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Short communication

Prediction of retention times for anions in linear gradient elution ion chromatography with hydroxide eluents using artificial neural networks

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Abstract

The feasibility of using an artificial neural network (ANN) to predict the retention times of anions when eluted from a Dionex AS11 column with linear hydroxide gradients of varying slope was investigated. The purpose of this study was to determine whether an ANN could be used as the basis of a computer-assisted optimisation method for the selection of optimal gradient conditions for anion separations. Using an ANN with a (1, 10, 19) architecture and a training set comprising retention data obtained with three gradient slopes (1.67, 2.50 and 4.00 mM/min) between starting and finishing conditions of 0.5 and 40.0 mM hydroxide, respectively, retention times for 19 analyte anions were predicted for four different gradient slopes. Predicted and experimental retention times for 133 data points agreed to within 0.08 min and percentage normalised differences between the predicted and experimental data averaged 0.29% with a standard deviation of 0.29%. ANNs appear to be a rapid and accurate method for predicting retention times in ion chromatography using linear hydroxide gradients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Gradient elution; Neural networks, artificial; Mobile phase composition; Retention prediction; Inorganic anions; Organic acids

1. Introduction

Ion chromatography (IC) is a widespread separation technique used predominantly for the separation of low molecular mass ionic species. Separations can be performed either by isocratic elution or by gradient elution [1]. The advantage of gradient elution is its ability to separate both weakly retained and strongly retained analytes in the same run. With

isocratic elution a compromise must be made between resolution of the weakly retained analytes and the total length of the experiment.

Traditionally the use of gradient elution in IC has been minimal due to the fact that conductivity detection is normally used and this detection mode is sensitive to changes in eluent composition. In non-suppressed IC the use of a so-called isoconductive gradient [2] in which the conductances of the starting and finishing eluents used to create the gradient are equal partially overcomes this difficulty, but the variation in eluotropic strength for isoconductive

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gradients is quite limited. In suppressed IC, the use of gradient elution relies on the availability of suppressors with sufficient capacity to ensure that the background conductance of the suppressed eluent remains essentially constant over the course of the gradient. Hydroxide eluents are most suitable for gradient elution in suppressed IC because hydroxide is a weak ion-exchange displacing ion and therefore can be used to separate weakly retained analytes, but at high concentration can also be used to elute strongly retained analytes. Moreover, hydroxide is readily suppressed to water, giving a low background conductance and hence high detection sensitivity. When prepared by the dissolution of alkalis, hydroxide solutions invariably are contaminated by carbonate produced from absorption of atmospheric carbon dioxide. This carbonate interferes with hydroxide gradient elution by accumulating at the head of the separation column and then being eluted progressively later in the course of the gradient, thereby producing a sloping baseline. However in recent years the generation of hydroxide solutions which are free of carbonate has been made possible through the use of electrolysis devices, such as the Dionex Eluent Generator (EG40). Here, the gradient is generated not by the traditional method of mechanical mixing of solutions, but rather by varying the electrolysis current at fixed eluent flow. The more widespread use of hydroxide gradients has established the need for rapid methods to select the optimal gradient conditions for a desired separation.

Optimisation of the eluent conditions in IC can be carried out by a number of methods, with the two most commonly used approaches being Simplex and interpretive optimisation. Simplex optimisation involves running an initial set of experiments and then using a suitable algorithm to define subsequent experimental parameters which seek to produce an improved output (in this case, a better separation). Experiments continue until an optimal condition is reached. This strategy can frequently require a very large number of experiments to identify the optimal conditions. On the other hand, interpretive optimisations require only a minimal number of initial experiments to be conducted, after which the retention data are fitted to an appropriate model that describes the relationship between the experimental parameters and retention factor. This then allows

retention factors to be predicted for any of the experimental conditions within the defined search area. The reliability of the predicted retention factors varies with the complexity of the retention model used and in general, more complex retention models will give more reliable results, but will also increase the number of initial experiments required.

