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Comparison of prediction power between theoretical and neural-network models in ion-interaction chromatography

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Abstract

The separation by ion-interaction chromatography (IIC) of metal complexes having single and double charges has been studied in order to compare the prediction power of soft (neural-network) and hard modelling (IIC equation). The two approaches have been used to model the retention behaviour as a function of the composition of the mobile phase. With ion-interaction mobile phases, the parameters involved included the concentrations of ion-interaction reagent, organic modifier and ionic strength. From a set of 69 experimental design points (the different mobile phase compositions at which capacity factors are measured), one test set of ten design points and ten training sets, containing from 59 to 11 design points, have been extracted. Chromatographic and chemometric considerations for the selection of the data sets and minimum number of observations required have been discussed. The study showed that the IIC equation predicted more accurately when few experimental data were available, while a similar prediction power was obtained with both models when the number of data was more than 17. Nevertheless the neural-network accounted for a greater versatility without the need to develop an equation. © 1998 Elsevier Science B.V.

Keywords: Neural network; Retention models; Ion-interaction; Retention prediction; Metal complexes

1. Introduction

Ion-interaction chromatography [1] is a widespread chromatographic method that allows the separation of neutral, polar and charged analytes. The general operative mode includes a reversed-phase column dynamically coated with a lipophilic ion, the ion-interaction reagent, added to the eluent. The eluent consists of a water–organic solvent mixture at a defined ionic strength. The adsorbed ion-interaction reagent imparts a charge to the stationary phase, causing it to behave as an ion-exchanger.

The interpretation of retention behaviour and the optimization of separation performed with such a technique shows some difficulties owing to the different parameters that significatively influence retention (concentrations of ion-interaction reagent, organic modifier and ionic strength).

The theories which are able to describe quantitatively analyte retention behaviour in ion-interaction chromatography are useful for obtaining numerical equations in order to clarify the mechanisms involved and for their modelling.

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In previous papers [2,3] we developed an IIC equation, which can account for the main variables considered by stoichiometric [4–16] and thermodynamic methods, including electrostatic theories [17–26], usually used in the literature on ion-interaction chromatography. It describes the simultaneous effect of the concentration of ion-interaction reagent, organic modifier and salt on k' for (-1) and (-2) charged analytes and predicts k' values for every mobile phase composition.

The above-mentioned approaches represent hard-modelling methods. As an alternative, soft modelling, such as neural networks, can be used in order to yield sufficiently accurate prediction results.

The interest in neural-networks has greatly increased in the last years. Artificial neural-networks are computational simulations of the biological neural-networks. Different types of neural-networks are developed to simulate different tasks of the human brain: classification, pattern recognition, modelling [27–29]. In analytical chemistry neural-networks have been applied to spectroscopy [30,31] and electrochemistry [32]. In the chromatography, neural-networks are used for peak tracking in HPLC optimization [33], response surface modelling of linear and nonlinear changing capacity factors in HPLC optimization [34], assessment of chromatographic peak purity in HPLC using photodiode-array detection [35]. Most frequently used in analytical applications are back-propagation neural-networks that are able to model the relation between data into their connection weights. In no case has the neural-network been used for modelling and prediction of data in ion-interaction chromatography.

Both hard- and soft-modelling methods are useful tools for the optimization of mobile phase composition and for the achievement of the best chromatographic conditions in the separation of a well defined mixture of analytes.

The aim of this work is to compare the prediction power of soft (neural-network) and hard modelling (IIC equation applied with a multivariable nonlinear regression method) when used in ion-interaction chromatography, with respect to the mobile phase parameters. The chromatographic system chosen involved the separation of (-1) and (-2) charged analytes (Cu^{2+} and Co^{2+} complexed with Acid Alizarin Violet N to form sulphonated metal complexes) using a octyl silica based column. The mobile phase contained tetrabutylammonium hydroxide (TBA) as ion-interaction reagent, methanol as organic modifier and sodium nitrate as ionic strength modifier. The performance of both models has been evaluated at different number of experimental data. The minimum number of observations required to predict k' values with the minimum error has also been evaluated.

2. Experimental

The chromatographic system used was a Varian LC 9010 liquid chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Rheodyne injection valve (100-μl sample loop inserted), a Kontron (Kontron Instruments, Milan, Italy) UV–Vis spectrophotometric detector and a Axxiom Chromatography 727 Data System (Axxiom Chromatography, Calabasas, CA, USA). The separation column was a LiChrosorb RP-8 (10 μm) (250×4 mm I.D.), coupled with a LiChroCART 100 RP-8 (5 μm) guard column (4×4 mm I.D.), both obtained from Merck (Darmstadt, Germany). An Orion digital pH meter (Orion, Cambridge, MA, USA) was used for pH measurements.

