



# Methodology for porting retention prediction data from old to new columns and from conventional-scale to miniaturised ion chromatography systems

Boon K. Ng<sup>a</sup>, Robert A. Shellie<sup>a</sup>, Greg W. Dicoski<sup>a</sup>, Carrie Bloomfield<sup>a</sup>, Yan Liu<sup>b</sup>, Christopher A. Pohl<sup>b</sup>, Paul R. Haddad<sup>a,\*</sup>

<sup>a</sup> Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Private Bag 75, Hobart 7001, Australia

<sup>b</sup> Dionex Corporation, PO Box 3603, Sunnyvale, CA 94088-3603, USA

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## ABSTRACT

Several procedures are available for simulating and optimising separations in ion chromatography (IC), based on the application of retention models to an extensive database of analyte retention times on a wide range of columns. These procedures are subject to errors arising from batch-to-batch variability in the synthesis of stationary phases, or when using a column having a different diameter to that used when the database was acquired originally. Approaches are described in which the retention database can be recalibrated to accommodate changes in the stationary phase (ion-exchange selectivity coefficient and ion-exchange capacity) or in the column diameter which lead to changes in phase ratio. The entire database can be recalibrated for all analytes on a particular column by performing three isocratic separations with two analyte ions. The retention data so obtained are then used to derive a “porting” equation which is employed to generate the required simulated separation. Accurate prediction of retention times is demonstrated for both anions and cations on 2 mm and 0.4 mm diameter columns under elution conditions which consist of up to five sequential isocratic or linear gradient elution steps. The proposed approach gives average errors in retention time prediction of less than 3% and the correlation coefficient was 0.9849 between predicted and observed retention times for 344 data points comprising 33 anionic or cationic analytes, 5 column internal diameters and 8 complex elution profiles.

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## 1. Introduction

In recent times there has been an increasing focus on research associated with the miniaturisation of liquid chromatography (LC) techniques. This impetus has been supported by the wider availability of smaller diameter columns from commercial vendors and has been driven in part by an increased awareness of enhancing detection limits, reducing running costs, maintaining continuous instrument operation, and minimising environmental pollution by solvents and chemicals. Importantly, the use of miniaturised systems often opens opportunities for achieving faster separations, and thus it supports a long-standing goal of research in separation science. In the simplest instance miniaturisation is achieved by using narrow-bore separation columns, leading to micro-, capillary-, and nano-scale chromatography. The use of narrow-bore columns is a recognisable intermediate technique between conventional LC and microchip separations [1] and the concepts that lead to narrow-bore LC will be useful in designing approaches for even

smaller scale separations. In miniaturising any method, it is important to understand the effect that changing the column dimensions has on the integrity of the existing separation.

When scaling down from conventional scale to narrow-bore LC, it is often desirable to keep the separation selectivity constant, because a great deal of effort will have typically gone into optimising and validating the existing method. However, the goal of using the miniaturised separation system may also be to reduce the analysis time. Miniaturising the separation system can also result in changes in the overall peak distribution pattern of analytes if proper precautions are not taken. Several years ago Blumberg, and Klee described the concept of method translation for gas chromatography (GC) [2,3], which permits the variation of components (columns, carrier gases, detectors, etc.) and method parameters (pressures, temperature program, etc.) without affecting the peak elution pattern. Recently, Guillarme et al. described an approach for method transfer in LC [4]. Although this approach works well for translation to shorter columns [4], if the column inner diameter or the diameter of the packing material is altered, then changes in overall separation selectivity will be observed. Guillarme et al. attributed this to dwell volume effects [4]. However, it is our experience that selectivity changes exist even if careful attention is paid to correcting dwell volume and we attribute such selectivity changes

\* Corresponding author. Tel.: +61 3 6226 2179; fax: +61 3 6226 2858.

E-mail addresses: [Paul.Haddad@utas.edu.au](mailto:Paul.Haddad@utas.edu.au), [helen.barnard@utas.edu.au](mailto:helen.barnard@utas.edu.au) (P.R. Haddad).

to changes in phase ratio. This rationale is consistent with the theory supporting method translation in GC, which clearly states that phase ratio is a non-translatable parameter [2,3]. If the phase ratio in a liquid chromatography system cannot be maintained by careful scaling of both the column internal diameter and the stationary phase particle diameter, then method re-optimisation will usually be required to compensate for any selectivity changes introduced as a result of miniaturisation.

We have significant interests in ion chromatography (IC) separations as well as in the miniaturisation of IC systems and during the last decade we have developed strategies for retention prediction and the simulation and optimisation of IC that involve variations of the linear solvent strength retention equation [5–10]. These strategies are based on a database of carefully measured isocratic and linear gradient retention data obtained for a wide range of analytes and IC columns, with these data being “embedded” into the software used to simulate separations. These data were acquired some years ago using conventional 4 mm diameter IC columns, and through the use of a range of mathematical algorithms these data permit the calculation of retention times for analytes on 4 mm diameter columns using eluent compositions which are isocratic, linear gradient, or multi-step elution profiles comprising a combination of the two. Rapid *in silico* optimisation of IC separations can therefore be undertaken. A freeware version of this simulation software is available [11].

