are far from each other on the map. It means that without the previous knowledge of the individual coefficients of regression the evaluation of the similarities or dissimilarities between the variables is subjected to error when both negative and positive correlations occur between the members of the original data matrix. Theoretically, this discrepancy can be avoided by using only the absolute values for the constructing of the map and dendogram.

The objectives of the present study were the determination of the binding of nonsteroidal anti inflammatory drugs to a model protein by HPLC, to elucidate the relationship between the strength of binding and the physicochemical parameters of solutes, and the comparison of the efficacy of the traditional and modified nonlinear mapping technique and cluster analysis for this purpose.

EXPERIMENTAL

The common and IUPAC name and the provenance of solutes are compiled in Table 1. They were dissolved in water. In the case of sparingly-soluble samples the dissolution was facilitated by adding a low amount of methanol.

Zein coated silica was prepared by dissolving 0.5 g of zein in 200 mL n-propanol - water 7:3 vol/vol 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) was added and the mixture was stirred for two hours at the same temperature; than the solvents were removed under vacuum. The zein coated silica was dried in vacuum oven at 70°C. A column of 150 x 4 mm I.D. was filled with a Shandon (Pittsburgh, PA, USA) analytical pump using 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 Instr., Cambridge, UK), a Valco injector (Valco Inc., Houston, TX, USA) with a 20 μL sample loop, and a Waters 740 integrator (Water-Millipore Inc., Milford, MA, USA). Elution was performed with distilled water, the flow-rate was 1 mL min^{-1} and the detection wavelength was set to the UV maximum of solutes (see Table 2). The column was not thermostated; each determination was run at ambient temperature (22-24°C).

Each retention time was determined by three consecutive injections. The dead volume of the system was measured by injecting 1% $NaNO_3$. The log k'_w values and the standard deviation was calculated. It was supposed that higher log k'_w value indicates higher affinity to the protein on the silica surface, therefore it can be used as a quantitative indicator of the strength of drug protein interaction. To find the correlation between the strength of interaction and the physicochemical parameters of drugs PCA was applied.[15] The log k'_w values and the physicochemical parameters of drugs were the variable and the drugs the observations. The physicochemical parameters were: Van der Waals surface (VdWsurface),[16-19] water accessible surface (SASsurface),[16-19] Van der Waals volume (VdWvolume),[18] water accessible volume (SASvolume,[18] polarizability,[20] refractivity,[21,22] lipophilicity (log P),[23] total energy, binding energy, heat of formation, energy of the higher occupied molecular orbit

Table 1

Commercial and IUPAC Names of Nonsteroidal Anti-Inflammatory Drugs

No. Of Drugs	Commercial Name	IUPAC Name	Provenience
1	Acetysalicylic acid	2-(Acetyloxy)benzoic acid	Polfa, Starogard, Poland
2	Azapropazone dihydrate	4-(Dimetnylamino)-9-methyl--2-propyl-1H-pyrazolo[1,2-a][1,2,4]benzotriazine-1,3(2H)-dione	DuPont, Pharma, Bad Germany
3	Diclofenac sodium	2-[(2,6-Dichlorophenyl}amino] benzeneacetic acid sodium salt	Polfa, Starogard, Poland
4	Phenazone	1,2-Dihydro-1,5-dimethyl-2-phenyl-3H pyrazol-3-one	Caesar & Loretz GmbH, Hilden, Germany
5	Fenbufen	τ-Oxo[1,1'-biphenyl]-4-butanoic acid	POCh, Gliwice, Poland
6	Phenol		POCh, Gliwice, Poland
7	Phenyl-butazone	4-Butyl-1,2-diphenyl-3-5-pyrazolidinedione	Polfa, Warszawa, Poland
8	Ibuprofen	α-Methyl-4-(2-methylpropyl) benzeneacetic acid	Polfa, Pabianice, Poland
9	Indometacin	1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid	Polfa, Krakow, Poland
10	Ketoprofen	3-Benzoyl-α-methyl-benzeneacetic acid	Polfa, Krakow, Poland
11	Ketorolac trometamol	±-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid	Polfa, Starogard, Poland
12	Mefenamic acid	2-[(2,3-Dimethylphenyl)-amino] benzoic acid	Polfa, Pabianice, Poland
13	Naproxen	(S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	Polfa, Pabianice, Poland
14	Niflumic acid	2-[(3-Trifuloromethyl)phenyl] amino]-3-pyridine-carboxylic acid	Laboratoires UPSA Ruiel-Malmaison, France
15	Piroxicam	4-Hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothioazine-3-carboxamide-1,1-dioxide	Polfa, Starogard, Poland
16	Salicylic acid	2-Hydrxybenzoic acid	Cefarm, Gdansk, Poland
17		α-2-Pyrazylidine-α-cyano -N-butyl acetamide	
18		α-6-chloro-2-pyrazylidine-2-cyano-N-iospropyl acetamide Sodium salt	
19		α-2-pyrazylidine-α-cyano-N-isobutyl acetamide	
20		α-2-pyrymidylidine-α-cyano-N-isopropyl acetamide	