4-Hydroxy-3-(2-hydroxynaphthylazo)-benzenesulphonic acid (Acid Alizarin Violet N) was obtained from Aldrich and tetrabutylammonium hydroxide (TBA) was a Fluka product (Buchs, Switzerland). Acetic acid, sodium hydroxide, sodium nitrate, methanol and standard metal solutions (Cu^{2+}, Co^{2+} at 1000 mg/l) were Merck analytical-grade products.

Columns and tubings were cleaned daily with a methanol–water (80:20, v/v) solution for 30 min and with pure methanol for 10 min at a flow-rate of 1.0 ml/min. Eluents were prepared daily with high-purity water obtained from a Milli-Q System (Millipore, Bedford, MA, USA) and contained tetrabutylammonium hydroxide, sodium nitrate, acetate buffer (40 m*M*), methanol as required (see below) and 1.6 μ*M* Acid Alizarin Violet N, in order to prevent metal–chelates dissociation. Aqueous pH was adjusted to 5.5 by adding NaOH. Eluents were filtered through a 0.45-μm membrane filter (Millipore HAWP 04700) and degassed under vacuum before use. Columns were then conditioned with the

mobile phase solution. Standard metal solutions were obtained by diluting proper quantities of the metal stock solutions with 1.0 m*M* Acid Alizarin Violet N. pH was adjusted in order to match that of the eluent. The eluent flow-rate was 1.0 ml/min and the wavelength detection was chosen at 270 nm according to chelates and ligand absorption behaviour. Column dead volume, determined from the unretained peak of water, was 2.8 ml for the chromatographic conditions chosen.

2.1. Selection of design

The experimental design has been planned in order to describe the chromatographic behaviour in a multi-dimensional space: k' versus concentration of TBA, nitrate and methanol, studying a wide range of concentrations which cover the usual working conditions. The eluent concentrations of NO_3^-, (counterion), TBA and CH_3OH were varied in order to obtain regularly spaced design points on the three planes surfaces (TBA vs. NO_3^-, TBA vs. CH_3OH, NO_3^- vs. CH_3OH) and a few other points. In this way 76 retention data for Cu^{2+} and Co^{2+} complexes were obtained.

To select an optimum experimental design one is engaged in the construction of designs that give the best performance on a certain statistical criterion. One such criterion is the minimal variance of the estimated parameters

$$var(\hat{\beta}) = \sigma^2(X'X)^{-1} \quad (1)$$

where X is the matrix of parameter coefficients, or model matrix, and σ^2 is the measurement variance.

The D-criterion minimizes the volume of the hyperellips that gives a combined confidence interval of all parameters. This D-criterion is used most often. A D-optimal design is achieved when $|(X'X)|^{-1}$ is minimized.

The main difference between optimum experimental designs for linear and nonlinear models is their dependence on model parameter values. For linear models the estimation of optimal designs only depends on the type of model. For nonlinear models the optimum design depends on the true parameter value. The contradiction lies in the fact that the design usually is constructed to be able to estimate these parameters. To obtain optimum designs for nonlinear models a different strategy must be used.

The variance for estimated nonlinear model parameters is:

$$var(\hat{\beta}) = \sigma^2(F'F)^{-1} \quad (2)$$

where F is the Jacobian matrix, which must be evaluated for the estimated parameters $\hat{\beta}$ and σ^2 is the measurement variance. The Jacobian matrix is defined as: $F = \partial f(X,\Theta)/\partial\Theta$. F is an $n^* p$ matrix (n is the number of design points and vector Θ contains p parameters).

To obtain a D-optimal design, initial values of the parameters have to be available. The D-optimal design is used to perform experiments and on these experiments parameter values are estimated. If these values are significantly different from the initial parameters, the new values can be used in the search for a new optimal design.

Owing to the complexity of this procedure, the selection of different designs was based on the following approach. From the total of 76 points 7 points were removed as outliers of the design, leaving 69 points. These points lay outside the feasible region as indicated by Fig. 1 and were removed because it is to be expected that due to their great distance from the center of the design they will have disproportionally high leverage values.