In the present manuscript we address the question of how the existing retention database can be implemented to permit prediction of retention times on new columns which may exhibit batch-to-batch variability, and also in miniaturised IC column formats using columns having diameters different to that which was used to acquire the original retention data. We describe this procedure as “porting”, wherein the retention database is recalibrated to account for stationary phase and column differences. Porting leads to substantially improved accuracy of predicted retention times and hence more reliable *in silico* optimisation of IC separations.

## 2. Experimental

### 2.1. General

The isocratic and gradient retention data used in this study had been collected previously [6]. It is important to note that these isocratic and gradient data were acquired at different times using different instruments and columns from different manufacturing batches. Any comparisons of data made between the isocratic and gradient measurements will therefore include variability between instruments and column batches. Statistical analyses of the performance of the various retention models were carried out using Microsoft Excel.

### 2.2. Reagents and solutions

Test solutions were prepared by dissolution of analytical grade salts obtained from Sigma-Aldrich (St. Louis, MO, USA) in Milli-Q water (18.2 M $\Omega$ ; Millipore, MA). The compositions of the various test mixtures containing the analyte ions and their concentration used are shown in the figure legends.

### 2.3. Instrumentation

The capillary-IC experiments were performed using a Dionex (Sunnyvale, CA, USA) ICS-5000 ion chromatograph (consisting of an AS autosampler, ICS5000 detector/chromatography module, ICS-5000 dual gradient pump, and ICS-5000 eluent generation module). All other analyses were performed on a Dionex DX-600 ion chromatograph (consisting of an AS50 autosampler, AS50 thermal

compartment, GP50 gradient pump, EG40 eluent generator, and CD25A suppressed conductivity detector). Details of the columns used in this study are provided in Table 2. A flow-rate of 1.0 mL/min was used for the 4 and 5 mm i.d. columns, 0.36 mL/min with 3 mm i.d. columns, 0.25 mL/min with 2 mm i.d. columns, and 0.01 mL/min with 0.4 mm i.d. columns.

Each eluent composition was generated electrolytically by an eluent generation module. All eluents were produced as potassium hydroxide or methane sulfonic acid (MSA) in Milli-Q water (18.2 M $\Omega$ ) obtained from a Millipore Academic Gradient water purification system attached to a Millipore Elix system (Millipore, MA, USA).

Retention data for the anion-exchange separations were collected at a column temperature of 30 °C and data for the cation-exchange separations were collected at a column temperature of 40 °C. The analytes were detected using suppressed conductivity detection. Suppression of the eluents was achieved using 2 mm or 4 mm Dionex ASRS II or CSRS II membrane suppressors operated in the autosuppression recycle mode. An ACES suppressor was used for capillary IC experiments.

## 3. Results

### 3.1. Effects of altering column diameter

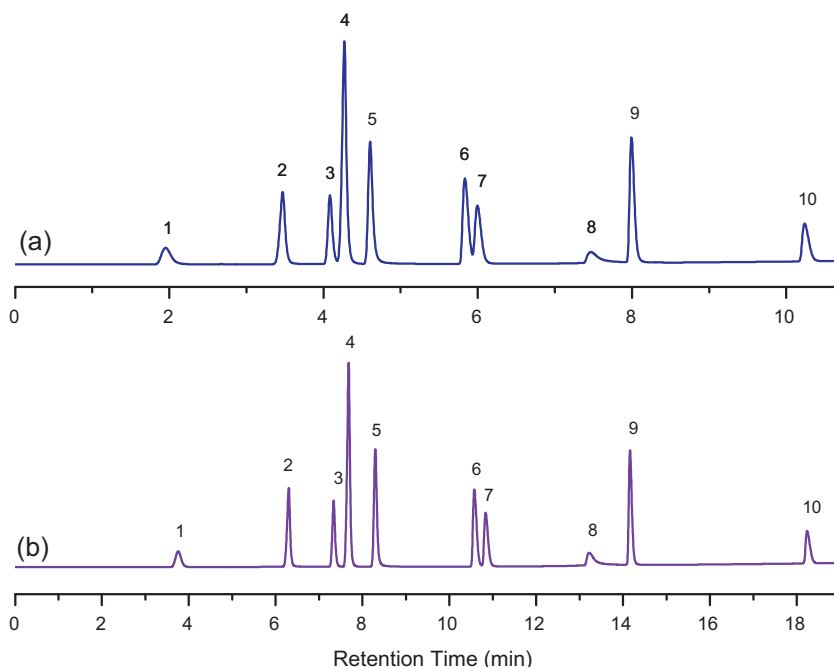
In principle, an isocratic separation method developed for a column of a particular diameter can be translated to a column of a different diameter by simply adjusting the eluent flow-rate in proportion to the square of the radius of the two columns. In the case of gradient separations or when using complex elution profiles which comprise both isocratic and gradient steps, it is also necessary to normalise the elution profile to account for the change in void volumes resulting from changing the column diameter. Thus, if the column diameter is halved, the flow-rate should be reduced by a factor of 4. However, this simple method translation is valid only when altering the column diameter causes no change in the fundamental parameters which govern the separation.

