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Artificial neural networks for computer-aided modelling and optimisation in micellar electrokinetic chromatography

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Abstract

The separation process in capillary micellar electrochromatography (MEKC) can be modelled using artificial neural networks (ANNs) and optimisation of MEKC methods can be facilitated by combining ANNs with experimental design. ANNs have shown attractive possibilities for non-linear modelling of response surfaces in MEKC and it was demonstrated that by combining ANN modelling with experimental design, the number of experiments necessary to search and find optimal separation conditions can be reduced significantly. A new general approach for computer-aided optimisation in MEKC has been proposed which, because of its general validity, can also be applied in other separation techniques.

Keywords: Neural networks, artificial; Optimisation; Computer modelling; Experimental design; Metal complexes

1. Introduction

In micellar electrokinetic chromatography (MEKC), a surfactant is added to the mobile phase and the resultant micelles act as a pseudo stationary phase. The technique was developed by Terabe et al. [1]. MEKC is used for a great variety of analytes, particularly neutral compounds where the high efficiency of capillary electrophoresis (CE) separations can be applied. Recently, an extensive review on the separation of metal ions and metal-containing species by MEKC, including utilisation of metal ions in the separation of other species, has been published by Haddad et al. [2].

The explanation of migration behaviour in MEKC and the optimisation of separation is often difficult because there are many parameters that are known to influence the migration process and the mathematical description of these parameters can be quite complex. Various ''hard'' (i.e., physico-chemical) models for MEKC have been examined recently [3–10]. If an appropriate model is chosen from those available, computer optimisation of composition of the background electrolyte can be performed and/or the migration behaviour of analytes can be predicted. In spite of the demonstrated success of this approach, the process can be very laborious if many parameters are involved because numerous experimental points are then necessary to derive the parameters of the model.

Over the last decade, increased attention has been paid to the applications of ''soft'' models in chemistry. Soft models can be defined as approaches in which an explicit mathematical model is neither formulated nor used. The prime example of such an approach is the use of artificial neural networks

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(ANNs). ANNs can be applied to various problems in chemistry as reviewed recently [11,12] and to process different chemical information using association, classification, mapping, modelling, etc. In separation science, ANNs have already been used in CE [13,14] and for the optimisation of chiral separations [12,15]. A survey of the various approaches to optimisation of CE can be found in a recent review [16].

Büterhorn and Pyell [17] published a computer-aided optimisation of resolution in MEKC but neither a hard model nor an empirical model (equation) was used. ANNs have been applied for peak tracking in high-performance liquid chromatography (HPLC) optimisation [18], response surface modelling in HPLC optimisation [19], assessment of chromatographic peak purity [20], or for deconvolution of overlapping peaks [21]. Marengo et al. [22] studied the possibility of using ANNs to investigate the effect of five factors in ion-interaction chromatography and Sacchero et al. [23] have made a comparison of the prediction power between theoretical and neural-network models in ion-interaction chromatography. However, only in some publications [13–15] have attempts to achieve optimisation been performed, with ANNs normally being used only for modelling purposes. Recently, an extensive use of ANNs for modelling in ion chromatography has been presented [24]. The use of the combination of ANN and experimental design for optimisation has been first proposed in CE by Havel and co-workers [14,15,22]. Marengo et al. [22] also used ANNs and experimental design but only for decreasing the number of experiments for modelling purposes. Applications of experimental designs in CE have been recently reviewed by Altria et al. [25].

In this work the aims were to examine the modelling capabilities of the soft model-ANN approach in MEKC, with comparison to hard models, and the use of ANNs in combination with suitable experimental designs to facilitate the optimisation and/or prediction of electrophoretic mobilities in MEKC.

2. Theory

2.1. Theory of ANNs

While ''hard'' models in chemistry require formulae, mathematical equations and the knowledge or determination of the values of physico-chemical constants on which these equations are based, ''soft'' models (such as ANNs) consist only of arrays of simple activation units linked by weighted connections. The basic processing unit in an ANN is called a node or a simulated neuron [26]. A complete ANN is composed of multiple layers of neurons arranged so that each neuron in one layer is connected with each one in the next layer.