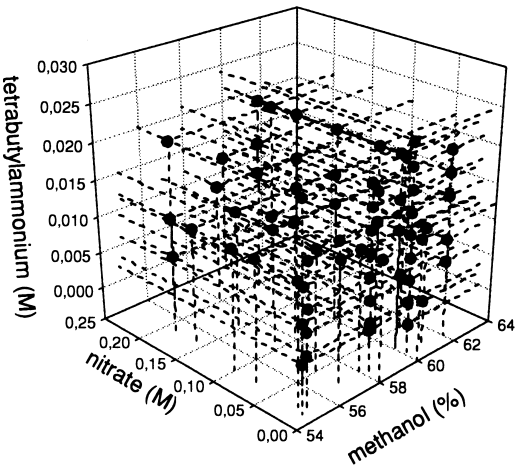

Fig. 1. Design of the 69 mobile phases studied as a function of tetrabutylammonium, nitrate and methanol concentrations.

The independent test-set (ten design points) and training-sets were selected to cover the whole design space in such a way that points have equal distance to each other and that the extreme vertices are occupied by design points. To test how far we can reduce the remaining data-set without losing on the prediction performance, 10 training-sets were created of 59, 51, 43, 35, 27, 19, 17, 15, 13 and 11 design points. The 69 retention data and the mobile phases selected for test and training sets are shown in Table 1.

2.2. Neural networks

The neural network used in this paper is of the backpropagation neural-network (BNN) type. The neural-networks are usually built as a feed-forward layered structure of neurons. The network usually consists of three layers: the input layer, the hidden layer and the output layer. In our work the first layer, the input layer, consists of three neurons representing the fraction of organic modifier, the counter-ion concentration and the concentration of ion-interac-

Table 1
Retention data of Cu- and Co-complexes obtained at 69 eluent compositions[a]

Methanol (%)	Nitrate (*M*)	TBA (*M*)	*k*′Cu	*k*′Co	Methanol (%)	Nitrate (*M*)	TBA (*M*)	*k*′Cu	*k*′Co
60.0	0.0000	0.012	0.87	6.08	58.0	0.0000	0.012	1.03	7.86
54.8	0.0120	0.012	1.70	26.10	58.0	0.0120	0.012	1.03	7.45
60.8	0.0120	0.012	0.80	4.75	55.0	0.0220	0.012	1.55	16.32
57.0	**0.0233**	**0.012**	**1.27**	**10.67**	58.0	0.0238	0.012	0.92	5.79
60.0	0.0250	0.012	0.74	4.50	58.0	0.0476	0.012	0.90	5.18
60.0	0.0500	0.012	0.73	3.31	57.0	0.0510	0.012	1.05	6.63
55.0	0.0722	0.012	1.25	9.70	55.0	0.1000	0.012	1.21	8.90
60.0	**0.1000**	**0.012**	**0.69**	**2.56**	58.0	0.1010	0. 012	0.77	3.32
57.0	**0.1020**	**0.012**	**0.91**	**4.89**	57.0	0.1490	0.012	0.86	3.91
55.0	0.1500	0.012	1.08	6.50	60.0	0.1500	0.012	0.65	2.20
55.0	**0.1778**	**0.012**	**0.99**	**5.23**	60.0	0.2000	0.012	0.63	1.50
58.0	**0.2024**	**0.012**	**0.71**	**2.38**	60.0	0.2500	0.012	0.65	1.57
60.0	0.0120	0.000	0.26	0.12	60.0	0.0120	0.003	0.54	1.36
60.0	0.0120	0.006	0.66	1.95	60.0	0.0120	0.009	0.79	3.48
60.0	0.0120	0.012	0.83	4.36	60.0	0.0120	0.015	1.06	6.28
60.0	**0.0120**	**0.018**	**1.06**	**7.29**	60.0	0.0120	0.021	1.19	9.31
60.0	0.0120	0.024	1.18	10.43	60.0	0.0250	0.016	0.88	5.32
60.0	0.0250	0.022	1.09	7.79	60.0	0.0500	0.016	0.77	3.96
60.0	0.0500	0.022	0.90	4.97	60.0	0.1000	0.016	0.73	3.03
60.0	0.1000	0.022	0.84	4.28	60.0	0.1500	0.016	0.68	2.27
60.0	0.1500	0.022	0.70	2.50	60.0	0.2000	0.016	0.65	2.02
60.0	**0.2000**	**0.022**	**0.71**	**2.70**	58.0	0.0120	0.003	0.66	2.51
58.0	0.0120	0.006	0.83	3.94	58.0	0.0120	0.009	0.91	5.74
58.0	0.0120	0.015	1.25	10.37	58.0	0.0120	0.018	1.32	11.89
58.0	0.0120	0.021	1.53	16.35	62.0	0.0120	0.009	0.58	1.95
62.0	**0.0120**	**0.006**	**0.48**	**1.33**	62.0	0.0120	0.018	0.84	4.66
62.0	0.0120	0.021	0.92	5.69	62.0	0.0120	0.015	0.69	3.30
55.0	0.0120	0.003	0.89	4.68	55.0	0.0120	0.006	0.99	5.64
55.0	0.0120	0.009	1.40	13.63	55.0	0.0120	0.015	1.71	20.23
56.0	**0.0420**	**0.004**	**0.74**	**2.75**	61.0	0.0420	0.004	0.42	0.84
56.0	0.0420	0.020	1.53	16.11	56.0	0.2080	0.020	1.03	5.50
61.0	0.2080	0.020	0.66	1.95	56.0	0.2080	0.004	0.68	1.70
61.0	**0.2080**	**0.004**	**0.37**	**0.81**	61.0	0.0420	0.020	0.81	4.10
58.0	0.0120	0.002	0.52	1. 39	54.8	0.0120	0.002	0.71	2.14
					60.8	0.0120	0.002	0.39	0.65