Before considering miniaturising an ion-exchange separation it is therefore pertinent to evaluate how modifying column dimensions might affect the separation. Retention in ion-exchange chromatography is described by the equation [12,13]:

$$\log k = \frac{1}{y} \log(K_{A,E}) + \frac{x}{y} \log\left(\frac{Q}{y}\right) + \log\left(\frac{w}{V_M}\right) - \frac{x}{y}[E^y] \quad (1)$$

where  $k$  is retention factor,  $K_{A,E}$  is the ion-exchange selectivity coefficient,  $Q$  is the effective ion-exchange capacity of the stationary phase (generally expressed as equiv/g),  $w$  is the mass of the stationary phase (g),  $V_M$  is volume of the eluent species (L), and  $[E^y]$  is the concentration of the eluent competing ion (mole L<sup>-1</sup>),  $x$  is the effective charge of the analyte, and  $y$  is the effective charge of the eluent competing ion.

The total ion-exchange capacity of a column can be calculated from  $Q$  and  $w$ . However, altering the total ion-exchange capacity does not influence  $k$  provided that  $Q$  remains unaltered and the same isocratic or normalised eluent composition is used. This is illustrated in Fig. 1, which shows the separation of a mixture of fluoride, chlorite, bromate, chloride, nitrite, nitrate, chlorate, carbonate, sulfate, and phosphate using a 250 mm  $\times$  4 mm i.d. IonPac AS11 analytical column (Fig. 1(a)) or two IonPac AS11 analytical columns joined in series (Fig. 1(b)). A three-step complex elution profile was used for each separation, with the eluent profile adjusted for the different void volumes of each separation system. The total ion-exchange capacity differs for each chromatogram but since the same stationary phase material has been used, retention factors and separation selectivities are identical.



**Fig. 1.** Comparison of the separation of inorganic anions using (a) a single 250 mm  $\times$  4 mm i.d. IonPac AS11 column and (b) two 250 mm  $\times$  4 mm i.d. IonPac AS11 columns connected in series. Solute identities: 1-fluoride, 2-chlorite, 3-bromate, 4-chloride, 5-nitrite, 6-nitrate, 7-chlorate, 8-carbonate, 9-sulfate and 10-phosphate where each component was prepared in 10 ppm using a complex eluent profile starting at 0.50 mM for 2 min, 0.50–5.00 mM over 5 min, 5.00–38.25 mM over 15 min at 2 mL/min and 30 °C on the AS11 4 mm column.

Reducing column inner diameter will invariably lead to a small change in the ratio  $w/V_M$  (which is defined as the phase ratio) due to the increased influence of wall effects in packed columns of small diameter. In such situations simple method translation will not be possible. To illustrate this effect, chromatograms of suitable test solutes were collected using a 250 mm  $\times$  4 mm i.d. IonPac AS19 analytical column and a 250 mm  $\times$  2 mm i.d. IonPac AS19 analytical column. In this experiment both column formats (4 mm and 2 mm i.d.) were packed with identical 7.5  $\mu$ m stationary phase material so the two column formats will have slightly different phase ratios due to wall effects. The retention factors for the two separations are shown in Table 1. Relative retention between the two columns should be constant since the same stationary phase is used and this is illustrated in Table 1, with the exception of fluoride, for which minor error in retention time or the void time would significantly alter  $k$  for such a weakly retained analyte. However, differences in retention will be further exacerbated by the use of a multi-step gradient, and since such operation is common in IC, this is a serious consideration. The inability to achieve direct method translation from conventional scale to miniaturised IC means that an eluent profile which has been optimised on a 4 mm column will generally require further optimisation when translated to a column of smaller diameter.

**Table 1**

Retention factors for the IonPac AS19 4 mm i.d. and 2 mm i.d. columns using 25 mM hydroxide as eluent. The void times were 2.785 min for the 4 mm i.d. column operating at 1 mL/min and 2.868 min for the 2 mm i.d. column operating at 0.25 mL/min. Void times were measured by first turning the suppressor off and a water injection made. The void time was indicated by the resultant water dip.

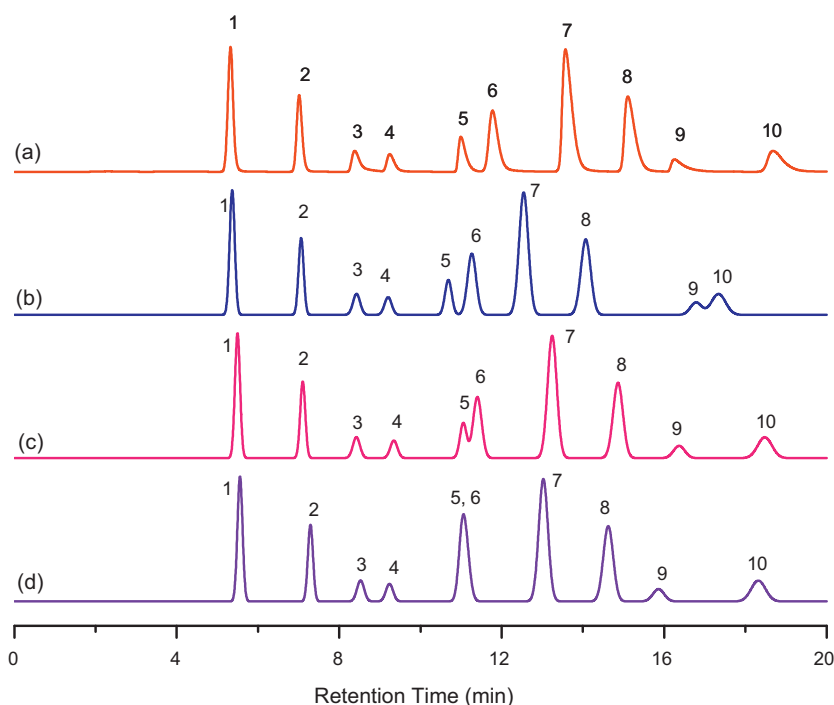
| Anion       | $k$ (4 mm i.d.) | $k$ (2 mm i.d.) | $k_{2\text{mm}}/k_{4\text{mm}}$ |
|-------------|-----------------|-----------------|---------------------------------|
| Fluoride    | 0.28            | 0.30            | 1.06                            |
| Nitrate     | 1.53            | 1.25            | 0.82                            |
| Thiocyanate | 5.95            | 4.53            | 0.76                            |
| Perchlorate | 6.89            | 5.16            | 0.75                            |

