A Computer-Based Undergraduate Exercise Using Internet-Accessible Simulation Software for the Study of Retention Behavior and Optimization of Separation Conditions in Ion Chromatog...

4 AUTHORS, INCLUDING:

Paul R Haddad
University of Tasmania
508 PUBLICATIONS **10,711** CITATIONS

SEE PROFILE

Greg W Dicinoski
University of Tasmania
55 PUBLICATIONS **843** CITATIONS

SEE PROFILE

Waters Symposium ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━

A Computer-Based Undergraduate Exercise Using Internet-Accessible Simulation Software for the Study of Retention Behavior and Optimization of Separation Conditions in Ion Chromatography

W

Paul R. Haddad,* Matthew J. Shaw, John E. Madden, and Greg W. Dicinoski
Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, Private Bag 75, Hobart 7001, Tasmania, Australia; **Paul.Haddad@utas.edu.au*

Ion chromatography (IC) is a term to refer to a collection of chromatographic techniques used mainly for the separation of inorganic anions and cations and low molecular weight water-soluble organic acids and bases. The range of chromatographic techniques covered by IC includes ion-exchange chromatography, reversed-phase ion-interaction chromatography, and ion-exclusion chromatography, but the majority of IC separations are performed by ion-exchange chromatography using specialized stationary phases.

IC methods employing ion exchange can be divided somewhat arbitrarily into two main groups, largely on the basis of historical development and commercial marketing influences. These groups of methods are referred to as "nonsuppressed ion chromatography" and "suppressed ion chromatography". Nonsuppressed IC comprises all those methods in which an ion-exchange column is used to separate a mixture of ions, with the separated analytes being passed directly to the detector. In suppressed IC an additional device, called the suppressor, is inserted between the ion-exchange separator column and the detector. The function of the suppressor is to modify both the eluent and the analyte to improve the detectability of the analytes with a conductivity detector.

Full details of the many stationary phases and eluents used in IC can be found in monographs (1–3) or a useful and succinct overview of the technique can be obtained from recent review and summary articles (4–6). These literature sources reveal that the most common and simplest form of IC involves the separation of inorganic anions on a suppressed IC system using specialty ion-exchange stationary phases, employed in conjunction with conductivity detection. In such a case, the eluent is normally composed of sodium salts of carbonate, bicarbonate, or hydroxide, used individually or in combination.

Teaching IC in the laboratory is a challenging task. Earlier published experiments on IC have used the analysis of a particular sample (e.g., determination of phosphate in cola, ref 7 or the determination of sulfur in coal, ref 8) to illustrate the utility of the technique. A suitable experiment should also seek to demonstrate the effects of changing the eluent composition on the retention behavior of analyte anions, but this requires the column to be equilibrated with each new eluent composition. A typical IC column takes about 30 min to equilibrate, so it becomes impractical to explore the effects of more than a few eluent compositions in a typical laboratory exercise. Our approach to this problem has been to create computer software ("Virtual Column 2"; ref 9) that simulates retention behavior in IC in a manner similar to that used previously in high-performance liquid chromatography (10). However, this software has the unique characteristic that it is based on a large data bank of experimentally measured retention times rather than relying on some form of algorithm to predict retention from a theoretical standpoint. The outcome of this is that the retention behavior generated by the software is very close (typically within 1%) to that exhibited in practice. The software therefore provides an ideal tool for students to see the effects of changing eluent compositions in IC without the necessity to spend large quantities of time acquiring retention data experimentally.

The exercise illustrates the major characteristics of retention behavior, peak-shape effects, and pH effects evident in IC using common stationary phases and eluents. The exercise can be completed within a four-hour laboratory period and if desired, can be followed by hands-on use of the instrument for analysis of a particular sample. Other advantages of virtual experiments in teaching have been discussed recently (11).