[a] Mobile phases included in the test-set but not in the training-sets are in bold type.

tion reagent. Information in a BNN is stored in weights which are connections between neurons in successive layers. The activation, Net_j of neuron j is defined as the sum of the weighted input signals x_i to that neuron

$$Net_j = \sum_i w_{ji}x_i + bias_j \quad (3)$$

where w_{ji} is the weight connection of neuron j in the actual layer from neuron i in the previous layer and $bias_j$ is the bias of neuron j. This activation is transformed to the output of the neuron by means of the activation function which is symmetrical sigmoid for the hidden nodes

$$y_j = \frac{2}{1 + e^{-Net_j}} - 1 \quad (4)$$

It has been found, however, from preliminary experiments, that for the output nodes a linear transfer function gave a better performance:

$$y_j = Net_j \quad (5)$$

where y_j has the same meaning as in Eq. (4).

The number of input nodes equals the number of elements of the input vector x_p in our case the concentration of counter-ion, ion-interaction reagent and the fraction of organic modifier. The number of output nodes equals the number of elements of response vector y_p (i.e. the number of solutes in the chromatographic sample); the number of hidden nodes has to be optimized.

According to the back-propagation algorithm, x_p is propagated through the network to the output layer. The errors between the output response vector y_p and the expected response vector t_p, are used to correct the weights to decrease the output error [34].

2.3. Software

The artificial neural network was written in C based on an algorithm which adjusts the learning rate factor and the momentum factor during training [34]. The optimum number of hidden nodes was determined by the algorithm of Yasui [36], which eliminates redundant hidden nodes by lateral inhibition.

The multi-variable regression in IIC equation and graphic elaborations were performed using SIGMAPLOT software for Windows (Jandell Scientific). The multi-variable nonlinear regression analysis is based on the Marquardt–Levenberg algorithm and allows the determination of the IIC equation parameters by iterative calculations.

3. Results and discussion

3.1. Neural-networks

To test the prediction performance of the neural network, MSEP (mean square error of prediction) values were calculated according to the following procedure. Because the variance of the capacity factors is heteroscedastic, the capacity factors are logarithmically (ln) transformed before modelling in order to get a homogeneous variance in y. The input values are scaled between 1 and -1 (to bring the values of the input variables into the dynamic range of the sigmoid transfer function) and the responses are scaled between 0.6 and -0.6. The number of input nodes equals the number of components in the eluent and the number of output nodes equals the number of solutes in the chromatographic sample.

The performance of the network depends on the number of hidden nodes, the number of cycles to train the network and the random initial values of the weight vectors. The number of hidden nodes is optimized for each of the ten data-sets by eliminating redundant hidden nodes by lateral inhibition [36]. The number of hidden nodes varies between 4 and 5 depending on the structure of the data set. However, as this number of hidden nodes is only an estimation, the procedure for optimizing the number of cycles during training was also performed with a network containing one hidden node less or one hidden node more than the number found by lateral inhibition. The number of cycles of each of these networks was optimized. The network with the lowest mean test error (see below) was selected.

To obtain the optimal number of training cycles for every network and data-set, cross validation by the leave one out method (LOOM) has been used to control overfitting [34]. Fig. 2 shows a typical example. The mean test error resulting from LOOM is used to obtain the optimum number of cycles, which is 500 in this example. After 500 cycles overfitting occurs as can be seen from the increase of

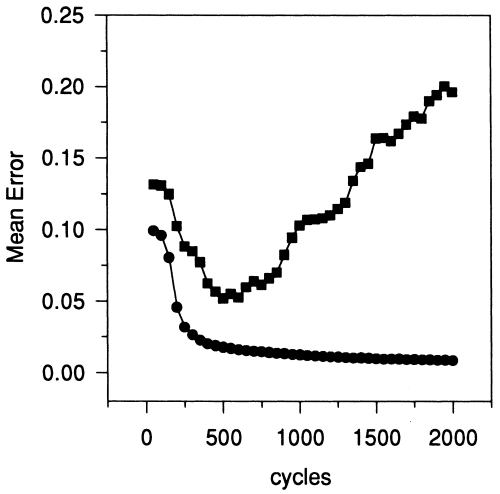

Fig. 2. Plot of the mean training error (●) and the mean test error (■). The mean training error is used to monitor the convergence of the network; the mean test error is used to obtain the optimum number of training cycles and prevent overfitting. For explanation see text.