### 3.2. Effects of column variability

It was recently shown that it is possible to simulate IC gradient separations using a small number of isocratic retention data [6]. In this case, a database of retention factors for a particular stationary phase and eluent competing ion had been acquired previously and this database was used to predict gradient retention data. Fig. 2 illustrates the predictive capability of this approach for a three-step complex elution profile applied to the separation of inorganic cations. Fig. 2(a) shows the experimentally obtained separation, while Fig. 2(b) shows the predicted separation. Comparison of the two chromatograms shows that correlation between experimental and predicted retention times was good for the first 5 analytes, but was less satisfactory for the later-eluted analytes. The simulation relies on two prediction coefficients ( $a_i$  and  $b_i$ ) that are normally estimated on the basis of experiments in which the retention factor ( $k_i$ ) of each analyte is measured at a limited number of isocratic eluent compositions and the resultant data fitted to Eq. (2) (which is a simplification of Eq. (1)) [6].

$$\log k_i = a_i - b_i \log [E_m^y] \quad (2)$$

The derived values of  $a_i$  and  $b_i$  can then be used to predict retention factors for any desired isocratic, gradient, or multi-step eluent composition [6]. The simulated chromatogram shown in Fig. 2(b) was derived using this process by applying the isocratic retention database embedded in the Virtual Column Separation Simulator [14]. These embedded data were acquired on columns which were manufactured 6 years ago and the observed differences between Fig. 2(a) and (b) can therefore be attributed to batch-to-batch variability in column manufacture arising from changes over time to the synthetic procedures. Therefore, there is a clear need to either update the retention database to reflect changes in column manufacture or to establish a means by which the existing retention data can be utilised for retention simulation on columns which have been manufactured more recently.



**Fig. 2.** (a) Experimental chromatogram on a 5 mm CS16 column for a 10-component mixture consisting of 1-lithium(I) (5 ppm), 2-sodium(I) (5 ppm), 3-ammonium (5 ppm), 4-ethylammonium (5 ppm), 5-potassium(I) (5 ppm), 6-magnesium(II) (10 ppm), 7-calcium(II) (10 ppm), 8-strontium(II) (10 ppm), 9-cesium(I) (15 ppm) and 10-barium (15 ppm) using a complex eluent profile starting from 30 to 35 mM over 3 min, 35–50 mM over 3 min, 50–60 mM over 3 min, 60–80 mM over 3 min followed by 80–95 mM over 12 min at 1 mL/min and 40 °C. (b) Simulated separation using embedded retention data obtained on a 5 mm CS16 column. (c) Simulated separation using new retention coefficients obtained on the same 5 mm CS16 column used in (a). (d) Simulated separation using retention coefficients ported from the embedded database after recalibration.

The chromatogram shown in Fig. 2(c) was simulated using retention data obtained from a new series of isocratic experiments performed on the same column as used for Fig. 2(a). These experiments involved the measurement of retention times for each analyte at three eluent concentrations, followed by derivation of new  $a_i$  and  $b_i$  values from Eq. (2). This leads to a substantial improvement in the match between simulated and experimental results. Although the approach of using fresh retention data prior to each simulation clearly generates accurate prediction of retention times, implementation of this approach would require that considerable experimentation be performed prior to simulation and optimisation of separations. A more attractive option is to modify the existing retention database to enable it to be used for more accurate predictions. Essentially, this can be accomplished if the  $a_i$  and  $b_i$  values for each analyte in the database can be updated for all new columns with minimal experimentation.

### 3.3. Recalibration of the retention database

Retention data were measured experimentally using a new 5 mm CS16 column and 13 representative analyte cations (ammonium, barium(II), calcium(II), cesium(I), ethylammonium, lithium(I), magnesium(II), manganese(II), methylammonium, potassium(I), rubidium(I), sodium(I) and strontium(II)).  $a_i$  and  $b_i$  values were calculated for these analytes and compared with those for the same analytes in the Virtual Column Separation Simulator embedded retention database. The  $a_i$  and  $b_i$  values obtained by both approaches were found to be highly correlated, regardless of analyte charge, as follows:

$$a_{\text{new}} = 0.617 \pm 0.007 a_{\text{embedded}} \quad (R^2 = 0.9976) \quad (3)$$

$$b_{\text{new}} = 0.923 \pm 0.003 b_{\text{embedded}} \quad (R^2 = 0.9984) \quad (4)$$