Theory

A brief summary of the theory of retention in IC is relevant. The equilibrium shown in eq 1 depicts the ion-exchange displacement of an eluent ion E^{y-} from the resin phase (denoted by the subscript r) of an anion-exchange material by an analyte ion A^{x-} initially present in the eluent phase (denoted by the subscript m). The outcome of this equilibrium is the binding of the analyte by the stationary phase.

$$y A_m^{x-} + x E_r^{y-} \rightleftharpoons y A_r^{x-} + x E_m^{y-} \tag{1}$$
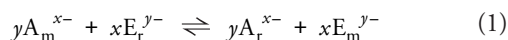
From this equation and invoking a number of basic chromatographic principles, the following model can be derived

$$\log k_A' = \frac{1}{y} \log(K_{A,E}) + \frac{x}{y} \log\left(\frac{Q}{y}\right) + \log\left(\frac{w}{V_m}\right) - \frac{x}{y} \log\left[E_m^{y-}\right] \tag{2}$$

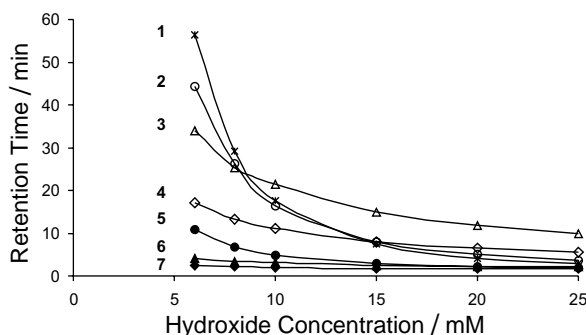where, k'_A is the retention factor of the analyte, $K_{A,E}$ is the

Figure 1. The effect of hydroxide concentration on the retention of analytes for the AS11 column: (1) phosphate, (2) thiosulfate, (3) thiocyanate, (4) iodide, (5) sulfate, (6) bromide, and (7) chloride.
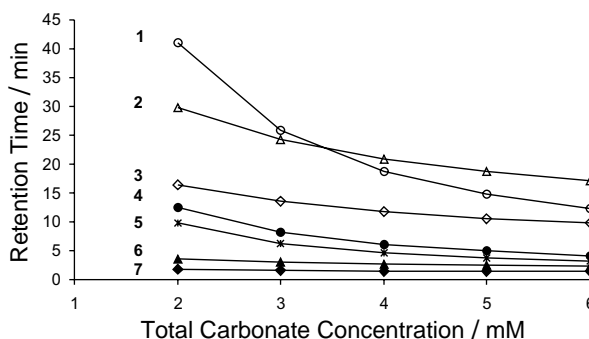

Figure 2. The effect of total carbonate concentration (for eluents containing 1:1 carbonate:bicarbonate) on the retention of analytes for the AS4A-SC column: (1) thiosulfate, (2) thiocyanate, (3) iodide, (4) sulfate, (5) phosphate, (6) bromide, and (7) chloride.

ion-exchange selectivity constant between the analyte and the eluent competing ion, Q is the effective ion-exchange capacity of the stationary phase, w is the mass of the stationary phase, and V_m is the volume of the eluent phase. This equation predicts a linear relationship between the logarithm of the retention factor and the logarithm of the eluent concentration. Furthermore, the slope of this linear relationship is equal to the negative ratio of analyte to eluent charge. Equation 2 can be simplified by collecting together all of the constant terms to give:

$$\log k_A' = C - \frac{x}{y} \log \left[E_m^{y-} \right] \qquad (3)$$

The values of x and y in eq 3 are straightforward when the eluent contains a single competing ion. However, a major uncertainty arises in determining the charge on the eluent anion, y, when the eluent contains more than one competing ion. This is the case when a mixture of carbonate ($y = 2$) and bicarbonate ($y = 1$) is used as eluent, which is a very common occurrence in IC. There are two distinct approaches to applying eq 3 to this situation.

In the first approach (termed the "dominant equilibrium approach") it is assumed that the most highly charged competing ion in the eluent is solely responsible for the elution of the analyte, even when relatively large quantities of less charged competing ions are present. Thus, for the case of the carbonate–bicarbonate eluents, only CO_3^{2-} is considered to exert an influence on the retention of analytes and $y = 2$ in eq 3. The second approach (termed the "effective charge approach") assumes that all charged competing ions in the eluent are responsible for the elution of the analyte, in proportion to their charges. The charge on the competing ion (y) in eq 3 is equated to the "effective charge" (y_{eff}) of the eluent, which is the sum of the mole fractions of eluent species present multiplied by their charge. Thus for the carbonate–bicarbonate eluent, y_{eff} is calculated from:

$$y_{eff} = \alpha_1 + 2\alpha_2$$

$$\alpha_1 = \frac{\left[HCO_3^- \right]}{C_T} = \frac{K_{a1} \left[H^+ \right]}{\left[H^+ \right]^2 + K_{a1} \left[H^+ \right] + K_{a1} K_{a2}} \qquad (4)$$

$$\alpha_2 = \frac{\left[CO_3^{2-} \right]}{C_T} = \frac{K_{a1} K_{a2}}{\left[H^+ \right]^2 + K_{a1} \left[H^+ \right] + K_{a1} K_{a2}}$$

where, α_1 is the mole fraction of bicarbonate in the eluent, α_2 is the mole fraction of carbonate in the eluent, and C_T is the total concentration of carbonate in the eluent.

Experimental

The results for this exercise have been generated using a freeware version of Virtual Column 2, which can be downloaded at the Virtual Column Web site (12). This freeware edition is capable of demonstrating retention behavior and optimizing eluent conditions for the best separation available for any combination of 22 analytes on a Dionex AS4A-SC column using carbonate–bicarbonate eluents and a Dionex AS11 column using hydroxide eluents. A complete set of instructions for the use of Virtual Column 2 is available at the Web site (12); with the aid of these instructions students can become familiar with the software in less than one hour. A personal computer with a 300 MHz processor or greater and Windows 95 or later system versions are required to perform this exercise. The data required for students to complete the exercise were collected as described below. All data could be collected comfortably within a period of three hours.

Retention data for the AS11 column with hydroxide eluents were determined from the Virtual Column software for the following analytes: phosphate, thiosulfate, thiocyanate, iodide, sulfate, bromide, and chloride. Retention times were measured for eluents comprising 6, 8, 10, 15, 20, and 25 mM hydroxide. For the AS4A-SC column, the same analytes were used with eluents of 2, 3, 4, 5, and 6 mM total carbonate at a 50% carbonate–bicarbonate ratio. A subset of analytes (fluoride, bromide, chloride, chromate, nitrite, nitrate, iodide, thiocyanate, sulfate, oxalate, phthalate, thiosulfate, and phosphate) was then selected from the data determined for the AS11 and AS4A-SC columns. For the AS11 column, a plot of log k' versus log[OH$^-$] was made for each analyte. In a similar manner, plots of log k' versus log[E] (where E is the eluent) were made for the AS4A-SC column using an eluent ratio of 50% (i.e., equal concentrations of carbonate
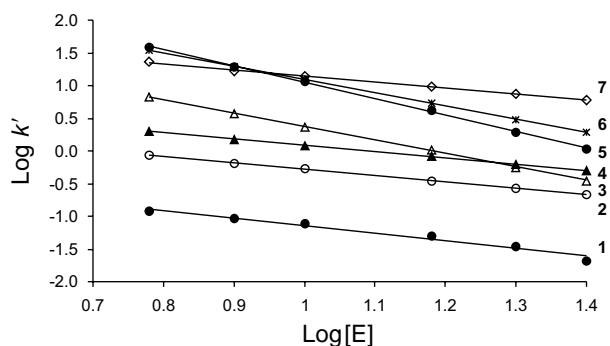
Figure 3. Plots of log[E] versus log k' for seven analytes on the AS11 column using hydroxide eluents: (1) fluoride, (2) nitrite, (3) sulfate, (4) nitrate, (5) phosphate, (6) chromate, and (7) thiocyanate.

Figure 4. Plots of log[E] versus log k' for seven analytes on the AS4A-SC column using carbonate–bicarbonate eluents containing 1:1 carbonate–bicarbonate: (1) fluoride, (2) nitrite, (3) nitrate, (4) phosphate, (5) sulfate, (6) thiosulfate, and (7) thiocyanate.

and bicarbonate) for total carbonate concentrations of 2, 3, 4, 5, and 6 mM.

Effects of peak asymmetry were investigated by selecting the AS11 column and the analytes chloride and nitrite. The asymmetry value for chloride was then changed from 1 to 2, 2.5, 3, 3.5, and 5 at a constant hydroxide concentration of 5.58 mM. A plot was then made of the asymmetry value versus the resulting resolution between chloride and nitrate.

