Correlation analysis in liquid chromatography of metal chelates

III. Multi-dimensional models in reversed-phase liquid chromatography

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(First received May **4th,** 1993; revised manuscript received June **7th,** 1993)

ABSTRACT

A number of multiparametric retention models for metal chelates in reversed-phase high-performance liquid chromatography (RP-HPLC) were developed and compared. The most significant structural descriptors of the chelates and mobile phase parameters were selected with the help of one-dimensional correlation analysis performed in this and previously published papers of this series. Log k' values for metal di-n-alkyldithiophosphates used as test solutes were determined using a C_{18} column and dioxane as organic modifier and subjected to multiple regression analysis. The predictive ability of the resulting multiparametric regression equations was evaluated in terms of their statistical significance. The most meaningful retention model is **described** by the linear regression equation $\log k' = 0.716 + (0.236 \pm 0.010)n_C - (0.040 \pm 0.018)E_n - (0.048 \pm 0.003)c + (0.029 \pm 0.010)Z$ ($R = 0.976$; S.D. = 0.111), where n_C is the carbon number, E_n is the orbital electronegativity of the metal atom, c is the volume concentration of the organic modifier and Z is the parameter of proton-donating ability of the mobile phase. The results confirm the modern representations of the separation mechanism for metal chelates of moderate polarity in RP-HPLC, present rather valuable sets of solute structural descriptors and eluent parameters to approximate the experimental retention values and open new possibilities in the application of multivariate statistical methods to interpret a large number of chromatographic data.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC), in spite of growing competition from other chromatographic methods, can be still considered the preferred mode for metal chelate separation by HPLC. In this respect, the problems of forecasting the chromatographic behaviour of chelates (including an a priori prediction of retention parameters), understanding the mechanism of chromatographic retention at a molecular level and optimizing the ligand selectivity are of considerable interest. Our previous investigations [1–5] showed that these problems can be solved by using one-dimensional correlation models of the retention–structure and retention-mobile phase composi-

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tion type. Using this approach, we have developed a mathematical model for a metal chelate in a given chromatographic system. This is just one of the ultimate goals of multiparametric equations derived and evaluated in this paper.

EXPERIMENTAL

Preparation of chelates

Metal dialkyldithiophosphates were prepared directly by mixing aqueous solutions of metal nitrate (cadmium, copper, mercury, nickel, lead or zinc) and the potassium salt of the corresponding reagent (dimethyl-, diethyl-, di-*n*-propyl, di-n-butyl-, di-n-hexyl-, di-n-octyl-, di-n-decyldithiophosphate).

Apparatus and chromatographic conditions

We used a Waters (Milford, MA, USA) high-performance liquid chromatograph consisting of a Waters 600E programmable multisolvent delivery system, a Waters 991 computer-controlled photodiode-array detector monitoring at 254 nm and a Waters 5200 printer-plotter. An ODS-Hypersil column (250 x 4 mm) (particle size 5 μm) was purchased from Österreichisches Forschungszentrum, Seibersdorf, Austria. 1,4-Dioxane of analytical reagent grade (Merck, Darmstadt, Germany) and double-distilled water were used as the solvent system providing the optimal resolution of metal dithiophosphates [3,4].

Solute and eluent parameters

The structural parameters calculated according to ref. 3 or taken from the literature are listed in Tables I-III. Parameters for the dioxane-water mixtures which were considered (see Table V) were taken from refs. 4 and 6-11.