Predictably, the  $b$ -coefficients, which reflect the ratio of the charge of the analyte to the charge of the competing ion in the

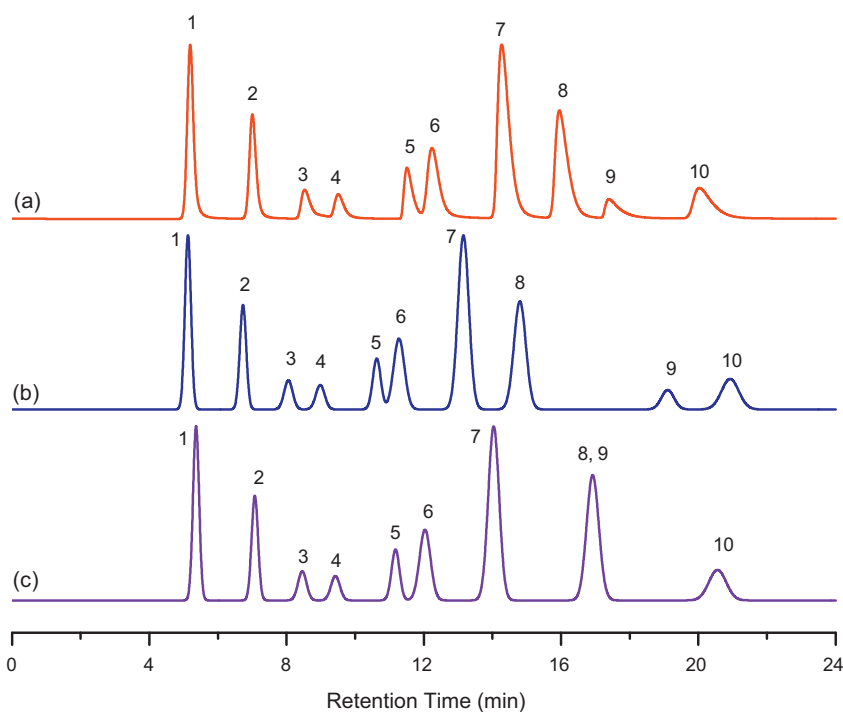
eluent were found to be essentially unchanged between the new data and the embedded database data. However, the  $a$ -coefficients, which reflect changes in  $K_{A,E}$  and  $Q$  (since the particle size and column diameter are constant), showed considerable change between the two data sets, although most importantly all analytes showed essentially the same degree of change (as reflected by the high correlation coefficient for the plot of  $a_{\text{new}}$  vs.  $a_{\text{embedded}}$ ). This creates the possibility for recalibration of the entire suite of cations based on experimental data for a very limited number of analytes.

To this end, barium(II) and methylammonium were selected as analytes for the recalibration experiments on the IonPac CS16 analytical column, although it is likely that the choice of cations is not particularly important. A mixture of these two analytes was analysed at three eluent strengths (25, 40, 62 mM MSA) and retention factors used to determine  $a_i$  and  $b_i$  values. These values were averaged and the ratio of new:embedded values was used to recalibrate the  $a_i$  and  $b_i$  values for all analytes in the embedded retention database. The recalibration factors are shown in the first line of Table 2 and are very similar to the factors in Eqs. (3) and (4) which were derived using all 13 analytes. A new separation was then simulated for the eluent conditions used in Fig. 2, leading to the simulated chromatogram shown in Fig. 2(d). Comparison

**Table 2**

Summary of porting equations used for CS16 cation-exchange and AS19 anion-exchange columns of various internal diameters. The equations for CS16 are used to update embedded retention data acquired using a 250 mm × 5 mm i.d. IonPac CS16 cation-exchange column. Those for AS19 are used to update embedded retention data acquired using a 250 mm × 4 mm i.d. IonPac AS19 anion-exchange column.

| Column      | Porting equation (a)                                   | Porting equation (b)                                  |
|-------------|--|---|
| 5 mm CS16   | $a_{\text{new}} = 0.624 a_{\text{embedded}} + 16.102$  | $b_{\text{new}} = 0.930 b_{\text{embedded}} + 0.005$  |
| 3 mm CS16   | $a_{3\text{mm}} = 0.634 a_{\text{embedded}} + 2.976$   | $b_{3\text{mm}} = 0.976 b_{\text{embedded}} + 0.112$  |
| 4 mm AS19   | $a_{\text{new}} = 0.830 a_{\text{embedded}} + 2.796$   | $b_{\text{new}} = 1.004 b_{\text{embedded}} - 0.024$  |
| 2 mm AS19   | $a_{2\text{mm}} = 0.567 a_{\text{embedded}} + 4.884$   | $b_{2\text{mm}} = 0.978 b_{\text{embedded}} - 0.013$  |
| 0.4 mm AS19 | $a_{0.4\text{mm}} = 0.687 a_{\text{embedded}} + 4.034$ | $b_{0.4\text{mm}} = 1.00 b_{\text{embedded}} - 0.012$ |



**Fig. 3.** (a) Experimental chromatogram on a 4 mm AS19 column for a 10-component mixture comprising 1-fluoride (5 ppm), 2-chlorite (5 ppm), 3-bromate (5 ppm), 4-chloride (5 ppm), 5-nitrite (5 ppm), 6-chlorate (10 ppm), 7-bromide (5 ppm), 8-nitrate (5 ppm), 9-carbonate (15 ppm) and 10-sulfate (10 ppm) using a complex eluent profile starting at 3.75 mM for 0.8 min, 3.75 mM to 33.75 mM over 10 min, 33.75–99.75 mM over 4 min at 1 mL/min and 30 °C on the AS19 4 mm column. (b) Simulated separation using embedded retention data obtained on a 4 mm AS19 column. (c) Simulated separation using retention coefficients ported from the embedded database after recalibration.

of Fig. 2(a) and (d) shows that this process provided a simulated chromatogram which matched closely with the experimental chromatogram, except for potassium and magnesium for which co-elution was predicted when in fact separation was achieved in practice.