In this work, multilayered feedforward neural networks were used, which employed the algorithm of backpropagation (BP) of errors and the generalised ''delta rule'' [16,17] for the adjustment of the connection weights (further called BP networks). BP networks comprise one input layer, one (or possibly several) hidden layer(s) and an output layer (see Fig. 1). The number of nodes in the input and output layers are defined by the complexity of the problem being solved. The input layer receives the experimental information [e.g., pH, concentration of the background electrolyte (BGE) components, etc.] and the output layer contains the response sought, for example the migration times, resolution of a defined peak pair, overall resolution, etc. The hidden layer encodes the information obtained from the input layer, and delivers it to the output layer.

The number of nodes in the hidden layer may be considered as an adjustable parameter [16]. Each neuron thus has a series of weighted inputs, w_{ij}, which may be either output from other neurons or input from external sources. Each neuron calculates a sum of the weighted inputs and transforms it by a transfer function,

$$\delta_j = \frac{1}{1 + e^{\frac{(1-x)}{\gamma}}} \quad (1)$$

where δ is the output from the jth neuron connected to ith neuron in the previous layer and γ is the gain determining the slope of the sigmoid transfer function, and x is given by

$$x = \sum_{i=1}^{n} w_{ij} o_i + \theta_j \quad (2)$$

where w_{ij} represents the weight applied to the connection from ith to jth neuron, o_i is the output from the ith neuron in the previous layer and θ_j is a bias term.

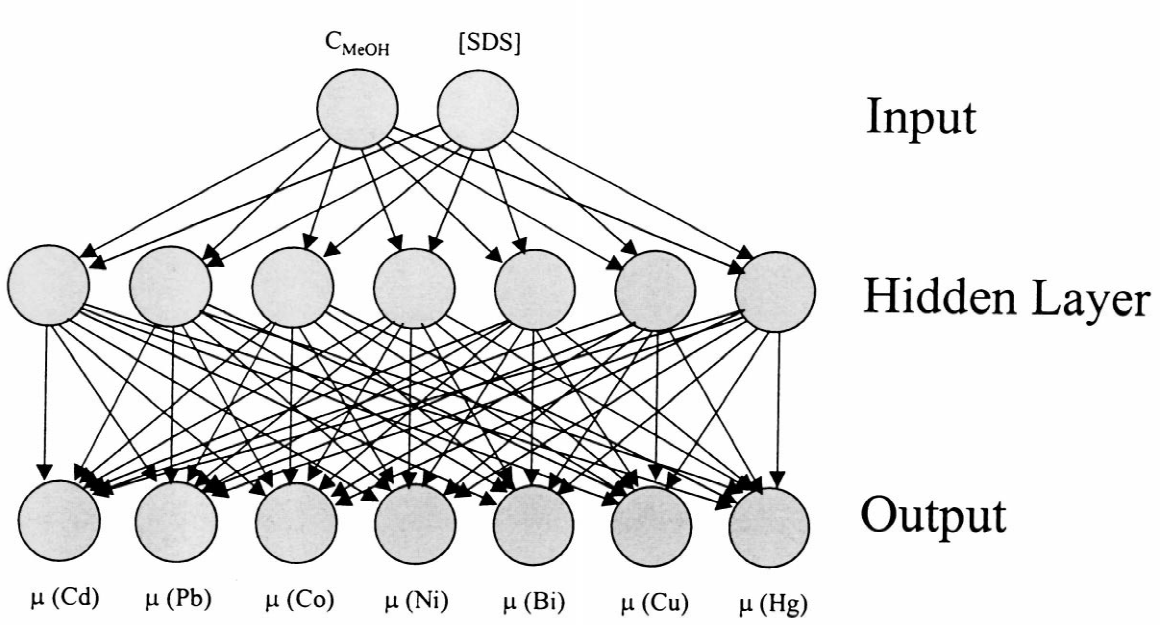

Fig. 1. ANN structure for the prediction of observed mobilities of seven metal–HEDTC complexes from two inputs (C_{MeOH} and [SDS]) and seven neurons in the hidden layer.