Compounds 17-20 were synthesized by Dr. H. Foks and Dr. B. Pilarski at the Department of Organic Chemistry, Medical University of Gdansk, Poland.

Table 2

Detection Wavelength, Log k'_w Values and Relative Standard Deviation (R.S.D. %) of Nonsteroidal Anti-Inflammatory Drugs on a Zein-Coated Silica HPLC Column*

No. of Drugs	Detection Wavelength (nm)	Log k'_w	R.S.D. %
1	254	-08.11	0.86
2	254	0.507	0.55
3	254	0.863	0.71
4	254	1.010	0.32
5	280	0.594	0.48
6	254	-0.571	0.57
7	254	0.297	0.73
8	225	0.320	0.98
9	254	0.972	0.44
10	254	-0.024	0.62
11	254	0.012	0.39
12	285	1.342	0.53
13	235	-0.033	0.79
14	285	0.956	0.84
15	360	0.774	0.49
16	254	-0.144	0.75
17	254	0.553	0.56
18	254	0.517	0.67
19	310	0.235	0.97
20	254	0.177	0.61

* Numbers refer to nonsteroidal anti-inflammatory drugs in Table 1.

(HOMO), energy of the lower unoccupied molecular orbit (LUMO), dipole moment, minimum charge of the atoms, and maximum charge of the atoms. These parameters were computed by HyperChem 5.01 with ChemPlus Extension (Hypercube Inc., Waterloo, Ont., Canada).

Structures were first optimized using molecular mechanic calculations.[24] The molecular modelling structural descriptors (energetic parameters) were computed using semi empirical calculation method Austin Model 1.[25-27] The limit of the variance explained was set arbitrarily to 99%. The two dimensional nonlinear map and cluster dendogram of the PC loadings and variables were also calculated. The inclusion of non-linear mapping technique and cluster analysis in the evaluation was motivated by the consideration that each of them

Table 3

Similarities and Dissimilarities Between the Physicochemical Parameters of Nonsteroidal Anti Inflammatory Drugs and their Retention on Protein-Coated Silica Column*

No. of Component	Eigenvalue	Variance Explained (%)	Sum of Variance Explained (%)
1	8.57	53.54	53.54
2	2.59	16.20	69.74
3	1.38	8.61	78.35
4	1.18	7.40	85.75
5	0.97	6.09	91.84

	Principal Component Loadings No. of Principal Component				
Parameters	**1**	**2**	**3**	**4**	**5**
Log k'_w	0.65	-0.10	0.53	-0.22	0.03
VdWsurface	0.99	0.05	-0.03	0.09	0.01
SaSsurface	0.97	0.05	-0.02	0.18	0.02
VdWvolume	0.99	0.06	0.04	0.04	0.02
SASvolume	0.99	0.06	-0.02	0.11	0.02
Polarizability	0.99	0.12	-0.02	0.00	0.02
Refractivity	0.99	0.02	-0.02	-0.01	0.03
Log P	0.36	0.76	-0.15	-0.18	-0.20
Total energy	-0.92	0.10	0.10	-0.06	0.21
Binding energy	-0.97	-0.11	0.11	-0.08	-0.10
Heat of Formation	-0.13	-0.28	-0.31	0.48	0.70
HOMO	0.27	-0.08	0.75	0.31	0.46
LUMO	-0.35	0.65	-0.13	-0.40	0.20
Dipol moment	-0.22	-0.35	0.49	0.50	-0.37
Maximum charge	0.28	-0.77	-0.31	-0.43	-0.03
Minimum Charge	0.28	0.85	0.20	0.35	0.08