Deriving multiparametric retention models

Retention values (logarithms of capacity factors) were related to solute and eluent parameters by means of multiparametric regression analysis processed with the program SigmaPlot (Jandel Scientific, USA) on a personal computer. The equations derived were tested according to the requirements of a meaningful correlation

TABLE I

PARAMETERS FOR METAL ATOMS

Metal	Electronegativity (E_n) [6]	Hydrophobic increment (Δ_M)
Cd	−1.15	0.105
CU	-1.60	0.300
Hg	-4.40	0.756
Ni	-0.70	0.010
Pb	-1.25	0.210
Zn	-0.60	-0.010

TABLE II

PARAMETERS OF DIALKYLDITHIOPHOSPHATE LIGANDS

Ligand	Carbon number	Hydrophobic constant (f)	Molar volume (ml/mol)
Methyl	1	2.808	139.56
Ethyl	2	4.916	180.48
Propyl	3	7.024	262.32
Butyl	4	9.132	303.24
Hexyl	6	13.348	385.08
Octyl	8	17.564	466.92
Decyl	10	21.780	548.76

TABLE III

MOLECULAR PARAMETERS OF METAL DIALKYL-DITHIOPHOSPHATES

Ligand	Metal atom					
	Cd	Cu	Hg	Ni	Pb	Zn
Molecular connectivity index						
Methyl	21.24	18.68	24.34	18.68	24.15	18.98
Ethyl	23.59	21.03	26.69	21.03	26.50	21.33
Propyl	25.59	23.03	28.69	23.03	28.50	23.33
Butyl	27.59	25.03	30.69	25.03	30.50	25.33
Hexyl	31.59	29.03	34.69	29.03	34.50	29.33
Octyl	35.59	33.03	38.69	33.03	38.50	33.33
Decyl	39.59	37.03	42.69	37.03	42.50	37.33
Stability constant (log β_n)						
Butyl	8.5	15.1	29.6	5.9	12.3	7.9
Distribution constant (log K_D)						
Ethyl	0.170	0.369	1.217	0.148	0.285	0.011

A.R. Timerbaev et al. I J. Chromatogr. 648 (1993) 307–314

309

analysis, taking into account the correlation coefficient, standard deviation, significance level of the whole equation, t-test value and number of data points used to derive the equation.

RESULTS AND DISCUSSION

General conclusions from single-parametric correlation analysis

In earlier papers of this series [3,4], we have confirmed that one-dimensional retention models can be widely applied in RP-HPLC of neutral chelates. Non-polar sulphur-containing metal chelates such as dialkyldithiocarbamates and dialkyldithiophosphates were used as test solutes. The results of these studies can be briefly summarized as follows.

Retention-structure **dependences** observed for metal chelates (at least for those that are sufficiently stable in a chromatographic process) can be well described at the quantitative level in terms of linear free-energy relationships [3]. However, the polyfunctional structure of these solutes means that three types of structure-dependent parameters have to be taken into account as variables of the corresponding models. All the structural descriptors already studied and subdivided into three groups are collected in Table IV. Obviously, the structural complexity of chelates makes the task of correlation analysis more complicated than that for organic substances; in addition, the selection of the parameters is more critical (see below) and requires a knowledge of some special aspects of coordination chemistry.

The most recent application of correlation analysis to RP-HPLC of metal chelates was the correlation of **chelate** retention with the mobile phase composition and physico-chemical characteristics of solvents [4]. We found that the effect of the mobile phase can be evaluated by a variety of macroscopic and microscopic (molecular) parameters of binary water-organic mixtures listed in Table V, the former providing more reliable predictions of retention data. An analysis of linear retention models derived allowed us to conclude that, according to the solvophobic theory of RP-HPLC, the retention of metal chelates is governed mainly by the solvophobic

TABLE IV

STRUCTURAL PARAMETERS OF METAL CHELATES APPLICABLE TO DESCRIPTION OF THE RETENTION BEHAVIOUR IN RP-HPLC

Parameter	Refs.
(A) Molecular parameters	
Distribution constant (log K_D)	2, 3
Stability constant (log β_n)	1, 3
Molecular connectivity index (χ)	3, 5
Molar volume (V_m)	3
Solubility in the mobile phase	12
(B) Parameters of metal atom	
Effective charge, ρ_M	3
Electronegativity	3
Ratio of electronegativity to ionic radius	3, 13
Orbital electronegativity (E_n)	1, 3
Distribution coefficient	14, 15
Metal increment in the distribution constant (A,)	2, 3
(C) Ligand parameters	
Carbon number of alkyl homologies (n_C)	3, 15
Induction constant (a*)	3
Steric constant (E,)	3
Hydrophobic constants, π and f	3
Molecular connectivity index of the ligand	3, 5
Molar volume of the ligand	3