BP networks operate in a supervised learning mode. In the first step called the ''training phase'', known data are given to the network (a series of experiments coupling parameters and response values constituting the learning set). Using the BP algorithm connection weights w_{ij} are iteratively adjusted until the output values equal or are insignificantly different to the experimental values. This adjustment is carried out by minimising residuals $(t_{ij}-o_{ij})$, i.e., the difference between the calculated (target t_{ij}) and desired output (o_i) values and searching for the minimum of the total sum of squares (TSS) of the deviation $(t_{ij}-o_{ij})$ for the n patterns of the learning set (Eq. (3));

$$\mathrm{TSS}=\sum_{i=1}^{n}(t_{ij}-o_{ij})^2=\min \tag{3}$$

2.2. Experimental designs

Experiments for determination of the optimal conditions for separations should be made according to a suitable experimental design. These have been described elsewhere [26,27] and we will only summarise briefly here what will be applied in this work. The basic experimental designs are full factorial designs in which each factor is investigated at several levels, the most common being a two-level factorial design, characterised by the orthogonality of the factors. In this work we mainly used a two-level factorial design, which requires 2^n experiments, (where n is the number of factors being investigated) with the addition of a central point to cover the possibility of non-linearity.

3. Data description and computation

The PDP ANN computational package [17] was used in this work with processing being performed on a Pentium-PC compatible computer. BP networks having three layers were created with this program and optimisation of the parameters for the networks was then carried out by systematically varying the values of the parameters until the ''best'' network performance was achieved.

Different data sets were used to study the applications of ANNs to the prediction of the best experimental conditions for MEKC separations. Recently, Breadmore et al. [3] derived and validated a hard model to describe and predict the observed mobilities of anionic metal complexes, when two

electrolyte parameters (concentration of methanol and surfactant) were varied. Complexes formed using two ligands were examined, bis(2-hydroxyethyl)dithiocarbamate (HEDTC) and *trans*-1,2-diamino-*N*,*N*,*N′*,*N′*-cyclohexanetetraacetic acid (CDTA). These same data will be examined for use with ANNs.

For ANN modelling, the observed mobilities of several metals were considered, in addition to the resolution between two metal complexes and also the overall normalised resolution product [28] of the entire separation. The effects of adjustable parameters (scaling of the data, momentum, learning weight, number of hidden nodes and the number of learning cycles) were also studied. Momentum and adaptive learning rate were used in order to improve the performance, whereby the momentum prevents sudden changes in the direction by taking into account the preceding correction of the weights. However, this lowers the sensitivity of the neural network to small details in the error surface. The adaptive learning rate attempts to keep the learning step-size as large as possible, such that the process keeps converging. The use of an adaptive learning rate leads to a lower training time, but if it is set too high, the error in prediction soon starts to oscillate or increase. Further details of the process can be found in previous communications in this field [13,15,17,24].

4. Results and discussion

The use ANNs for modelling in MEKC will be examined for several previously studied cases and the results of the ANN modelling approach will be compared to those of hard models reported for each case by Breadmore et al. [3]. Training and general application of the ANN will be considered using the slightly anionic complexes of HEDTC, with the more significantly charged complexes of CDTA then being used to apply the ANN procedures.

4.1. ANN modelling of migration behaviour of selected metal complexes in MEKC

The first step is to search for the architecture of the ANN which enables sufficiently accurate modelling of the migration behaviour of the metal complexes in micellar solutions, that is, to model the response surface, $\mu_{ob} = f([SDS], C_{MeOH})$, where μ_{ob} is the observed mobility of the metal complex, [SDS] is the molar concentration of SDS and C_{MeOH} is the concentration of methanol (%, v/v) in the BGE. From the wide range of metal complexes for which data are available [3], we have quite arbitrary selected Ni(II)– and Cu(II)–HEDTC complexes. The data for these complexes consisted of pairs of input ([SDS] and C_{MeOH}) and one output value (the observed mobility), Table 1. Different ANN structures with various numbers of neurons in one hidden layer were constructed and after the training phase (using seven experimental points) the quality of the fit examined using the entire 50 data points collected for each metal. This process showed that a simple ANN with one hidden layer was sufficient. The lowest necessary number of hidden nodes was then determined systematically in order to avoid problems with ''overtraining'' [24].