The first step in the development of an interpretive optimisation method is normally the selection of a suitable retention model. A large number of retention models exist for isocratic elution in IC [3–12] and these models have been analysed for their suitability in an interpretive optimisation process [13–16]. However, for gradient elution IC, the retention models that have been published are relatively complex [17,18] and are therefore unlikely to be easily implemented in interpretive optimisation. An alternative approach is the use of an artificial neural network (ANN) to predict the effects on retention caused by different gradient conditions. ANNs have already proved successful for predicting retention in isocratic elution IC and in fact have been shown to give superior performance to that exhibited by mathematical retention models of the type discussed earlier [16]. In this paper we examine the feasibility of using an ANN to predict retention times in gradient elution IC performed on a particular column (Dionex AS11) with the aim to develop a new “soft model” approach for computer aided interpretive optimisation of separations on this column.

2. Theory

ANNs consist of an array of simple activation units linked by weighted connections, see Fig. 1. The basic processing unit in an ANN is called a node, which is a simulated neuron. These nodes can form multiple layers arranged so that each node in one layer is connected with each node in the next layer, and so on. The entire group of layered nodes constitutes the complete ANN.

In this work we used a multi-layer perceptron feed-forward neural network using the Delta-bar-Delta variation of the back propagation of errors for the adjustment of the connection weights as the training scheme. A multi-layer perceptron network is composed of a collection of strongly interconnected

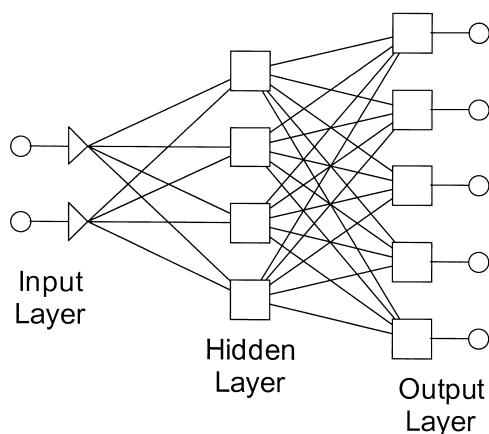


Fig. 1. Schematic representation of a typical ANN consisting of two input nodes, four hidden nodes (in a single layer) and five output nodes (2,4,5).

elementary units called nodes, which constitute the fundamental processing elements of an ANN [19,20]. The global architecture of a multi-layer perceptron network is made up of nodes arranged into one input layer, one output layer and at least one hidden layer. Any number of nodes can be used in the input, hidden and output layers, with the complexity of the experiment under study being the main determinant of the number of nodes. Each unit in a multi-layer perceptron network performs a biased, weighted sum of their inputs and passes this activation level through a transfer function to produce their output. Each unit in a particular layer is connected to every output of the previous layer and every input of the next layer, thus forming a feed-forward network. The input layer of the network receives input from the original data, while the output layer outputs its data as the results.

The benefits of an ANN over a physical retention model include its ability to accurately describe a response surface on the basis of a minimal set of data required for training and the fact that ANN modelling can be performed simultaneously over a range of analytes, eluents and stationary phases, whereas physical models normally require specific input parameters for each analyte, eluent and stationary phase. The disadvantages of ANNs are that no information on physico-chemical aspects (such as ion-exchange selectivity coefficients, adsorption coefficients, etc.) of the system under study are pro-

vided, and the more complex the response surface, the more data points are typically required for the training phase.

3. Experimental

3.1. Instrumentation

The chromatographic instrumentation consisted of a Dionex DX-500 ion chromatograph (Dionex Co., Sunnyvale, CA, USA), consisting of an IonPac AS11 anion separator column (250×4 mm ID), an IonPac ATC-1 Anion Trap Column and a ASRS-II self regenerating suppressor housed in an LC30 chromatography oven at 30°C, a model EG40 Eluent Generator, a model AS40 AutoSampler, a model ED40 electrochemical detector operated in the conductivity mode and a model GP40 gradient pump. The injection loop was 25 μ l. All samples were analysed in duplicate with a flow-rate of 1.0 ml/min.