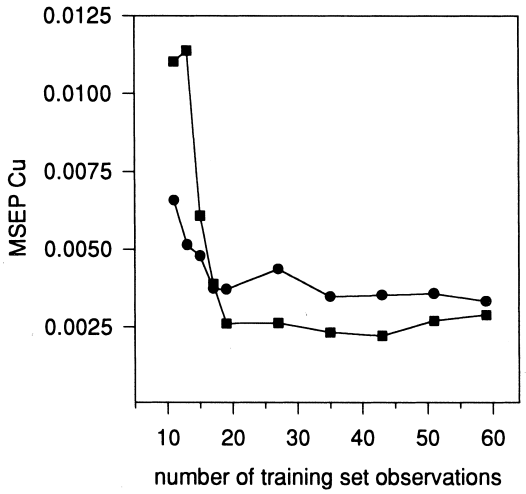

Fig. 3. Plot of MSEP of the test set (●=IIC equation and ■=neural-network) against the number of design points for the Cu-complex.

the mean test error while the training error is still decreasing. The optimum number of cycles varied from 400 to 1500 depending on the structure of the training set and the network. At the optimum number of cycles MSEP values were calculated from the predicted k' of the independent test set.

$$\mathrm{MSEP}_j = \frac{1}{tp}\sum_{n=1}^{tp}(y_{nj} - t_{nj})^2 \qquad (6)$$

In Eq. (6) y_{nj} represents the predicted k' and t_{nj} the measured k' of solute j of the nth observation of the test set with tp observations. The whole procedure for optimizing the number of hidden nodes and the number of training cycles was repeated three times for every data set starting the neural network at different random initial values of the weight vectors.

The MSEP values predicted by the three neural network configurations of the 10 training sets are plotted in Figs. 3 and 4 against the number of design points of the training sets. In the same figures the MSEP values, referring to the IIC equation, are shown (see discussion below).

Table 2 shows the relative standard deviations of the mean MSEP values for the Cu complex and the Co complex of the independent test set. The different initial values of the weight vectors and the different number of hidden nodes and number of training cycles obtained in the optimization of the network result nevertheless in a considerable standard deviation of the mean MSEP.

Additional experiments with networks with an optimized number of hidden nodes and cycles, but

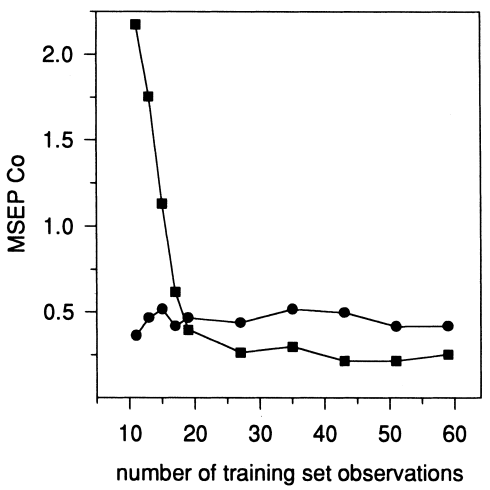

Fig. 4. Plot of MSEP of the test set (●=IIC equation and ■=neural-network) against the number of design points for the Co-complex.

Table 2
Standard deviations of MSEP with neural network started with three different initial values of the weights

Dataset (no. points)	Standard deviation (%)	
	Cu	Co
59	7.6	11
51	4.8	9.9
43	29	20
35	15	9.2
27	6.8	8.1
19	23	37
17	36	56
15	58	89
13	40	35
11	84	90

different initial weight vectors gave comparable results.

The relative prediction error can be calculated from the differences of the predicted and the measured k' values. The errors are given as percent values of the relative differences, according to the expression

$$\text{error}(\%) = \frac{|k'_{\text{meas}} - k'_{\text{calc}}|}{k'_{\text{meas}}} 100$$

The overall relative prediction error of the neural network for Cu-complex is about 5% for 59 to 17 design points and then increases to 10% at 11 design points. The prediction error for the Co-complex is about 10–12% for 59 to 15 design points and increases to 25% at 11 design points.