* Results of Principal Component Analysis.

are theoretically similar, they calculate and visualize the relative distances between the members of the matrix. The iteration of the nonlinear map was carried out to the point when the difference between the two last iterations was lower than 10^{-8}. As the matrix of PC loadings contained negative values too, nonlinear mapping and cluster analysis was also performed by using the absolute values of PC loadings. In order to control the reliability of the results of nonlinear mapping and cluster analysis linear correlations were calculated between the log k'_w values and each physicochemical parameters.

RESULTS AND DISCUSSION

The detection wavelength, log k'_w values and the relative standard deviation are compiled in Table 2.

The data in Table 2 indicate that the retention on drugs on the protein-coated silica column shows considerable variation. As the retention in water is related to the strength of protein drug interaction, the differences in log k'_w suggest that the strength of interaction marked depends on the chemical structure of the drug. The relative standard deviation is low in each instance indicating the good reproducibility of retention time and the stability of the protein-coated silica column. The results of PCA are summarized in Table 3.

Five principal components explain the majority of variance indicating that the 16 original variables can be substituted by 5 background (abstract) variables with only 8% loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility. The log k'_w values, together with the sterical, energetical, and polarity parameters, have high loading in the first PC indicating the marked influence of these physicochemical parameters on the strength of protein drug interaction. Interestingly, the hydrophobicity of drugs has a low loading in the first PC suggesting that role of apolar, hydrophobic forces is negligible in the protein drug interaction. The regression coefficients entirely support the previous conclusions (Table 4).

The two dimensional nonlinear maps calculated from the original PC loadings and from the absolute values of PC loadings are shown in Fig.1. Maps show marked differences in the distribution of variables indicating the considerable impact of the modification of the mode of calculation. Physicochemical parameters 9 and 10 (total energy and binding energy) are far away from log k'_w value (point 1) on map A calculated from the original PC loadings. It can be concluded, erroneously, that these physicochemical parameters are not correlated with the protein drug interaction. However, the data in Table 4 clearly show that the relationship between the log k'_w value and variables 9 and 10 is significant but the negative.

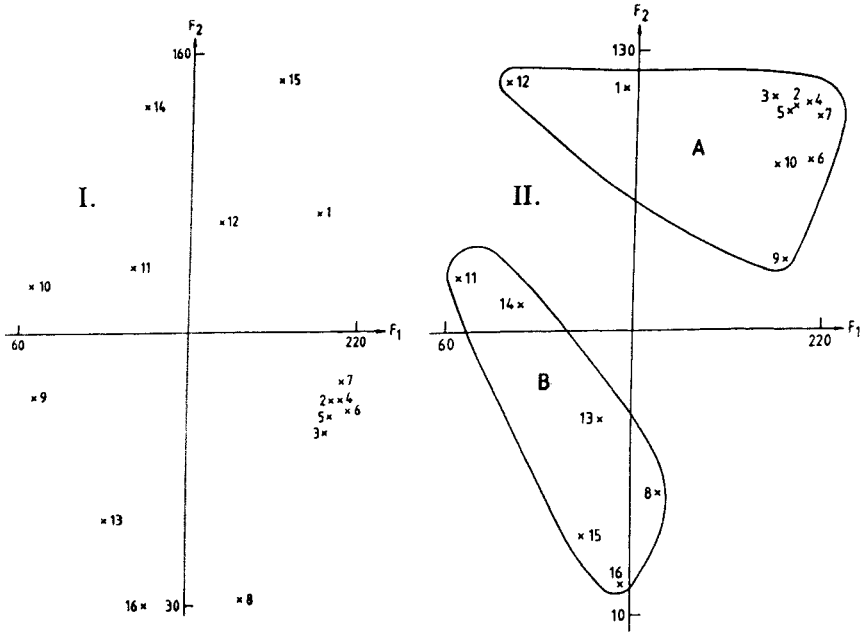

Figure 1. Two-dimensional nonlinear map calculated from the original PC loadings (I) and from the absolute values of PC loadings (II). A = number of iterations: 142; maximum error: $3.71.10^{-2}$. B = number of iteraitons: 113; maximum error: $1.34.10^{-2}$. 1 = log k'_w; 2 = Vwsurfac; 3 = SASsurfac; 4 = VdWvolume; 5 = SASvolume; 6 = Polarizability; 7 = Refractivity; 8 = Log P; 9 = Total energy; 10 = Binding energy; 11 = Heat of formation; 12 = HOMO; 13 = LUMO; 14 = Dipol moment; 15 = Maximum charge; 16 = Minimum charge.