The results of such a search for the two metal complexes of Ni(II) and Cu(II) are given in Table 2 which shows that for $n \geq 4$–5 the TSS value is

Table 1
Selected points from ANN application (2.7.7) to the observed mobilities of Ni(II)–HEDTC complex[a]

C_{MeOH} (%, v/v)	[SDS] (m*M*)	μ_{ob} (ANN) (10^{-9} $m^2/V s$)	μ_{ob} (Exp) (10^{-9} $m^2/V s$)	Error (%)
2	20	20.76	19.90	4.13
6	30	20.01	21.80	8.98
10	10	7.49	8.70	16.11
12	10	6.90	7.60	10.07
12	30	15.83	16.40	3.63
14	20	10.12	10.00	1.47
16	10	5.02	5.20	3.64

[a] Only seven were used for ANN training. Total number of points = 51. Average error = 5.67%, maximum error = 16.11%.

Table 2
Change in TSS for Cu(II)– and Ni(II)–HEDTC complexes with increasing number of hidden neurons in the hidden layer

No. of hidden neurons	TSS for Cu(II) (·10 000)	TSS for Ni(II) (·10 000)
1	38.63	29.80
2	38.62	29.40
3	5.40	5.21
4	3.30	5.08
5	3.36	4.82
6	3.00	5.10
7	3.41	3.72
8	3.33	3.30
12	1.80	3.18

almost constant. Surprisingly, the overtraining (increase of TSS, unconvergence or oscillations) was not observed up to $n=12$. It can be concluded that for the case studied, migration behaviour for a single metal complex can be modelled with sufficient accuracy (TSS = 5% rel.) using a (2,*n*,1) architecture, where $n=4$–12.

4.2. ANN modelling of the migration behaviour of several metal complexes in MEKC simultaneously

The results obtained above demonstrate the modelling power of ANN for a single metal complex and provided encouragement to examine the modelling of all metal complexes simultaneously. Investigation of several ANN architectures showed that excellent agreement could be obtained using a (2,7,7) neural network. Fig. 1 shows the architecture used, and Table 1 shows selected data and Fig. 2 shows the response surface for the Ni(II)–HEDTC complex over the defined area with the solid points representing the experimentally observed mobilities of the complex at these points. The surface was constructed via ANN modelling using a training set of seven points and then predicting intermediate points.

The quality of the fit between experimental and predicted mobilities obtained with ANN modelling can be visualised in Fig. 2 which shows acceptable scattering of the experimental points from the ANN constructed surface. A more quantitative assessment