Since this recalibration approach potentially increases the portability of the original embedded Virtual Column Separation Simulator data, the word “porting” is used to describe this approach. The porting process was repeated for the separation of anions. A test set of analytes comprising acetate, bromate, bromite, carbonate, chlorate, chloride, chlorite, chromate, fluoride, formate, iodide, methane sulfonate, nitrate, nitrite, oxalate, phosphate, propionate, sulfate, thiocyanate and thiosulfate was separated on a new 4 mm AS19 column. The chromatogram obtained appears as Fig. 3(a). The  $a$ - and  $b$ -coefficients were determined from the new retention data and were compared with the values for these coefficients in the embedded data set. A correlation plot of measured  $a$ - and  $b$ -coefficients for the new column versus the embedded  $a$ - and  $b$ -coefficients exhibited very strong correlation as shown below:

$$a_{\text{new}} = 0.809 \pm 0.001 a_{\text{embedded}} \quad (R^2 = 1.000) \quad (5)$$

$$b_{\text{new}} = 0.994 \pm 0.001 b_{\text{embedded}} \quad (R^2 = 0.9998) \quad (6)$$

Again, this process was repeated using only two representative anions (chloride and thiosulfate), with the result being shown in the third row of Table 2. The porting equations which use only two analytes for updating the embedded 4 mm AS19 retention data are again similar to Eqs. (4) and (5) which were based on 20 analytes.

Fig. 3(b) shows the simulated chromatogram obtained using  $a$ - and  $b$ -coefficients from the embedded retention data, and Fig. 3(c) shows the chromatogram simulated using the porting equations shown for the 4 mm AS19 column in Table 2. All three chromatograms in Fig. 3 are quite similar, which indicates that there has been little change in the stationary phase characteristics for this column.

#### 3.4. Prediction of retention for different column diameters

The above porting process compensates primarily for changes in the  $a_i$  term of Eq. (2) and thereby addresses changes in  $K_{A,E}$ ,  $Q$ ,  $w$ , and  $V_M$ . Of these parameters,  $K_{A,E}$  and  $Q$  can be expected to be related only to the stationary phase, while  $w$  and  $V_M$  can be expected to be related only to the column packing process, the particle diameter, and to the column diameter. Since all of the porting calculations performed thus far have been applied to columns of the same diameter, they relate only to changes in  $K_{A,E}$  and  $Q$ . However, it should also be possible to apply the same procedure to recalibrate the retention database to simultaneously accommodate changes in both the characteristics of the stationary phase and the physical characteristics of the column.

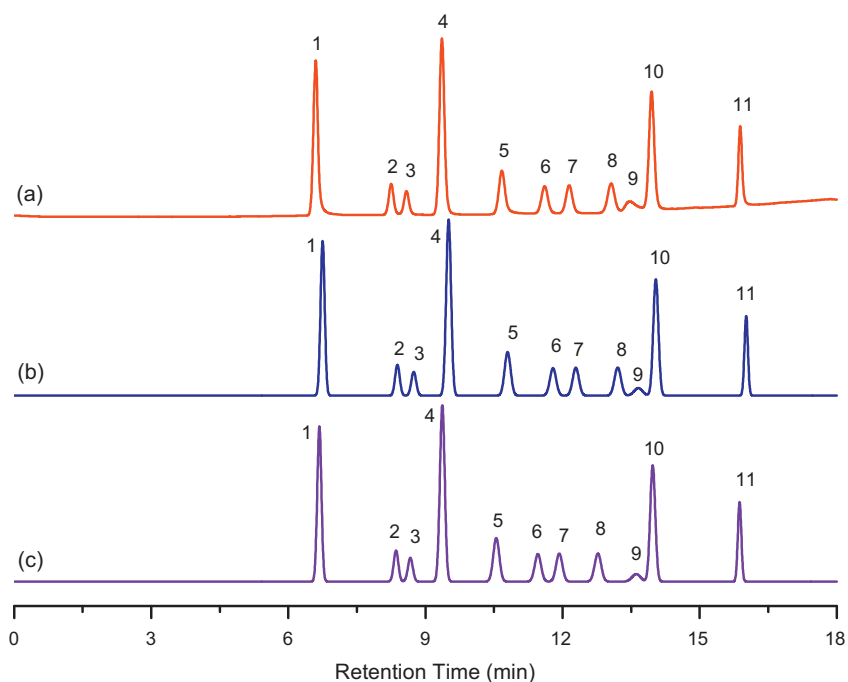
In order to investigate the validity of porting retention data between columns of differing diameter, a set of  $a$ - and  $b$ -values was calculated from retention data acquired for a 3 mm i.d. IonPac CS16 column using ammonium, barium(II), calcium(II), cesium(I), ethylammonium, lithium(I), magnesium(II), manganese(II), methylammonium, potassium(I), rubidium(I), sodium(I) and strontium(II) as analytes. These data were compared with the 5 mm prediction coefficients for the same analytes. The hypothesis that porting can be applied to different internal diameter columns is supported by the strong correlation between both sets of coefficients:

$$a_{3 \text{ mm}} = 0.624 \pm 0.007 a_{5 \text{ mm}} \quad (R^2 = 0.9976) \quad (7)$$

$$b_{3 \text{ mm}} = 0.899 \pm 0.008 b_{5 \text{ mm}} \quad (R^2 = 0.9909) \quad (8)$$