effect or, in other words, by the hydrophobic, bulk-dependent nature of a molecule, whereas specific intermolecular interactions in the mobile phase (mostly hydrogen bonds involving solute

TABLE V

PARAMETERS OF BINARY WATER-ORGANIC MOBILE PHASES USED IN RP-HPLC OF METAL CHELATES [4]

(A) Macroscopic parameters
Volume concentration of an organic modifier (c)
Molar concentration of an organic modifier (log C)
Hydrophobicity (log P_s)
Methylene selectivity (log α_{CH_2})
Surface tension (γ)
Viscosity (η)
Dielectric constant (ε)

(B) Molecular parameters
Solvent dipolarity/polarizability by Kamlet-Taft (π^*)
Acceptor strength by Dimroth-Reichardt (E_T)
Proton-donating ability by Kosower (Z)
Solvent strength by Brounstein (S)

molecules and proton-donor eluent molecules) are responsible for the differences in the retention and hence the separation.

However, the predictive value of the aforementioned one-dimensional relationships is restricted because of the limited nature of the approach itself, which takes advantage of only one of the possible parameters. In practice, the retention behaviour of a metal chelate quantified by log k' depends on *all* possible parameters characterizing its structure, the mobile phase nature and the chromatographic system as a whole. Therefore, only a **multiparametric** approach for the correlation analysis of retention dependence on both structure and **chromato**graphic experimental conditions can provide a sufficiently complete answer. This approach necessarily includes choosing the most informative sets of chelate structural parameters and parameters of the **chromatographic** system (or of a mobile phase at a given stationary phase), taking into account the most likely retention mechanism, and the search for a number of multiparametric models based on various parameter sets and providing the most adequate description of experimental data. **Statistically** most significant models will characterize the retention behaviour of a given class of chelates and, thereby, represent a certain mathematical pattern of the solutes in RP-HPLC.

Selection of parameters

In this paper, we shall first discuss the choice of the most significant parameters to be used as variables for the multiple linear regression analysis. **The** first selection principle utilized is to reject some less important parameters based on results obtained earlier in our one-dimensional correlations. Then, to facilitate the development of multivariate models, the set of the parameters is further reduced using one-dimensional correlation analysis for particular chelates and the separation system studied in this work. Finally, the rest of the most valuable parameters will be grouped as "solvophobic" and "hydrogen bonding" terms.

Of the molecular structural descriptors listed in Table IV, we do not consider here molar volume, which shows a poor correlation with log

k' values owing to inaccuracy in the evaluation of the metal increment, and solubility, which requires special experiments to be performed. The retention values of metal dithiophosphates (as the chelates of the S,S-type) in RP-HPLC did not correlate with the effective charge or **elec**tronegativity of a metal atom, or with the ratio of electronegativity to metal ion radius. This is because specific electrostatic interactions in the chromatographic system do not significantly influence the retention behaviour of these chelates. Data on distribution coefficients are not available, and their experimental evaluation is out with the scope of this work. The application of electronic and steric constants of alkyl **sub**stituents, characterizing the hydrogen bonding capability of donor atoms, is limited by the comparatively small number of carbon atoms in a ligand (not more than four atoms). Both types of hydrophobic constants well describe the hydrophobic nature of a chelate molecule in **RP**-HPLC. However, since the Rekker constants *(f)* can more accurately evaluate the **hydropho**bicity, we give preference to this parameter.