Optimization of eluent composition was performed on the AS4A-SC column with carbonate–bicarbonate eluents and the AS11 column with hydroxide eluents using fluoride, formate, bromate, chloride, nitrite, bromide, chlorate, and nitrate as analytes. The global optimal separation was determined by Virtual Column 2 using the default peak asymmetry and peak area values. The 'Find fastest chromatogram' option was also used in conjunction with a minimum resolution of 1.0.

Discussion

Analyte Retention
As a Function of Eluent Concentration

The most important parameter for manipulating the retention of analytes to achieve the desired separation is through changing the eluent composition, for example, by altering the eluent concentration or pH. The retention behavior on the AS11 column with a hydroxide eluent (hydroxide being a weak ion-exchange competing anion) is shown in Figure 1. The data demonstrate that analyte retention decreased as the concentration of competing ion in the eluent was increased. The decreases in retention were more rapid for the multivalent ions (phosphate and thiosulfate) than for univalent ions. This behavior is typical in IC and can be explained in terms of the retention models (see below).

The retention behavior of analytes on the AS4A-SC column using eluents comprising equimolar mixtures of carbonate and bicarbonate, but with varying total carbonate concentration, is shown in Figure 2. Similar retention trends to those shown in Figure 1 are again evident. A comparison of Figures 1 and 2 shows that a less concentrated carbonate-based eluent was required to elute the analytes in a comparable time period than for the hydroxide eluent. Although
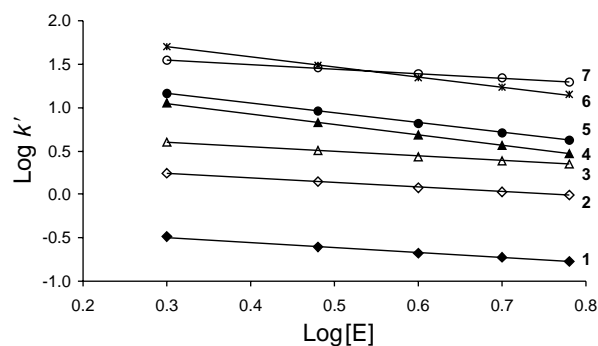
the ion-exchange capacity of the stationary phases must be taken into account (AS11: 4.5 meq/column, AS4A-SC: 20 meq/column) it can generally be seen that the electroselectivity of the eluent competing ion exerts the greatest effect upon analyte retention, with eluents of greater charge having a stronger affinity for the stationary phase, thereby becoming stronger eluents.

Prediction of Analyte Retention
Using Theoretical Models

The ability to predict retention using a theoretical model can lead to a simplified approach to method development and selection of the optimal eluent composition for a desired separation. The retention data presented in Figures 1 and 2 were plotted as log k' versus log[E] (in accordance with eq 3) and the resultant plots are shown in Figures 3 and 4. Data for the correlation coefficient and slopes of these plots for all analytes are listed in Table 1.

As shown in Table 1, the data have a high degree of linearity, in accordance with eq 3. The slopes of these plots can now be compared with the predictions made in the various models outlined in the Theory section. In the case of the AS11 data, the eluent contains only a single competing ion (hydroxide), so that $y = 1$ and the theoretical slopes predicted by eq 3 can be calculated readily and are listed in Table 1. Agreement between the predicted (eq 3) and experimental slopes is good (< 5% error), with the exception of fluoride and phosphate, and this demonstrates two important points. The modeled slope for fluoride differs from the actual value simply because the accurate measurement of retention factors is difficult for weakly retained analytes that are eluted close to the void volume. The behavior of phosphate is influenced by its acid–base dissociation as a function of eluent concentration (and hence pH). Retention data were obtained over a hydroxide concentration of 6–25 mM, corresponding to eluent pH values of 11.8–12.4. pK_{a3} for H_3PO_4 is 12.3 (13), therefore the charge on this anion will change over this pH range from −2.24 to −2.56. Equation 3 does not consider such changes to the analyte charge so it is to be expected that there would be poor agreement between the predicted and experimental slopes. This behavior will be evident for any analyte that undergoes acid–base dissociation over the eluent pH range.