3.2. IIC equation

As previously discussed [2,3], ion pair reagent Q^+, added to the mobile phase, is adsorbed, as charged and neutral ion pairs (QX^- and Q_2X, respectively) with analyte X^{2-}, onto the stationary phase. The resulting equilibria and their constants are:

$$A_s + [Q^+] + [X^{2-}] \rightleftarrows (QX^-)$$

$$K_1 = \frac{(QX^-)}{A_s[Q^+][X^{2-}]} \quad (7)$$

$$A_s + 2[Q^+] + [X^{2-}] \rightleftarrows (Q_2X)$$

$$K_2 = \frac{(Q_2X)}{A_s[Q^+]^2[X^{2-}]} \quad (8)$$

where A_s is the number of free adsorption sites on the lipophilic stationary phase, according to Xianren and Baeyens [15], while the round and the square brackets refer to stationary and mobile phases, respectively.

In absence of the ion pair reagent, the anionic analyte can be adsorbed on to the stationary phase according to the following adsorption equilibria.

$$A_s + [X^{2-}] \rightleftarrows (X^{2-}), \qquad K_3 = \frac{(X^{2-})}{A_s[X^{2-}]} \quad (9)$$

Every adsorbed ion pair can exchange its anion with the other ones present in the mobile phase. For a general ion C^-, the involved equilibria and their constants are:

$$(QX^-) + [C^-] \rightleftarrows (QC) + [X^{2-}]$$

$$K_{c1} = \frac{(QC)[X^{2-}]}{(QX^-)[C^-]} \quad (10)$$

$$(Q_2X) + 2[C^-] \rightleftarrows (Q_2C_2) + [X^{2-}]$$

$$K_{c2} = \frac{(Q_2C_2)[X^{2-}]}{(Q_2X)[C^-]^2} \quad (11)$$

Introducing the adsorption capacity of column k_0, defined as the amount of adsorbed species and free sites still available [15], Eq. (12) can be derived:

$$k_0 = A_s + (Q_2X) + (QX^-) + (X^{2-}) + (QC) + (Q_2C_2) \quad (12)$$

Obtaining A_s, (QX^-), (X^{2-}), (QC) and (Q_2C_2) from the equilibria written above, it is possible to rewrite Eq. (12) as:

$$\frac{(Q_2X)}{[X^{2-}]} = \frac{k_0K_2[Q^+]^2}{1 + [X^{2-}](\text{ads}) + (\text{exch})} \quad (13)$$

The terms within brackets, (ads) and (exch), represent the contributions of adsorption and ion-exchange respectively. Their expressions are:

$$(\text{ads}) = K_2[Q^+]^2 + K_1[Q^+] + K_3$$

$$(\text{exch}) = K_1K_{c1}[Q^+][C^-] + K_2K_{c2}[Q^+][C^-]^2$$

The capacity factor k' for the solute ion X^{2-} can be defined by Eq. (14):

$$k' = \phi K_d = \phi \frac{(Q_2X) + (QX^-) + (X^{2-})}{[X^{2-}]} \quad (14)$$

(ϕ = phase ratio, K_d = distribution coefficient).

Substituting (QX^-) and (X^{2-}) obtained by the previous equilibria it is possible to write an equation as a function of $(Q_2X)/[X^{2-}]$. Introducing ratio $(Q_2X)/[X^{2-}]$ as expressed by Eq. (13) and the contribution of organic modifier, k' can be written as:

$$k'_{X^{2-}} = \frac{(\text{ads})}{1 + [X^{2-}](\text{ads}) + (\text{exch})} be^{c\varphi} \quad (15)$$

where b includes the product $k_0\phi$. The values of b and c ($c<0$) are constant for a given ion pair reagent–organic modifier combination and for each solute; φ is the fraction of the organic modifier.

The equation describes the retention behaviour of doubly charged anionic analytes in ion-interaction chromatography in the presence of an ion-interaction reagent and a counter ion in an organic–water mixture.

The retention behaviour for a singly charged analyte is also described by Eq. (15) (K_2 and K_{c2} = 0).

The IIC Eq. (15) has been tested in the logarithmic (ln) form by multi-variable nonlinear regression. The analyte concentrations used in the calculations were $7.86\cdot10^{-5}$ M for the Cu complex and $8.48\cdot10^{-5}$ M for the Co complex, which represent the actual amounts injected into the separator column during the experimental work. It has been shown [14] that this term is negligible for small amounts of sample injected, as in the present case.