3.2. Reagents and procedures

Eluents were prepared using purified water from a Milli-Q system (Millipore, Bedford, MA, USA) containing a 0.45- μ m filter at the outlet. Sample solutions of fluoride, acetate, formate, bromate, chloride, nitrite, methanesulfonate, bromide, chlorate, nitrate, iodide, thiocyanate, succinate, sulfate, tartrate, oxalate, tungstate, phthalate, chromate, thiosulfate and phosphate were prepared by dissolution of analytical grade sodium salts in deionised water. Formate, nitrate, chromate, chloride, bromate, phthalate and nitrite salts were obtained from Ajax (Auburn, NSW, Australia), iodide, chlorate, phosphate, thiosulfate and tungstate salts from BDH Chemicals (Kilsyth, Vic., Australia), tartrate and oxalate salts from Mallinckrodt (Paris, KY, USA), fluoride and sulfate salts from Prolabo (Paris, France), bromide and methanesulfonate salts from Sigma (St. Louis, MO, USA) and thiocyanate and succinate salts from Aldrich (Milwaukee, WI, USA). The concentrations of the anions in the standard solutions varied from 0.5 mg/l to 10 mg/l. The retention time for each analyte was determined in duplicate using seven linear eluent gradients with the initial concentration of hydroxide set at 0.5 mM and

the final concentration at the end of the gradient being 40.0 mM, with the rate of change of concentration being 4.00, 3.33, 2.86, 2.50, 2.22, 2.00 and 1.67 mM/min, see Fig. 2. When duplicate measurements of retention time were found to be inconsistent (to three significant figures), then further measurements were taken to ensure repeatability. The dead time of the column was determined by injecting a sample of de-ionised water into the column and measuring the retention time of the negative water peak.

Statistical analysis of the performance of the ANN was carried out using retention data acquired directly from the system described above. All calculations were performed using Microsoft Excel 97 on a Pentium II 400 computer with 64 MB of SDRAM, running Windows NT workstation v4.0 sp5. The ANN was simulated using the Trajan Neural Network Simulator release 3.0D software package [21], operated on the same computer.

3.3. Training of the ANN

A minimum of two data points for each analyte anion was required to train the ANN. In the simplest configuration, the retention data obtained for the 1.67 and 4.00 mM/min gradients could be used, giving a two level two factor factorial design. However, a more statistically robust central two level two factor factorial design was used in which a further data point was added, namely the data from the 2.50 mM/min gradient.

An ANN with a (1,10,19) architecture was used; comprising one input node (gradient slope), 10

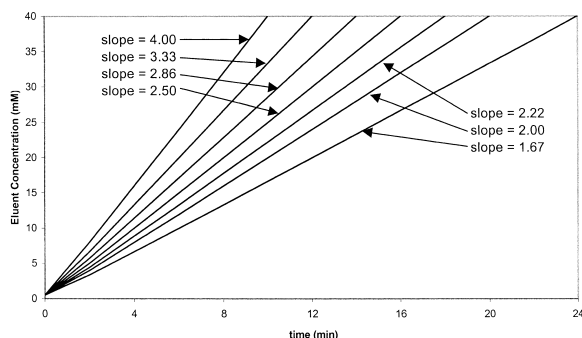


Fig. 2. Profiles of the seven hydroxide gradients used in this study.

hidden nodes and 19 output nodes (retention times for the 19 analytes). The ANN was trained using the Delta Bar Delta algorithm [21–23]. The following values for the training parameters were found to be optimal; initial learning rate, 0.1; learning rate increment, 0.01; learning rate decay, 0.8; smoothing, 0.5 and noise, 0. Training was conducted until the RMS of the training error fell below 0.0001. Pre/post processing of the data was set up to normalise the data between a maximum value of 0.6 and a minimum value of 0.4 in order to maximise the linearity of the transfer function.