The majority of mobile phase parameters presented in Table V can be used to describe the chelate retention in terms of one-dimensional linear models. Therefore, we have omitted only two of them at the stage of the initial choice. Although viscosity should reflect intermolecular solute-eluent interactions, non-linear **dependen**ces for log k' values of metal **dithiophosphates** were observed (for more details see ref. 4). Solvent polarity parameter E_T is sensitive only to the proton-donating properties of the mobile phase and, consequently, is linearly correlated with log k' values only for water-alcoholic eluents.

Table VI shows the statistical criteria of **one**-dimensional regression equations calculated from experimental retention data. Molar volumes show a slightly lower correlation with log k' values than carbon numbers and hydrophobic constants, while the last two parameters possess identical predictive value owing to the high intercorrelation between them. Therefore, we limited the ligand descriptors to the number of carbon atoms in alkyl substituents. Of the parameters reflecting the ability of the mobile

TABLE VI

AVERAGE CORRELATION COEFFICIENTS AND STANDARD DEVIATIONS FOR ONE-DIMENSIONAL CORRELATIONS

Statistical criteria calculated and averaged from six sets of retention data consisting of 28 and 21 data points for structural and eluent parameters, respectively.

Parameter	R	S.D.	Parameter	R	S.D.
n_C	0.9859	0.071	c	0.9890	0.080
f	0.9859	0.071	log C	0.9874	0.096
V_n	0.9829	0.076	log P_S	0.9801	0.121
Z	0.9890 0.9820	0.119 0.080	$\gamma\varepsilon$	0.9880 0.9871	0.089 0.086
S	0.9724	0.142	α_{CH_2}	0.9868	0.099

phase to participate in intermolecular interactions of different types, π^* and Z will be considered below as those that are characterized by higher correlation factors. The list of eluent macroscopic parameters has been reduced to the volume concentration of the organic modifier and surface tension of the mobile phase based on the same principle.

Deriving and estimation of multiparametric retention models

Following the separation mechanism described above, we subdivided selected solute and eluent parameters into "solvophobic" and "hydrogen bonding" terms and then composed a number of multiparametric retention models given by the following equation:

$$\log k' = a, + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_4$$

In this equation, the parameters x_1 and x_2 characterize the hydrophobicity and hydrogen bond accepting ability of the chelates, respectively; x_3 and x_4 are the corresponding terms of the mobile phase (the eluent property complementary to solute hydrogen bond accepting ability is hydrogen bond donating ability). Independent variables of the retention models subjected to multiple linear regression analysis are collected in Table VII. For convenient comparison, the characteristics of statistical significance for derived correlation equations are assembled in Table VIII together with intercepts and coefficients of the independent variables (only one, the "best", equation for each retention model is included).

TABLE VII

VARIABLES OF MULTIPARAMETRIC CORRELATION MODELS

Eqn.	x_1	x_2	x_3	x_4	Eqn.	x_1	x_2	x_3	x_4
1a	log	K_D –	c	π^*	1c	log	K_D –	γ	π^*
1b	log	K_D –	c	Z	1d	log	K_D –	γ	Z
2a	log K_D	E_n	c	π^*	2c	log K_D	E_n	γ	π^*
2b	log K_D	E_n	c	Z	2d	log K_D	E_n	γ	Z
3a	X	E_n	c	π^*	3c	X	E_n	γ	π^*
3b	X	E_n	c	Z	3d	X	E_n	γ	Z
4a	X	log β_n	c	π^*	4c	X	log β_n	γ	π^*
4b	X	log β_n	c	Z	4d	X	log β_n	γ	Z
5a	n_C	E_n	c	π^*	5c	n_C	E_n	γ	π^*
5b	n_C	E_n	c	Z	5d	n_C	E_n	γ	Z
6a	n_C, Δ_M	E_n	c	π^*	6c	n_C, Δ_M	E_n	γ	π^*
6b	n_C, Δ_M	E_n	c	Z	6c	n_C, Δ_M	E_n	γ	Z
7a	Δ_M	log β_n	c	π^*	7c	Δ_M	log β_n	γ	π^*
7b	Δ_M	log β_n	c	Z	7d	Δ_M	log β_n	γ	Z