In the case of the dual species carbonate–bicarbonate eluent species, the dominant equilibrium (DE) and effective charge approaches (EC) can be evaluated. For the former model, $y = 2$ and for the latter $y = 1.5$ (since the eluents used were all equimolar mixtures of carbonate and bicarbonate), giving the theoretical slopes listed in Table 1. While the experimental data showed good linearity, there was considerable disagreement between the experimental slopes and those predicted from either of the two retention models, with the experimental slopes generally being intermediate between the predictions of the models. The dominant equilibrium approach gave the best agreement for monovalent analytes, suggesting that the carbonate competing anion made a major contribution to the elution of these analytes. For divalent analytes, the observed slope values suggested that the carbonate competing anion is neither dominant in the elution process, nor participates proportionately to its charge. These results are typical of retention studies in IC. A further interesting point to note is that the observed slopes were greater for the hydroxide eluent on the AS11 column than for the carbonate eluent with the AS4A-SC column. This illustrates that singly charged competing ions cause the largest changes in analyte retention as a function of eluent concentration.

General conclusions that should be made from the data given in Table 1 and Figures 3 and 4 are that the theoretical models examined are sufficiently accurate to reliably predict retention factors for single species eluents but not for dual species eluents. However, the retention data do show good linearity for plots of log k' versus log[E], even when there are two competing ions present in the eluent. However, the slope for this relationship cannot be predicted reliably from these theoretical approaches.

Separation Selectivity

A notable effect evident from Figures 1–4 is the relatively long retention of the univalent anions thiocyanate and iodide, and again this behavior is typical in IC. It might be anticipated that all monovalent anions would behave similarly, but the actual behavior can be explained in terms of the polarizability of the analytes, which causes them to interact strongly with the quaternary ammonium cation groups on the ion-exchange packing. This arises as a result of their large hydrated radii compared with other monovalent ions, which leads to a high affinity towards the stationary phase since the cationic charge on the resin is capable of polarizing them and thereby increasing their interaction with the stationary phase. Other analytes in the database, as a general rule, show the expected increase in retention as a function of size and ionic charge.

Effects of Peak Asymmetry

Anions injected as a mixture onto a chromatographic column can have an impact upon each other with regard to their resolution. One significant parameter that needs to be considered is peak asymmetry; the effects of this parameter were investigated. An asymmetry value can be assigned to each peak by dividing the width of the trailing half of the peak by the width of the leading half of the peak. The default asymmetry values given in the database are those obtained from the experimental data derived in compiling the retention databases that underlie Virtual Column 2, and the user can choose to vary the degree of asymmetry for any analytes, or to ignore entirely the effects of peak asymmetry.

The resolution (R_s) value for chloride and nitrate, with the peak asymmetry for chloride (the first eluted peak) be-

Table 1. Comparison between Experimentally-Derived Slopes and Predicted Slopes for log[E] versus log k' Using Single Eluent Species (Eq 3), Dominant Equilibrium (DE), and Effective Charge (EC) Models, for Analytes on the AS11 and AS4A-SC Columns

Solute ion	AS11			AS4A-SC			
	Experiment		Theory	Experiment		Theory	
	Slope	r^2	Eq 3	Slope	r^2	DE	EC
Fluoride	-1.15	0.9739	-1	-0.58	0.9958	-0.5	-0.66
Bromide	-0.96	0.9998	-1	-0.50	0.9998	-0.5	-0.67
Chloride	-1.00	0.9993	-1	-0.54	0.9993	-0.5	-0.67
Nitrite	-0.97	0.9998	-1	-0.52	0.9992	-0.5	-0.67
Nitrate	-0.97	0.9998	-1	-0.52	0.9993	-0.5	-0.67
Iodide	-0.94	0.9999	-1	-0.50	1.0000	-0.5	-0.66
Thiocyanate	-0.93	0.9986	-1	-0.52	0.9993	-0.5	-0.66
Sulfate	-2.06	0.9999	-2	-1.13	0.9999	-1	-1.33
Chromate	-2.03	1.0000	-2	-1.12	1.0000	-1	-1.33
Oxalate	-2.04	0.9999	-2	-1.13	0.9999	-1	-1.33
Phthalate	-2.03	0.9999	-2	-1.12	0.9990	-1	-1.33
Thiosulfate	-2.06	0.9999	-2	-1.15	0.9990	-1	-1.33
Phosphate	-2.50	0.9992	-2[a]	-1.21	0.9998	-1[a]	-1.33[a]

[a]The charge on phosphate ion is taken as -2 for these calculations.

ing varied from 1 (a symmetrical or Gaussian peak) to 5 (a strongly tailed peak) is shown in Figure 5. The data were obtained for the AS11 column using 5.58 mM hydroxide as eluent. The data in Figure 5 demonstrate that only a slight degree of peak tailing can have a significant detrimental impact upon the resolution. Considering the fact that many analytes in IC show asymmetrical peaks, it becomes evident that possibility of peak tailing should form part of the determination of optimal separation conditions.