4. Results and discussion

The retention data obtained for the range of eluent concentrations and analytes studied are shown in Table 1. The eluent gradients of 1.67, 2.50 and 4.00 mM/min were used as the training set and the trained ANN was then used to predict retention times for all seven eluent gradients. This gave a total of $7 \times 19 = 133$ predicted retention times for the ANN. Table 2 shows the calculated retention times for the 19 analytes. Fig. 3 shows the predicted and experimental retention times for chloride, and it can be seen that agreement between these two parameters was very good.

From these data the percentage-normalised difference ($\%d_i$) between the measured and predicted retention times for a particular ion (i) were calculated using the following equation:

$$\%d_i = \frac{t_{R_{\text{exp}}} - t_{R_{\text{pred}}}}{t_{R_{\text{exp}}}} \cdot 100 \quad (1)$$

where $t_{R_{\text{exp}}}$ is the experimentally determined retention time and $t_{R_{\text{pred}}}$ is the predicted retention time. Table 3 shows the percentage-normalised difference values for all 19 analytes. If the values of the percentage-normalised differences for eluent gradients used for training (i.e. 1.67, 2.50 and 4.00 mM/min) are excluded, the average of the remaining values in Table 3 is 0.29 with a standard deviation of 0.29. The highest percentage-normalised difference was 0.88% for phosphate for the 3.33 mM/min gradient, which corresponds to a difference of 0.07 min between the calculated and experimentally de-

Table 1

Retention times (min) for 19 analytes on a Dionex AS11 IonPak column with an eluent flow-rate of 1.00 ml/min using a Dionex EG40 eluent generator to create hydroxide gradients of varying slopes. The void time was 1.42 min

| Slope (mM/min) | 4.00 | 3.33 | 2.86 | 2.50 | 2.22 | 2.00 | 1.67 |
|--|------|------|------|------|-------|-------|-------|
| Fluoride | 2.24 | 2.28 | 2.32 | 2.36 | 2.40 | 2.43 | 2.48 |
| Acetate | 2.30 | 2.35 | 2.40 | 2.44 | 2.48 | 2.52 | 2.58 |
| Formate | 2.43 | 2.52 | 2.57 | 2.62 | 2.67 | 2.72 | 2.80 |
| Methansulfonate | 2.54 | 2.60 | 2.67 | 2.74 | 2.79 | 2.85 | 2.94 |
| Bromate | 3.03 | 3.15 | 3.26 | 3.36 | 3.45 | 3.54 | 3.69 |
| Chloride | 3.15 | 3.28 | 3.40 | 3.51 | 3.62 | 3.71 | 3.89 |
| Nitrite | 3.39 | 3.56 | 3.69 | 3.82 | 3.94 | 4.06 | 4.26 |
| Bromide | 4.27 | 4.49 | 4.71 | 4.91 | 5.09 | 5.26 | 5.58 |
| Nitrate | 4.37 | 4.61 | 4.83 | 5.04 | 5.24 | 5.41 | 5.76 |
| Chlorate | 4.47 | 4.72 | 4.95 | 5.17 | 5.37 | 5.55 | 5.89 |
| System peak (CO ₃ ²⁻) | 4.95 | 5.37 | 5.76 | 6.14 | 6.5 | 6.85 | 7.49 |
| Iodide | 8.17 | 8.79 | 9.37 | 9.86 | 10.33 | 10.78 | 11.60 |
| Succinate | 4.72 | 5.11 | 5.48 | 5.83 | 6.17 | 6.49 | 7.09 |
| Tartrate | 4.94 | 5.36 | 5.77 | 6.16 | 6.51 | 6.86 | 7.43 |
| Sulfate | 5.37 | 5.85 | 6.31 | 6.75 | 7.16 | 7.56 | 8.31 |
| Oxalate | 5.67 | 6.19 | 6.68 | 7.16 | 7.60 | 8.03 | 8.85 |
| Tungstate | 6.56 | 7.20 | 7.80 | 8.37 | 8.92 | 9.45 | 10.44 |
| Phthalate | 7.18 | 7.89 | 8.58 | 9.23 | 9.85 | 10.44 | 11.59 |
| Phosphate | 7.36 | 8.19 | 8.96 | 9.69 | 10.42 | 11.10 | 12.44 |

rived retention times. The greatest absolute difference in retention times was 0.08 min, again for phosphate, but for the 2.00 mM/min gradient.