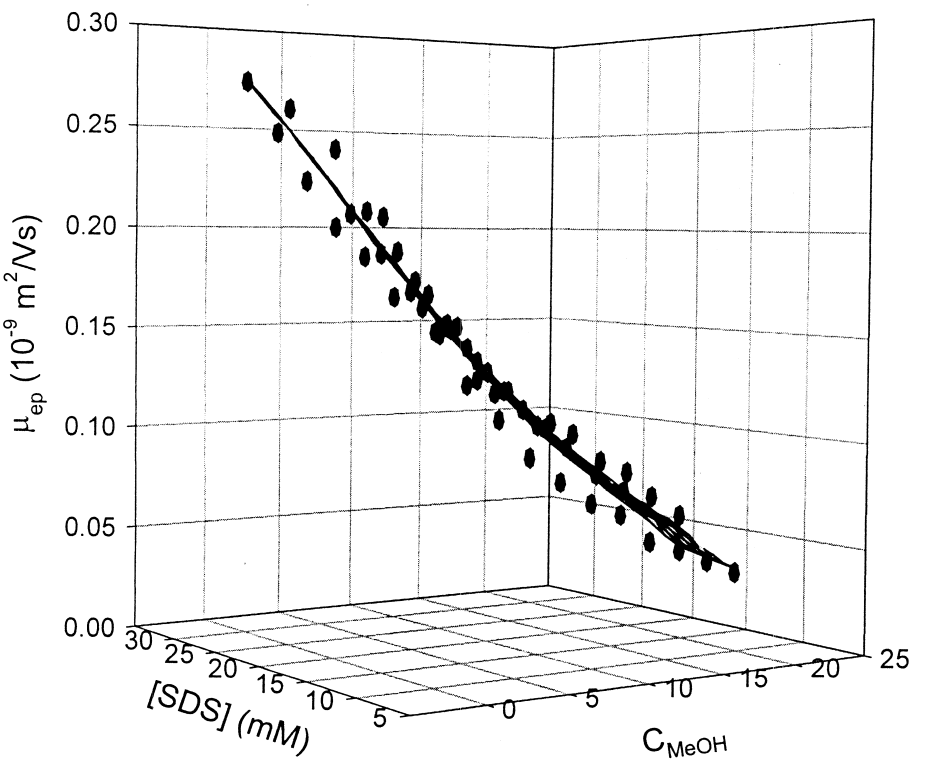

Fig. 2. Response surface for the observed mobility of the Ni(II)–HEDTC complex predicted from training of the ANN (2,7,1) using seven data points. Solid points are experimental values.

of the ability of the ANN to predict mobilities can be obtained by calculating the value of TSS. Moreover, the performance of the ANN can be compared to that of the hard model by again calculating TSS using the data obtained previously [3]. The values for TSS for all seven metal HEDTC complexes for all conditions were $89.6 \cdot 10^6$ for the (2,7,7) ANN and $65.2 \cdot 10^6$ for the hard model, which indicates a slightly better fit to the data using the hard model. However it is also important to recognise the versatility of the ANN approach. Using the hard model, specific information such as the mobility of the micelles at particular conditions, the value of the critical micelle concentration (CMC) and how the CMC changes with increasing concentrations of methanol were required before any predictions could be made. Furthermore, the knowledge that the complexes were very slightly charged and thus had their own electrophoretic mobility which would influence the separation was required. Using the ANN approach, none of this information was required before fitting the data. Further, the hard model can only be applied to one metal complex at a time since some of the parameters used in the model are specific to each complex and simultaneous optimisation requires significant computation time far exceeding that used in the ANN approach.

As ANNs can be successfully used to predict observed mobilities it is straightforward to use ANNs to predict mobilities and to therefore calculate resolution or some other parameter that can be used in optimisation strategies. A more interesting and demanding alternative is to examine the viability of using ANNs to directly model the desired parameter that is to be optimised.

4.3. ANN modelling of resolution behaviour of pairs of metal complexes and overall resolution in MEKC

Perhaps one of the simplest criterion for optimisation is the resolution between a given two peaks. Resolution between two peaks 1 and 2 is defined according to Eq. (4):

$$R_{1,2} = \frac{\mu_1 - \mu_2}{\frac{\mu_1 + \mu_2}{2}} \quad (4)$$

The observed mobilities and resolution between the components of two pairs of metal complexes [Bi(III) and Ni(II), and Pb(II) and Hg(II)] were modelled using an ANN design of (2,7,3). Excellent agreement with experimental data was obtained, which is a significant achievement considering the selectivity change that occurs between Ni(II) and Bi(III) over the BGE compositions studied (see Ref. [3] for a full discussion).

The ability to accurately predict electrophoretic mobilities and resolution between two metal–HEDTC complexes allows us to consider using an ANN in optimising the much more complex overall resolution of the separation for all seven metal complexes. The overall quality of the separation was judged by calculating the normalised resolution product, r [28]. Since values of r lie between 0 and 1, normalisation of the data was initially not performed. After training the ANN, it was found that for values of $r < 0.1$, agreement was very poor. Upon normalisation of the data, the TSS dropped by a factor of 10, giving a better overall fit for all of the data.

A similar process to that used to determine the required number of hidden neurons for modelling the observed mobility of the Ni(II) and Cu(II), was performed for the normalised resolution product. The best ANN structure was determined to be (2,12,1) but it should be noted, as before, no overtraining was observed even when more than 20 hidden nodes were used.

4.4. Prediction of resolution using experimental design and ANN modelling of overall resolution in MEKC

Successful modelling of migration behaviour and resolution makes it possible to use ANNs for method optimisation. This possibility was first examined using the HEDTC complexes and then applied to a second group of complexes, namely those with CDTA as the ligand.