TABLE VIII

PARAMETERS OF MULTIPARAMETRIC CORRELATION MODELS DERIVED FROM EQN. 1

Eqn.	a_0	a_1	a_2	a_3	a_4	n	R	S.D.
1c	9.09	0.528		−0.665	21.94	12	0.8878	0.158
2d	-7.10	3.22	0.837	-0.075	0.121	12	0.9136	0.127
3c	-0.718	0.061	0.033	-0.339	17.45	43	0.8716	0.200
4d	-12.98	-0.038	0.020	-0.163	0.247	14	0.8533	0.272
5b	0.716	0.236	-0.040	-0.048	0.029	43	0.9760	0.111
6c	-4.08	0.237; 0.275	0.008	-0.199	15.91	43	0.9736	0.117
7c	7.80	3.31	-0.094	-0.752	30.60	14	0.9143	0.193

It is seen that retention models based on n-octanol-water distribution constants, which correspond to eqns. 1 and 2 of Table VII, as well as on stability constants (eqns. 4 and 7), are of less importance than might be expected, mainly because of a small number of retention data used to derive regressions (only log K_D and log β_n values for the chelates of one ligand were available for calculations). This emphasizes the limiting nature of experimentally determined molecular descriptors as variables in a regression equation. On the other hand, the lack of reliable distribution and stability constant data (owing to the complexity of their experimental determination) makes worthwhile the solution of the reverse problem, i.e. evaluation of these parameters from chromatographic data.

The statistical goodness of eqn. 1, including only one solute parameter, log K_D, increases if the hydrogen bonding parameter, E_n, is introduced, as in eqn. 2 (see Figs. 1 and 2), but this increase is not very large. We attributed this to the fact that the distribution constant partly accounts for the input of specific electrostatic interactions to the RP-HPLC retention.

If molecular connectivity indices are considered instead of log K_D values, eqn. 3 results. We must admit that on the grounds of our earlier correlations we had expected better fitting of χ values to the retention models. This is probably because of some uncertainties resulting from a comparatively short set of test chelates of different metal atom nature considered here (the predictive potency of this molecular parameter for metal chelates with different alkyl substituents is beyond doubt [3]). When eqns. 4 and 7 are compared, it is quite evident that the contribution of the metal atom to molecular size (or hydrophobicity) is less well expressed by χ than by A,. However, χ parameters are more easily determined irrespective of the molecular complexity.

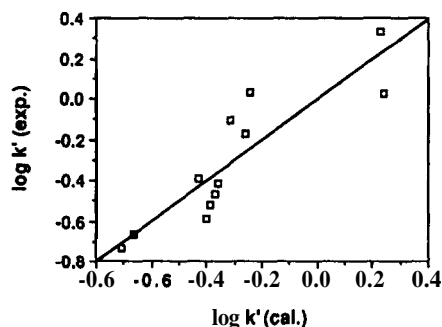The most meaningful models describing log k' are shown in regression eqns. 5 and 6. In these

Fig. 1. Relationship between the retention parameters of metal di-n-alkyldithiophosphates determined experimentally and calculated using eqn. Id.
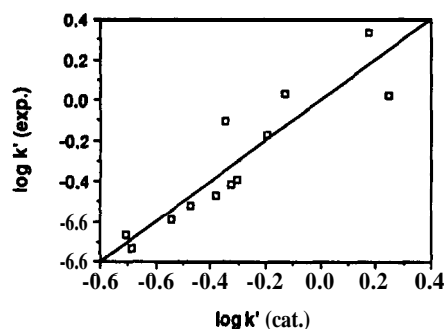