Again the above relationships can be approximated using only two analytes (barium(II) and methylammonium) at three eluent strengths (25, 40, 62 mM MSA). The resultant porting equation is shown in the second row of Table 2. Fig. 4(a) shows an experimental separation of 10 cations on a 3 mm CS16 column. A simulated separation using coefficients calculated from the embedded database of 5 mm CS16 retention data and a flow-rate adjusted to compensate

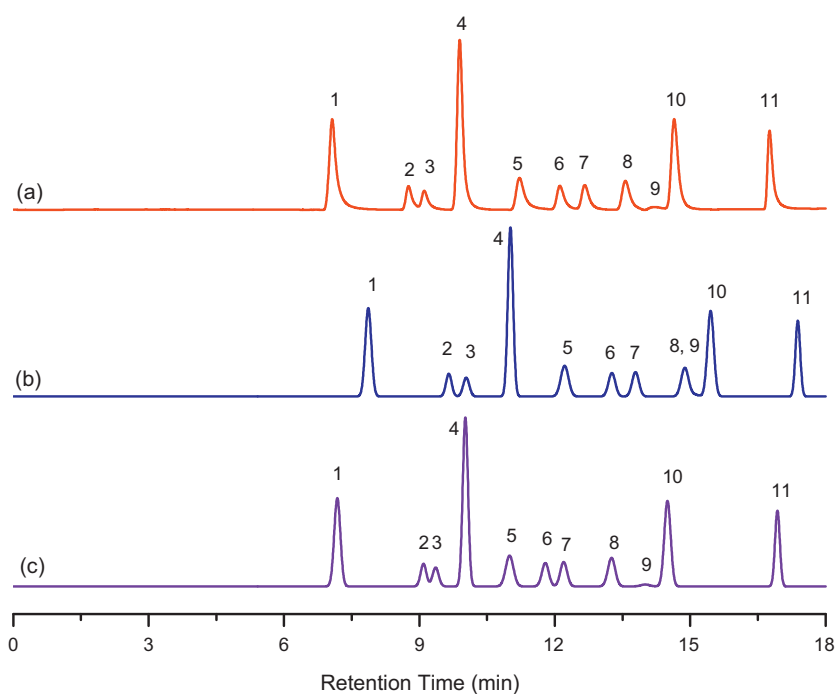




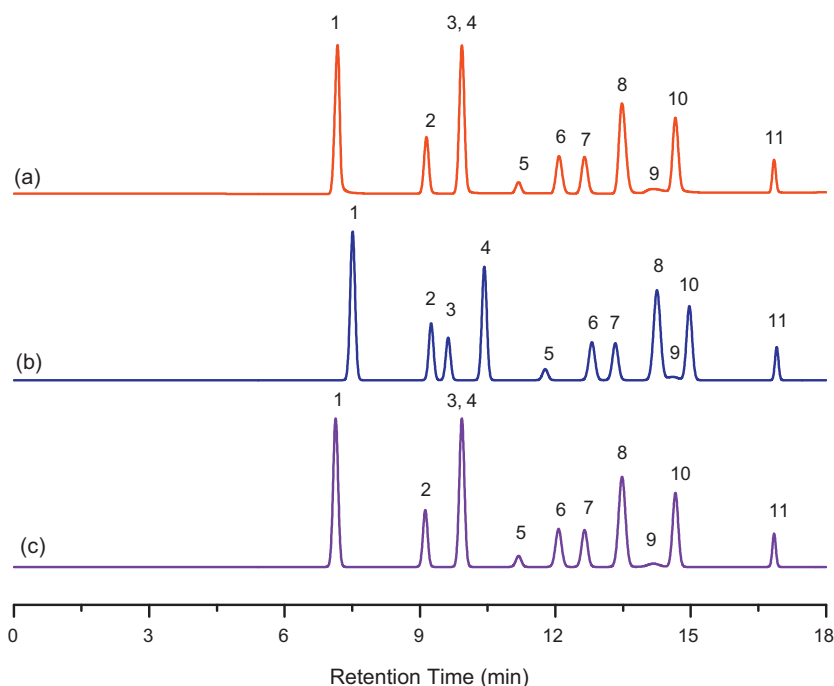
**Fig. 4.** (a) Experimental chromatogram on a 3 mm CS16 column for a 10-component cation mixture of the same composition as in Fig. 2 and using the same complex eluent profile at a flow rate of 0.36 mL/min and 40 °C. (b) Simulated separation using embedded retention data obtained on a 5 mm CS16 column. (c) Simulated separation using retention coefficients ported from the embedded database after recalibration.

for the changed column diameter is shown in Fig. 4(b). Comparison of this separation with Fig. 4(a) reveals significant differences, which can be attributed to differences in the phase ratio for each column. Fig. 4(c) shows the simulated separation obtained using the  $a$ - and  $b$ -coefficients calculated using the porting equation given in Table 2. The predicted retention times are now much closer to those in the experimental chromatogram, although co-elution of strontium(II) and cesium(I) is predicted erroneously.