Experimental error for the acquired data would be

expected to be of the order of 0.5%, primarily due to the errors in the flow-rate. Scrutiny of the errors listed in Table 3 shows that nearly all of the percentage-normalised differences are in fact within

Table 2

Retention times (min) for 19 analytes predicted using an ANN with a (1,10,19) architecture after training with a Delta Bar Delta algorithm using data in a central composite design

| Slope (mM/min) | 4.00 | 3.33 | 2.86 | 2.50 | 2.22 | 2.00 | 1.67 |
|--|------|------|------|------|-------|-------|-------|
| Fluoride | 2.24 | 2.28 | 2.32 | 2.36 | 2.40 | 2.43 | 2.48 |
| Acetate | 2.30 | 2.35 | 2.40 | 2.44 | 2.48 | 2.52 | 2.58 |
| Formate | 2.43 | 2.50 | 2.56 | 2.62 | 2.67 | 2.72 | 2.80 |
| Methansulfonate | 2.54 | 2.61 | 2.68 | 2.74 | 2.80 | 2.85 | 2.94 |
| Bromate | 3.03 | 3.15 | 3.26 | 3.36 | 3.46 | 3.54 | 3.69 |
| Chloride | 3.15 | 3.27 | 3.39 | 3.51 | 3.62 | 3.72 | 3.89 |
| Nitrite | 3.39 | 3.54 | 3.68 | 3.82 | 3.95 | 4.06 | 4.26 |
| Bromide | 4.27 | 4.49 | 4.70 | 4.91 | 5.11 | 5.28 | 5.58 |
| Nitrate | 4.37 | 4.60 | 4.82 | 5.04 | 5.25 | 5.44 | 5.76 |
| Chlorate | 4.47 | 4.71 | 4.95 | 5.17 | 5.38 | 5.57 | 5.89 |
| System peak (CO ₃ ²⁻) | 4.95 | 5.34 | 5.74 | 6.14 | 6.53 | 6.88 | 7.50 |
| Iodide | 8.17 | 8.76 | 9.32 | 9.86 | 10.37 | 10.82 | 11.60 |
| Succinate | 4.72 | 5.08 | 5.45 | 5.83 | 6.19 | 6.52 | 7.09 |
| Tartrate | 4.94 | 5.36 | 5.77 | 6.16 | 6.53 | 6.86 | 7.43 |
| Sulfate | 5.37 | 5.83 | 6.29 | 6.75 | 7.20 | 7.60 | 8.31 |
| Oxalate | 5.67 | 6.16 | 6.66 | 7.16 | 7.64 | 8.08 | 8.85 |
| Tungstate | 6.56 | 7.16 | 7.76 | 8.37 | 8.96 | 9.50 | 10.44 |
| Phthalate | 7.18 | 7.85 | 8.53 | 9.23 | 9.90 | 10.52 | 11.59 |
| Phosphate | 7.36 | 8.12 | 8.90 | 9.69 | 10.47 | 11.18 | 12.44 |

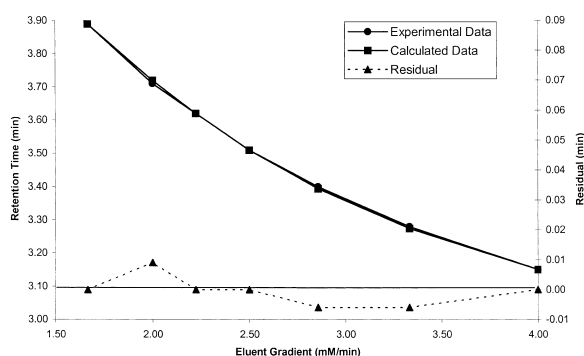


Fig. 3. Experimental and predicted retention times for chloride obtained at each gradient slope. Residuals of the experimental and ANN data for chloride ($t_{R_{exp}} - t_{R_{calc}}$).

the experimental error margin. It can also be seen that all the percentage-normalised differences are within 1% of their corresponding experimental values.