Optimization of Separation Conditions

The ability to scan retention data over a wide range of eluent compositions opens up the possibility of a computerised selection of the optimal separation conditions. Virtual Column 2 has the capacity to simulate all possible chromatograms within a specified search area of eluent compositions and to rank these chromatograms according to two optimization criteria. The first of these criteria is the minimum resolution criterion ($R_{s,min}$), which determines the value of R_s for the peak pair having the poorest separation in each chromatogram and selects the chromatogram with the highest value. An alternative optimization criterion is the normalized resolution product (r), defined by (1)

$$r = \prod_{i=1}^{n-1} \left(\frac{R_{s,i,i+1}}{\dfrac{1}{n-1} \displaystyle\sum_{i=1}^{n-1} R_{s,i,i+1}} \right)$$

where n is the number of peaks and $R_{s,i,i+1}$ is the resolution between peaks i and $i+1$. The normalized resolution product criterion gives a value of zero for a chromatogram that has one or more co-eluted peaks and a value of one for a chromatogram that has evenly spaced peaks.

Any set of analytes can be chosen for this part of the exercise, but the authors recommend using fluoride, formate, bromate, chloride, nitrite, bromide, chlorate, or nitrate as a good example to demonstrate the advantages of computer based optimization. For the AS4A-SC column the 'global optimum' separation under the minimum resolution criterion was produced using an eluent of 2 mM total carbonate–bicarbonate containing 10% carbonate ion, which gave $R_{s,min} = 1.042$ and the separation shown in Figure 6. An R_s value of 1.2 is usually deemed sufficient for quantification, so these conditions are not ideal, principally owing to the overlapping of the bromide, chlorate and nitrate peaks. However, this is the best separation that can be obtained with this combination of column and eluent and this point can be illustrated readily by viewing other possible chromatograms simply by clicking on any other part of the response surface.

The separation shown in Figure 6 required a total analysis time of 7.5 min. Faster separations could be sought provided a lower value of $R_{s,min}$ was acceptable. Using the 'find fastest chromatogram' option and setting a minimal acceptable R value of 1.0 yielded an analysis time of 7.0 min using an eluent of 2.08 mM total carbonate–bicarbonate containing 14% carbonate. Repeating the optimization using the normalized resolution product gave an optimal eluent comprising 2 mM carbonate–bicarbonate containing 90% carbonate and an analysis time of 3.5 min with $r = 0.237$. This is a dramatic

improvement in analysis time, but with a higher degree of overlap of the chlorate and nitrate peaks than in Figure 6.

Optimization of the same group of analytes on the AS11 column, again using the default asymmetry, peak area, theoretical plates, and void time gave the separation in Figure 7 using an eluent of 5.58 mM hydroxide, producing $R_{s,min} = 0.730$. The normalized resolution product criterion gave the
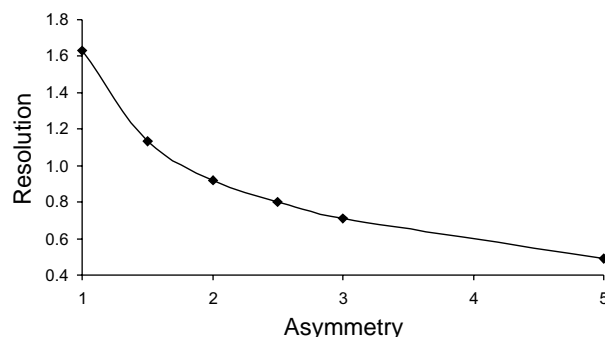