In using ANNs to find the optimum conditions, two methods were considered. The first involved finding the optimum separation conditions within a two-level factorial design with a central point (making five experimental points) covering the entire experimental area, that is, between 10–30 m*M* SDS and 0–20% (v/v) methanol. Using the normalised

resolution product as the criterion for evaluating the separation, an ANN structure of (2,12,1) was applied to the five data points defined by the experimental design. Values of *r* were then predicted over the entire space in intervals of 1 m*M* SDS and 1% (v/v) methanol. Using this design, the predicted optimum separation conditions were at 8% (v/v) methanol and 10 m*M* SDS. Retraining of the ANN using the experimental data obtained under these conditions gave a new optimum of 0% (v/v) methanol and 10 m*M* SDS. After retraining with this point (making a total of seven experiments), the optimum was established at 8% (v/v) methanol and 10 m*M* SDS, which are the same optimal conditions identified in the hard modelling approach [3].

The second approach used a smaller two-level factorial design that did not contain the global optimum. It was found that the starting points selected and the points used for retraining the ANN produced significantly different conditions for the optimum conditions. The area was defined between 12–20% (v/v) methanol and 10–30 m*M* SDS. This area contains several local maxima for the response criterion (*r*), but not the global maximum determined above (8%, v/v, methanol and 10 m*M* SDS). The first attempt to find the optimum involved training the ANN with five points (points 1–5 in Fig. 3a), and gave point 6 as the prediction of the conditions of optimum separation. The experimental data at this point were then included in the data set and the ANN retrained. After repeating this process, a total of nine experimental points were required to produce final optimum conditions of 14% (v/v) methanol and 10 m*M* SDS, which is not the global optimum, but a local optimum. As this process did not identify the global optimum, other strategies for selecting points for retraining the ANN were evaluated.

Fig. 3a shows the second optimisation path. In this method, the ANN was retrained using points constructed in two-level factorial design around the predicted point of optimum separation conditions (the center point). Thus, starting with points 1–5, point 6 is predicted. Creating a two-level factorial design around this point resulted in point 7 being included (other points of the two-level factorial design are outside the search space). Retraining the ANN, predicted point 8, etc. Repeating this process until a consistent optimum was found resulted in a total of 11 experiments to establish the optimum at