5. Conclusion

This study has shown that accurate prediction of

retention times for anionic analytes when eluted from an IC system using a hydroxide gradient can be achieved using an ANN having a (1, 10, 19) architecture and using retention data obtained at three gradient slopes as the training set. Whilst only the slope of the linear gradient was varied in this study, it is a relatively straightforward exercise to incorporate the starting and finishing eluent concentrations as further experimental variables and to modify the ANN architecture accordingly. We consider that this study has shown that an ANN could be used as the basis of a strategy for optimisation of gradient conditions in IC. Whilst linear gradients were utilised in this study because of their frequent usage in IC, the approach demonstrated can be applied also to non-linear gradients if gradients of this type are used to train the ANN.

Acknowledgements

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Table 3

Percentage-normalised differences ($\%d_i$) between the measured and predicted retention times^a

| Slope (mM/min) | 4.00 | 3.33 | 2.86 | 2.50 | 2.22 | 2.00 | 1.67 |
|------------------------------------|-------|-------------|-------------|-------|--------------|--------------|-------|
| Fluoride | 0.00 | -0.13 | -0.09 | 0.00 | 0.21 | 0.12 | 0.00 |
| Acetate | 0.00 | 0.00 | 0.17 | 0.00 | -0.04 | 0.12 | 0.00 |
| Formate | 0.00 | 0.83 | 0.35 | 0.04 | -0.11 | 0.00 | 0.00 |
| Methansulfonate | 0.00 | -0.38 | -0.22 | 0.04 | -0.29 | -0.04 | 0.00 |
| Bromate | 0.00 | 0.13 | 0.15 | 0.00 | -0.17 | -0.08 | 0.00 |
| Chloride | 0.00 | 0.18 | 0.18 | 0.00 | 0.00 | -0.24 | 0.00 |
| Nitrite | -0.03 | 0.59 | 0.24 | 0.03 | -0.18 | -0.07 | -0.02 |
| Bromide | 0.00 | -0.02 | 0.13 | -0.02 | -0.29 | -0.36 | 0.00 |
| Nitrate | 0.00 | 0.26 | 0.21 | 0.00 | -0.13 | -0.46 | 0.00 |
| Chlorate | 0.00 | 0.15 | 0.08 | -0.02 | -0.19 | -0.32 | 0.00 |
| System peak (CO_3^{2-}) | 0.02 | 0.48 | 0.33 | -0.02 | -0.38 | -0.39 | 0.01 |
| Iodide | 0.00 | 0.35 | 0.55 | 0.00 | -0.39 | -0.37 | 0.00 |
| Succinate | -0.02 | 0.51 | 0.47 | 0.03 | -0.28 | -0.42 | -0.01 |
| Tartrate | 0.00 | -0.02 | 0.09 | 0.00 | -0.29 | 0.00 | 0.00 |
| Sulfate | 0.02 | 0.34 | 0.32 | -0.03 | -0.49 | -0.54 | 0.01 |
| Oxalate | -0.02 | 0.52 | 0.37 | 0.03 | -0.53 | -0.65 | -0.01 |
| Tungstate | 0.02 | 0.62 | 0.51 | -0.02 | -0.45 | -0.52 | 0.00 |
| Phthalate | 0.00 | 0.52 | 0.54 | -0.01 | -0.52 | -0.77 | 0.00 |
| Phosphate | 0.03 | 0.88 | 0.71 | -0.04 | -0.48 | -0.72 | 0.00 |

^a Absolute values of $\%d_i$ above 0.5% are highlighted in **boldface**.

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