Next, the applicability of porting to a different diameter anion exchange column was explored. The IonPac AS19 stationary phase was selected for this purpose because it is available in a variety of column diameters (4 mm i.d., 2 mm i.d. and 0.4 mm i.d.). Chloride and thiosulfate were used to provide retention data to generate the porting equations for 2 mm and 0.4 mm AS19 columns (Table 2). Fig. 5(a) shows the experimental separation of 11 anions on a 2 mm AS19 column using a three step complex elution profile, while sim-



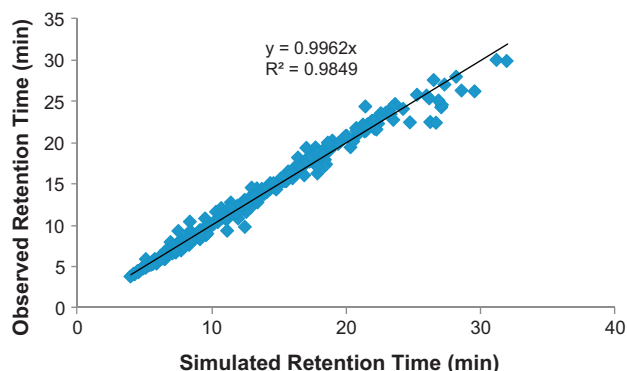
**Fig. 5.** (a) Experimental chromatogram on a 2 mm AS19 column for an 11-component anion mixture of the same composition as in Fig. 3, except for the inclusion of phosphate as peak 11 and using the same complex eluent profile at a flow-rate of 0.25 mL/min and 30 °C. (b) Simulated separation using embedded retention data obtained on a 4 mm AS19 column. (c) Simulated separation using retention coefficients ported from the 4 mm AS19 embedded database after recalibration.



**Fig. 6.** (a) Experimental chromatogram on a 0.4 mm AS19 column for an 11-component anion mixture of the same composition as in Fig. 3, except for the inclusion of phosphate as peak 11 and using the same complex eluent profile at a flow-rate of 0.01 mL/min and 30 °C. (b) Simulated separation using embedded retention data obtained on a 4 mm AS19 column. (c) Simulated separation using retention coefficients ported from the 4 mm AS19 embedded database after recalibration.

ulated separations calculated from the embedded 4 mm AS19 data (Fig. 5(b)) and from the porting equation derived from retention measurements of chloride and thiosulfate (Fig. 5(c)) are also shown. Fig. 6 shows chromatograms for experimental and simulated separations performed on a 0.4 mm AS19 column. In both figures, the simulated chromatograms obtained using the porting equations are a very close match for the experimental chromatograms.

Finally an extended series of separation conditions was employed to further validate the porting procedure and the results are presented in Fig. 7. These separations include retention simulations of 20 anions with three different column diameters and four different five-step elution profiles, together with 13 cations using two different column diameters over four different five-step elution profiles. A total of 344 retention data points are included



**Fig. 7.** Observed retention times versus simulated retention times of 20 anions (acetate, bromate, bromide, carbonate, chlorate, chloride, chlorite, chromate, fluoride, formate, iodide, methanesulfonate, nitrate, nitrite, oxalate, phosphate, propionate, sulfate, thiocyanate and thiosulfate) and 13 cations (ammonium, barium, calcium, cesium, ethylammonium, lithium, magnesium, manganese, methylammonium, potassium, rubidium, sodium and strontium) using 8 different complex elution conditions on five different internal diameter columns at four different flow-rates and two operating temperatures. A total of 344 data points are included on the graph.

in Fig. 7 and there is a high correspondence between predicted and observed retention times. It should be noted that the high correlation between simulated and experimental retention times observed in Fig. 7 confirms that Eq. (1) is valid for capillary columns. The porting procedure delivers an average of 3% error for simulating the retention behaviours of an extensive set of analytes and columns. It substantially improves the prediction accuracy of simulated separations in IC.

#### 4. Conclusions

In this study we have demonstrated that an extensive database of IC retention data obtained more than 5 years ago can be used for accurate prediction of retention times under complex elution conditions, using new columns. These new columns exhibit changes in ion-exchange capacity or selectivity coefficient for the packing material (resulting from changes in column chemistry arising from modifications in manufacturing procedures), or changes in phase ratio due to different column diameters. With minimal experimental data obtained on a desired column using just two analytes, the embedded retention data for all analytes on that particular column can be recalibrated to account for changes in selectivity coefficient and ion-exchange capacity. Moreover, the same process can be used to recalibrate embedded retention data so that it can be used for accurate simulation of separations on columns having different diameters to those used to compile the original database.

These processes permit accurate simulations of separations on a wide range of stationary phases and varying column diameters to be performed with minimal experimentation. In this way, method development in IC can be undertaken systematically and rapidly.

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