This finding supports our previous theoretical conclusions that the information contained in the two-dimensional nonlinear map may be misleading when both negative and positive correlations occur between the variables. The distribution of variables on the map calculated from the absolute values (Fig.1B) correspond to the data in Table 4.

Physicochemical parameters exerting a significant impact on the strength of protein drug interaction are near to the log k'_w values (Cluster A) while the other parameters are well separated forming a distinct cluster (Cluster B). The cluster dendograms entirely supports the conclusions drawn from the distribution of variables on the two dimensional nonlinear maps (Fig.2). Dendogram calculated from the absolute values of PC loadings more similar in its information content to the data in Table 4 than the dendogram calculated from the unmodified PC loadings.

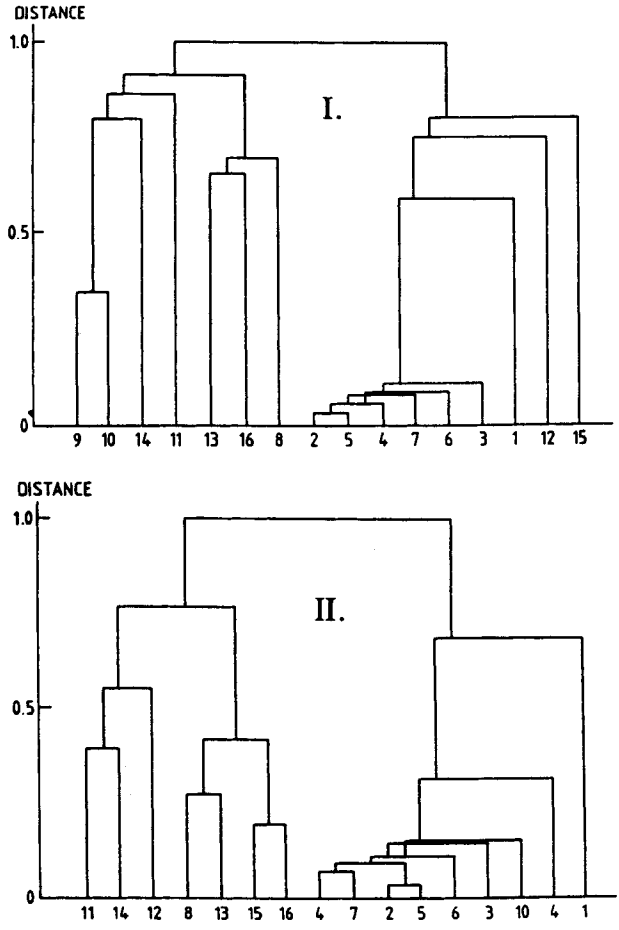

Figure 2. Cluster dendogram of original PC loadings (I) and the absolute value of PC loadings (II). Numbers refer to variables in Figure 1.

Calculations proved that the information contents of two dimensional nonlinear mapping and cluster analysis are highly similar. However, we strongly advocate the application of the two dimensional nonlinear mapping technique because the two dimensional map may contain more information than the one dimensional structure of clusters. Nonsteroidal anti-inflammatory drugs did not form distinct clusters either on the two dimensional map nor on the dendogram of PC components (data not shown).

Table 4

Regression Coefficients of the Relationships Between Log k'_w Values and the Individual Physicochemical Parameters*

Physicochemical Parameters	Regression Coefficient
VdWsurface	0.5568
SASsurface	0.5510
VdWvolume	0.5777
SASvolume	0.5615
Polarizability	0.5815
Refractivity	0.6058
Log P	0.2280
Total energy	-0.5676
Binding energy	-0.5297
Heat of formation	-0.1992
HOMO	0.5710
LUMO	0.2860
Dipol moment	0.2037
Maximum charge	0.2037
Minimum charge	-0.2197

* $r_{95\%} = 0.4329$.