Fig. 2. Relationship between the retention parameters of metal di-n-alkyldithiophosphates determined experimentally and calculated using eqn. 2d.
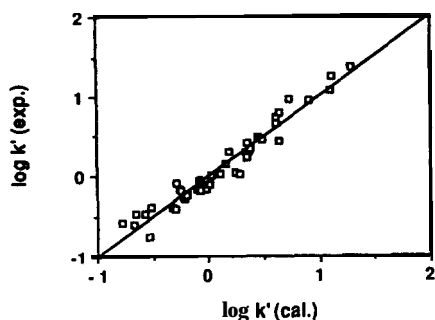
Fig. 3. Relationship between the retention parameters of metal di-n-alkyldithiophosphates determined experimentally and calculated using eqn. 5b.

equations we incorporated a combination of ligand and metal atom hydrophobic descriptors as a measure of the hydrophobicity of the whole molecule, thus yielding five-parametric models. Interestingly, the correlations obtained with eqn. 6 are not higher than those with eqn. 5, which includes only the ligand hydrophobic term. As a parameter of metal complex-forming ability [16], E_n reflects indirectly the ability of chelates to take part in hydrogen bonding interactions [1]. The predictive quality of both sets of retention models is illustrated in Figs. 3 and 4. It can be seen that fitted log k' values agree quite well with experimental data (the average difference between observed and predicted log k' values is 0.097 and 0.096 log units, respectively).

The quite good agreement between the four combinations of mobile phase parameters in Table VII indicates that we have achieved a
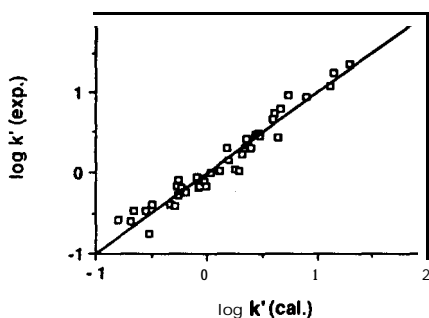

Fig. 4. Relationship between the retention parameters of metal di-n-alkyldithiophosphates determined experimentally and calculated using eqn. 6c.

rather precise algorithm for selection of the most valuable parameters. Note that the differences in correlation coefficients observed in eqns. 1, 2, 4 and 7 are not surprising in the light of the fact that only small subsets of log k' data could be used for these correlations. Thus, one can widely recommend volume concentration of the organic solvent, which provides predictions of log k' that are less dependent on organic modifier nature, as a measure of mobile phase solvophobic effect in relation to metal chelates. Both π^* and Z parameters are less universal since they are limited to mobile phases modified by aprotic solvents [4].

CONCLUSIONS

As demonstrated above, the multiparametric approach for analysing the dependence of chromatographic retention of metal chelates on structure and on the composition of the mobile phase provides an adequate description and rational interpretation of RP-HPLC data. Owing to sufficiently high statistical significance, correlation models derived here can be widely applied for forecasting the chromatographic behaviour, including prediction of retention data, and searching for optimal separation systems (including optimization of the chelating ligand). Evidently, further progress in coordination chemistry and accessibility of molecular modelling methods for metal chelates will result in more informative molecular descriptors, the shortage of which might hinder the practical application of retention models. Another promising approach in this direction consists in determinations of structural parameters from back-calculations of HPLC data. Also, taking into account the parameters of stationary phase materials is worthy of further investigations.

At the same time, meaningful retention models involving certain sets of structural and eluent parameters generally support the observed separation mechanism and confirm the dominant factors defining the intermolecular interactions of metal chelates of moderate polarity in RP-HPLC. Hence, the usefulness of multivariate correlation analysis in chromatography is

based on its ability to select the solute and chromatographic characteristics that significantly influence the retention and to analyse the retention mechanism. Thus, it can be considered an important prospective tool for investigating the chromatographic behaviour of metal complexes in other HPLC modes and related separation techniques (e.g., micellar electrokinetic chromatography or capillary zone electrophoresis) .

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