Figure 5. Effect of peak asymmetry on the resolution between chloride and nitrite on the AS11 column, with an eluent of 5.58 mM hydroxide.
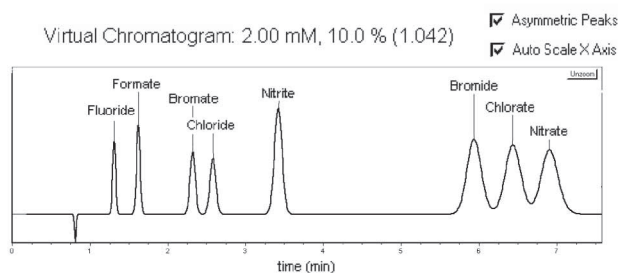

Figure 6. Virtual Column 2 screen capture illustrating the optimal isocratic separation of fluoride, formate, bromate, chloride, nitrite, bromide, chlorate, and nitrate on the AS4A-SC column. The optimal eluent composition was 2.08 mM total carbonate–bicarbonate containing 14% carbonate.
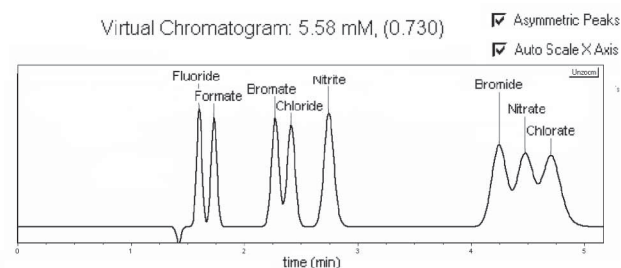

Figure 7. Virtual Column 2 screen capture illustrating the optimal isocratic separation of fluoride, formate, bromate, chloride, nitrite, bromide, nitrate, and chlorate on the AS11 column. The optimal eluent composition was 5.58 mM hydroxide.

same optimum (5.58 mM hydroxide) as the $R_{s,min}$ criterion. The poorer separation obtained with the AS11 column under isocratic conditions can be attributed to the fact that this column is designed more specifically for use with gradient elution.

Conclusions

Virtual Column 2 offers students the opportunity to explore a wide range of ion chromatographic behavior in a relatively short time. The exercise described is quite flexible and can be varied easily to fit into different time periods. The data presented to the user are based on experimental measurements and therefore illustrate actual retention behavior rather than contrived or modeled data. Virtual Column 2 is thus ideal as a tool for evaluation of theoretical models and to demonstrate the circumstances under which such models give good prediction of retention behavior. The software is easy to use and is available free of charge to interested users. It therefore provides a viable means for the teaching of IC in a laboratory context.

ᵂSupplemental Material

Follow-up questions to ask students after completing the exercise are available in this issue of *JCE Online*.

Literature Cited

1. Haddad, P. R.; Jackson, P. E. *Ion Chromatography: Principles and Applications;* Elsevier Science Publishers: New York, 1990.
2. Weiss, J. *Ion Chromatography,* 2nd ed.; VCH: New York, 1995.
3. Fritz, J. S.; Gjerde, D. T. *Ion Chromatography,* 3rd ed.; Wiley-VCH: New York, 2000.
4. Haddad, P. R. *Anal. Chem.* **2001,** *73,* 266A.
5. Haddad, P. R. In *Encyclopedia of Separation Science;* Wilson, I., Ed.; Academic Press: London, 2000; pp 696–705.
6. Pietrzyk, D. J. In *Encyclopedia of Analytical Chemistry;* Meyers, R. A., Ed.; Wiley: New York, 2000; pp 11360–11383.
7. Bello, M. A.; Gonzalez, A. G. *J. Chem. Educ.* **1996,** *73,* 1174.
8. Koubek, E.; Stewart, A. E. *J. Chem. Educ.* **1992,** *69,* A146.
9. Madden, J. E.; Shaw, M. J.; Dicinoski, G.W.; Haddad, P. R. *Anal. Chem.* **2002,** *74,* 6023.
10. Rittenhouse, R. C. *J. Chem. Educ.* **1988,** *65,* 1050.
11. Martinez-Jimenez, P.; Pontes-Pedrajas, A.; Polo, J.; Climent-Bellido, M. S. *J. Chem. Educ.* **2003,** *805,* 346.
12. Vitual Column Home Page. *http://www.virtualcolumn.com* (accessed Jun 2004). Full download instructions are provided on this site.
13. Aylward, G.; Findlay T. *SI Chemical Data,* 4th ed.; Wiley: New York, 1998; p 137.