On the basis of the IIC equation, from the experimental data, the adsorption constants, K_1, K_2, K_3, the ion-exchange constants, K_{c1}, K_{c2}, and the other parameters b and c were determined at each training set, for each analyte, by iterative calculations. Tables 3 and 4 show the values of the constants calculated for each training-set for Cu- and Co-complexes respectively. While the values of each parameters obtained for Co remain quite constant reducing the design points (Table 4), the same does not occur with parameters of Cu (Table 3). In fact, the reduction of the data set points from 27 to 19 causes a significant variation of the parameters, but the relative weight of the constants is not changed (e.g., among the adsorption constants K_1 is about 100-fold higher than K_3 both for data sets minor and major than 27 points).

The knowledge of the values of the parameters is of two-fold importance. It enables the evaluation of the weight of the equilibria involved in the whole retention mechanism [2,3] and it makes it possible to predict retention data at different elution conditions.

The values obtained were substituted in the IIC equation in order to predict the k' values of ten eluent compositions of test-set. This step is important

Table 3
Parameters of the IIC equation for Cu-complex, obtained by iterative calculation at different training sets

Dataset points	b	K_1	K_3	K_{c1}	c
59	$3.39\cdot10^4$	1.45	$1.21\cdot10^{-2}$	$1.35\cdot10^2$	−11.8
51	$2.58\cdot10^4$	2.15	$1.71\cdot10^{-2}$	88.6	−12.0
43	$2.21\cdot10^4$	1.88	$1.46\cdot10^{-2}$	95.6	−11.5
35	$3.41\cdot10^4$	1.16	$8.78\cdot10^{-3}$	$1.55\cdot10^2$	−11.4
27	$2.58\cdot10^4$	1.82	$1.37\cdot10^{-2}$	88.5	−11.7
19	0.207	$1.73\cdot10^5$	$1.00\cdot10^3$	$1.27\cdot10^{-3}$	−10.7
17	0.198	$2.04\cdot10^5$	$1.21\cdot10^3$	$1.11\cdot10^{-3}$	−10.8
15	0.123	$4.02\cdot10^5$	$1.93\cdot10^3$	$6.14\cdot10^{-4}$	−10.8
13	0.185	$2.11\cdot10^5$	$1.27\cdot10^3$	$8.93\cdot10^{-4}$	−10.8
11	0.130	$5.00\cdot10^5$	$1.95\cdot10^3$	$4.81\cdot10^{-4}$	−11.0

Table 4
Parameters of the IIC equation for Co complex, obtained by iterative calculation at different training sets

Dataset points	b	K_1	K_2	K_3	K_{c1}	K_{c2}	c
59	$1.16\cdot10^{4}$	$7.68\cdot10^{4}$	$3.74\cdot10^{6}$	29.8	$1.59\cdot10^{-2}$	$3.52\cdot10^{-10}$	−24.7
51	$8.52\cdot10^{3}$	$9.56\cdot10^{4}$	$5.52\cdot10^{6}$	37.5	$1.36\cdot10^{-2}$	$2.42\cdot10^{-10}$	−24.6
43	$6.73\cdot10^{3}$	$6.75\cdot10^{4}$	$3.58\cdot10^{6}$	28.0	$1.71\cdot10^{-2}$	$2.37\cdot10^{-10}$	−23.7
35	$4.92\cdot10^{3}$	$7.21\cdot10^{4}$	$3.39\cdot10^{6}$	28.0	$1.57\cdot10^{-2}$	$1.91\cdot10^{-10}$	−23.2
27	$9.10\cdot10^{3}$	$6.87\cdot10^{4}$	$3.69\cdot10^{6}$	28.0	$1.64\cdot10^{-2}$	$2.26\cdot10^{-10}$	−24.2
19	$5.69\cdot10^{3}$	$6.68\cdot10^{4}$	$7.90\cdot10^{6}$	102	$1.94\cdot10^{-2}$	$1.06\cdot10^{-10}$	−24.0
17	4280	$7.58\cdot10^{4}$	$1.06\cdot10^{7}$	131	$1.81\cdot10^{-2}$	$9.88\cdot10^{-11}$	−23.8
15	2090	$1.05\cdot10^{5}$	$1.90\cdot10^{7}$	216	$1.41\cdot10^{-2}$	$4.36\cdot10^{-11}$	−23.3
13	2750	$9.71\cdot10^{4}$	$2.03\cdot10^{7}$	162	$1.42\cdot10^{-2}$	$3.68\cdot10^{-11}$	−23.8
11	4380	$1.01\cdot10^{5}$	$1.96\cdot10^{7}$	134	$1.41\cdot10^{-2}$	$4.26\cdot10^{-11}$	−24.5

to test the reliability and the suitability of the equation developed. The MSEP values, calculated according to Eq. (6), are shown in Figs. 3 and 4 for the analytes considered.