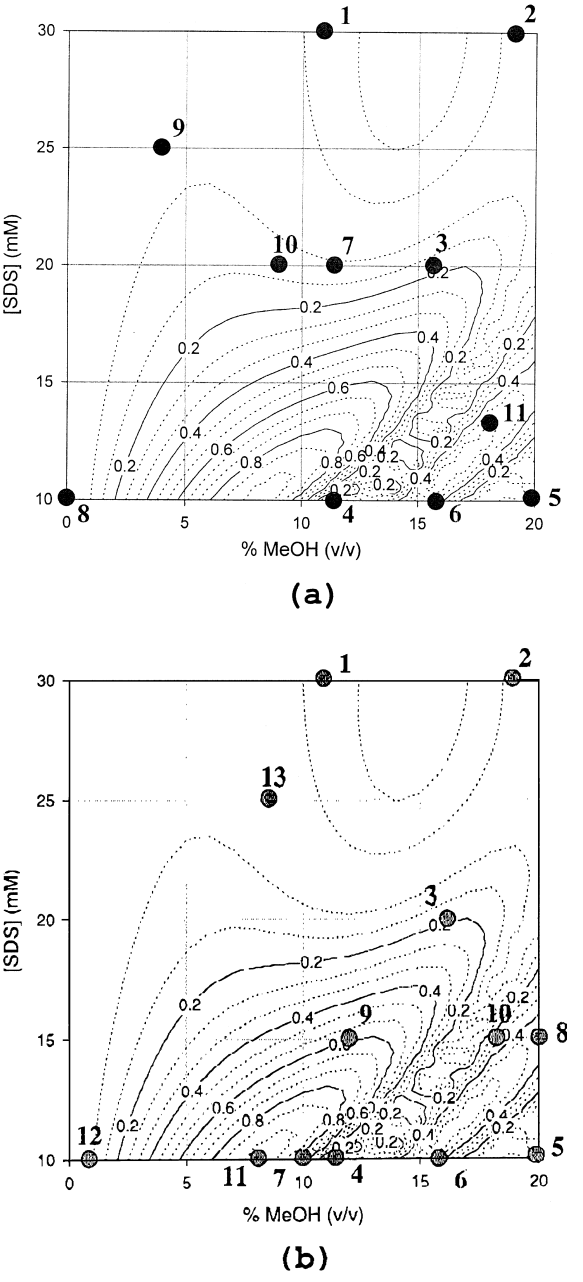

Fig. 3. Optimisation strategies employed to find the optimum conditions for the separation of seven metal–HEDTC complexes using an ANN (2,12,1) coupled with experimental design. (a) Using a two-level factorial design around the predicted point when retraining the ANN. The optimum predicted is at a C_{MeOH} of 18% (v/v) methanol and 13 m*M* SDS, with a total of 11 experiments. (b) Using a star design created around the predicted point when retraining the ANN. The optimum predicted is 8% (v/v) methanol and 10 m*M* SDS, with a total of 13 experiments required to reach the optimum.

18% (v/v) methanol and 13 m*M* SDS, which again was not the global optimum.

A further design, namely constructing a fractional star design around the point predicted as the best conditions for the separation, was then used and the global optimum was located within 13 experiments (Fig. 3b). This was by far the worst case encountered, and with all other designs tried, the use of the star design training around the predicted point was able to identify the global optimum using seven to nine experimental points.

It is important to recognise that in this case, the ANN structure that was used was (2,12,1) and that training with only five data points is not appropriate. However our focus was not in modelling the normalised resolution surface accurately, but in using ANN in conjunction with a suitable experimental design to optimise the separation by picking out general trends in the data and suggesting a ''better'' position to conduct the next experiment.

A procedure to optimise MEKC separations can be proposed based upon the above results: first, preliminary (screening) experiments are necessary to identify the region for the values of the factors. The experimental design is then selected and the required experiments undertaken. ANN modelling is used to predict interim values of the parameters under investigation, and experiment(s) are conducted under the predicted conditions and a new prediction is made. Experimentation is again undertaken at this point and the process repeated until no significant improvement is achieved. A further possibility is to construct a smaller experimental design around each predicted optimum in order to improve the ability of the ANN to locate the global optimum.

4.5. Application of ANN experimental design to metal–CDTA complexes

The proposed procedure was used to examine mobility data for a series of CDTA complexes of eight metal ions [3]. Preliminary screening experiments limited the experimental space to between 0 and 20% (v/v) methanol and 10–50 m*M* SDS. A two-level factorial design with a center point (requiring five experimental points), and an ANN structure of (2,7,8), gave an average error of 15%. Changing the design to a two-fold inclusive two-level factorial design (making a total of nine experimental points), reduced the average error to $<4\%$, which is within the error of reproducibility of CE.

In order to find the optimum separation conditions over the experimental space, several small two-level factorial designs (size 10 m*M* by 8%, v/v, methanol) of five points were used as starting points. ANN training with a structure of (2,12,1) was used, and star designs created around the predicted point, followed by retraining of the ANN was used. Optimum separations conditions of 2% (v/v) methanol and 50 m*M* SDS were typically obtained with eight experimental points, but as high as 11 were required for several starting point combinations.

5. Conclusions

The migration behaviour and the overall resolution of analytes in MEKC can be modelled with ANNs. Migration behaviour of individual metal complexes could be modelled accurately using an ANN with three or more hidden neurons, but modelling of seven metal complexes simultaneously required seven hidden neurons. A new optimisation procedure based on a combination of experimental design and ANN was proposed and for a MEKC separation of seven metal–HEDTC complexes and eight metal–CDTA complexes it was demonstrated that the combined use of a proper experimental design and ANN modelling could successfully reduce the number of necessary experiments.

The proposed ANN experimental design approach opens new possibilities for optimising not only MEKC but also some other separation methods. It exhibits the general advantage of ''soft model'' modelling, i.e., that there is no necessity to formulate an explicit mathematical model, without losing any precision.

The proposed strategy is under further development in both laboratories.

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