This finding indicates that more than one molecular substructure of drugs influence their capacity to bind to protein and the strength of interaction is the results of the interplay of various intermolecular forces. It can be concluded from the data that the binding of non steroidal anti inflammatory drugs to protein depends on the sterical and hydrophilic polarity parameters of the drugs; the role of hyrophobic forces is negligible. The use of the absolute values of PC loadings and components for the calculation of two dimensional nonlinear maps and cluster dendograms prevents the occurrence of errors originated from the positive and negative character of the relationships between the variables.

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REFERENCES

1. K. G. Miller, C. F. Poole, T. M. P. Pawlowski, Chromatographia, **42**, 639-646 (1996).

2. S. Wallerstein, T. Cserháti, E. Forgács, V. Kiss, J. Pharm. Biomed. Anal., **15**, 431-438 (1997).

3. M. Salo, H. Sirèn, P. Volin, S. Wiedmer, H. Vuorela, J. Chromatogr. A, **728**, 83-88 (1996).

4. C. Garcia-Jares, S. Garcia-Martin, R. Cela-Torrijos, J.Agric. Food Chem., **43**, 764-768 (1995).

5. K. Azzaoui, L. Morin-Allory, Chromatographia, **42**, 389-395 (1996).

6. E. Forgács, T. Cserháti, J. Liq. Chrom. & Rel. Technol., **19**, 1849-1858 (1996).

7. C. H. Lochmüller, B. Hansen, J. Chromatogr. Sci., **33**, 640-646 (1995).

8. C. Garcia-Jares, S. Garcia-Martin, N. Carro-Marino, R. Cela-Torrijos, J. Sci. Food Agric., **69**, 175-184 (1995).

9. M. Turowski, R. Kaliszan, C. Lüllmann, H. G. Genieser, B. Jastorff, J. Chromatogr. A, **728**, 201-211 (1996).

10. P. Karsnas, T. Lindblom, J. Chromatogr., **599**, 131-136 (1992).

11. R. Kaliszan, K. Osmialowski, B. J. Bassler, R. A. Hartwick, J. Chromatogr., **499**, 333-344 (1990).

12. T. Cserháti, J. Biochem. Biophys. Meth., **32**, 137-150 (1996).

13. J. W. Sammon, Jr., IEEE Trans. Comput., **C18**, 401-407 (1969).

14. W. R. Dillon, **Multivariate Analysis**. John Wiley and Sons, New York, 1984, pp.157-208.

15. K. V. Mardia, J. T. Kent, J. M. Bibby, **Multivariate Analysis**, Academic Press, London, 1979, pp. 213-254.

16. W. Hasel, T. F. Hendrickson, W. C. Still, Tet. Comput. Meth., **1**, 103-116 (1988).

17. W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, J. Am. Chem. Soc., **112**, 6127-6129 (1990).

18. N. Bodor, Z. Gabanyi, C. Wong, J. Am. Chem. Soc., **111**, 3783-3786 (1989).

19. A. Gavezotti, J. Am. Chem. Soc., **10**, 5220-5225 (1983).

20. K. J. Miller, J. Am. Chem. Soc., **112**, 8533-8542 (1990).

21. A. K. Ghose, G. M. Crippen, J. Chem. Inf. Comput. Sci., **27**, 21-35 (1987).

22. V. N. Viswanadhan, A. K. Ghose, G. N. Revankar, R. K. Robins, J. Chem. Inf. Comput. Sci., **29**, 163-172 (1989).

23. A. K. Ghose, N. N. Pritchett, G. M. Crippen, J.Comp. Chem., **9**, 80-90 (1988).

24. N. L. Allinger, J. Am. Chem. Soc., **99**, 8127-8134 (1977).

25. M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, J. Am. Chem. Soc., **107**, 3902-3909 (1985).

26. M. J. S. Dewar, K. M. Dieter, J. Am. Chem. Soc., **108**, 8075-8086 (1986).

27. J. J. P. Stewart, J. Comput. Aided Mol. Des., **4**, 1-105 (1990).

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