The comparison of the MSEP for Cu and Co shows a difference of two orders of magnitude between (−1) and (−2) charged analytes, whatever the model used may be. This is due to the uncertainty of the experimental data, that is higher for (−2) charged analytes which are more sensitive to the variations of eluent composition. In turn, this involves higher standard deviations for the Co-complex (8%), due to the preparation of the eluent, as compared to the Cu-complex (3%).

When the IIC equation is used for the modelling, MSEP values remain constant at every number of observations included in the training sets and is independent of the analyte charge. Such behaviour persists also when the observations are close to the number of parameters required by the equation (7 for doubly charged analytes and 5 for singly charged analytes). The prediction error for Cu is about 5% for 59 to 17 design points and then increases to 9% at 11 design points. The prediction error for the Co-complex is about 10% for all the training sets studied.

When the modelling is performed by neural-network, the MSEP values first decrease for both analytes and then become constant above 17 observations. Above this number of observations the MSEP is lower for the neural-network, indicating its best suitability in respect to the IIC equation model.

The different behaviour of MSEP calculated with neural-network and IIC equation is dependent on the capabilities of soft and hard modelling. The IIC equation, in facts, account for the most relevant physicochemical factors, neglecting minor effects of other variables. Nevertheless, the IIC equation is a theoretical model which contains the phenomenological description of the system. The modelling, in this case, consists in the calculation of the parameter values contained in the equation. As a consequence, few observations are enough to obtain a good predictive capability. Neural-network, being a soft model, is able to accommodate all types of linear and nonlinear relationships by learning the relationships from the data themselves. Therefore, it can reach a better accuracy in the prediction of retention behaviour when a sufficient number of observations is available.

4. Conclusions

This study compares the applicability of neural-network modelling with a theoretical retention model developed for data prediction in ion-interaction chromatography as a function of mobile phase components (ion pair reagent, counter ion and organic modifier concentrations). It can be stated from the prediction error that the neural network predicts the capacity factors of the Cu-complex better than the capacity factors of the Co-complex. As could be seen from Fig. 5, the study showed a higher accuracy

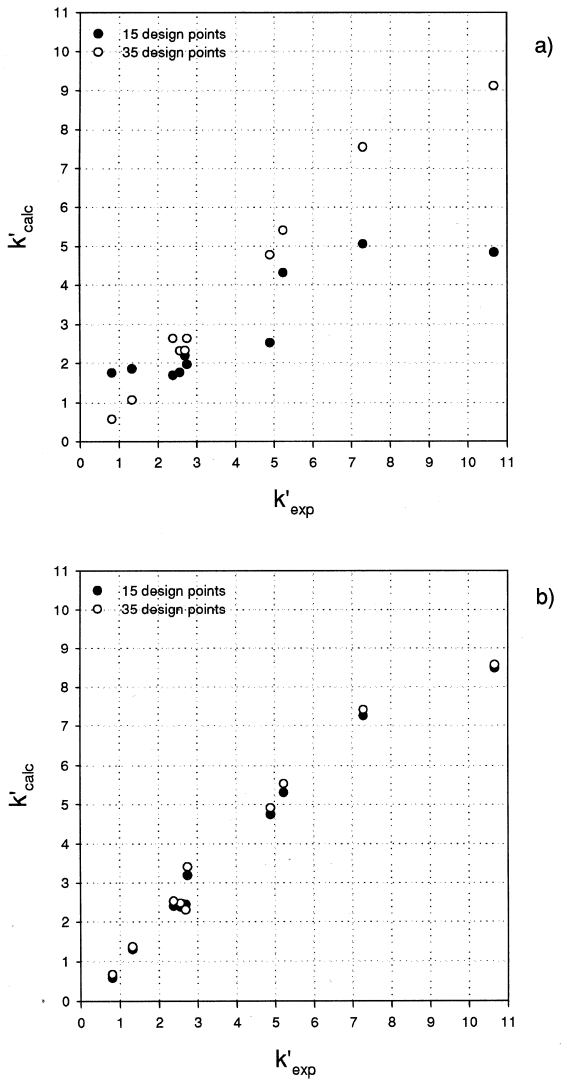

Fig. 5. Plot of predicted (k'_{calc}) versus experimental capacity factors (k'_{exp}) of the Co-complex at the test set design points obtained by neural network (a) and IIC equation (b) using 15 and 35 design points in the training sets.

of the IIC equation when few experimental data are available, while a similar prediction power is obtained with both models when the number of data is more than 17. Neural networks are specially useful when the relationships between retention behaviour and mobile phase parameters are unknown.

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