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Simulation and Optimization of Retention in Ion Chromatography Using Virtual Column 2 Software

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A new software package, Virtual Column 2, is described for the simulation and optimization of the separation of inorganic anions by ion chromatography (IC). The software uses a limited amount of experimental retention data acquired according to a correct experimental design to predict retention times for analytes over a designated search area of eluent compositions. The experimental retention data are used to solve a new retention model, called the linear solvent strength model, empirical approach (LSSM-EA), which then enables prediction of retention times for all eluent compositions in the search area. The theoretical development of LSSM-EA and the processes used for solving the equations are discussed. Virtual Column 2 can be used for eluents containing one or two competing ions, and the software contains retention databases for up to 33 analytes on the Dionex AS9A-HC, AS4A-SC, and AS14A analytical columns with carbonate−bicarbonate eluents and the Dionex AS10, AS15, and AS16 analytical columns with hydroxide eluents (results for the AS10 and AS15 columns are not discussed in the present study). Virtual Column 2 has been evaluated extensively and is shown to give predicted retention times that in most cases agree with experimentally determined data to within 5%. The software has uses in practical IC method development, education and training in IC, and refinement of existing IC methodology. A free version of this program is available by download at www.virtualcolumn.com.

It is an important facet of any chromatographic technique to optimize the separation in order to achieve a high sample throughput with the desired degree of resolution between analytes. Such optimization is of particular interest for chromatographic techniques in which manual development of methods is a time-consuming process. One example is ion chromatography (IC), in which equilibration of the ion-exchange stationary phase with a new eluent composition normally requires considerable time. For this reason, the simulation of anion separations by IC has received significant interest in recent years.[1,2]

The conventional approach to optimization in IC is to apply a suitable mathematical model to the prediction of retention times of analytes under a range of eluent conditions. The model is used to simulate the separations that can be achieved over a desired search area of eluent compositions, and a suitable optimization algorithm is then used to locate the eluent composition leading to the best separation. Previous research by the authors has focused on the development and comparison of a series of retention models for use in computer-aided optimization of IC.[3−5] The emphasis of this work was on determining which of the models provided the most accurate predictive capability, taking into account the degree of experimentation and computing requirements necessary to apply each model. This work has shown that simple empirical models and artificial neural networks provided better accuracy than more complex theoretical retention models.[3−6]

The linear solvent strength (LSS) model has been found to provide an excellent basis for the development of these empirical models. In the LSS model, direct proportionality is assumed between the logarithm of the retention factor of an analyte and the logarithm of the concentration of the competing ion in the eluent.[3] The LSS model further defines the slope of this relationship as the ratio of the analyte and eluent charges. However, the LSS model becomes difficult to apply when more than one competing ion is responsible for elution of analytes (e.g., when mixtures of carbonate and bicarbonate are used as eluents). Two modifications to the LSS model can be made under these circumstances.[7] The first is the dominant equilibrium approach, which assumes that only the eluent component having the higher ion-exchange selectivity coefficient (e.g., carbonate in the case of the carbonate−bicarbonate eluent) is responsible for elution of analytes. The second is the effective charge approach, which assumes that all eluent species contribute to analyte elution in proportion to their charge. The dominant equilibrium approach is usually inappropriate because all eluting species exert some influence on elution of the analytes, while the effective charge approach is typically inaccurate because the influences of eluent components are not usually in proportion to their charge. To

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overcome these problems, several complex retention models have been proposed,[8–16] but these invariably require the experimental determination of a large number of parameters before they can be applied and do not provide any significant advantage in terms of accuracy or precision. This makes them unsuitable for practical implementation in optimization procedures.

By contrast, the LSS model is relatively straightforward to use and requires minimal computing power and experiment to implement. Our previous studies[3–5] have shown that the linearities of the log k' versus log[eluent] plots in the LSS model are usually very high, but the slopes of these plots correlate poorly with theory. However, good predictability of retention data can be achieved if these slopes are measured experimentally from retention data obtained at eluent compositions representing the extremes of the range of eluent compositions to be studied. This approach, which we have termed the linear solvent strength model-empirical approach (LSSM-EA), enables very rapid and accurate calculation of retention data. In this paper, we describe the application of the LSSM-EA to the simulation and optimization of IC separations with carbonate−bicarbonate eluents, using a special-purpose software program called Virtual Column 2. The simulation and optimization of analyte retention in IC is desirable to assist rapid method development, for use in education and training of IC users, or for adaptation of existing separation methods to new samples. Until now there has been no software that meets these requirements.

THEORY

The LSS model can be given as[7]

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

where k'_A is the retention factor of the analyte, $K_{A,E}$ is the ion-exchange selectivity coefficient between the analyte and the eluent competing ion, x is the charge on the analyte, y is the charge on the eluent, Q is the effective ion-exchange capacity of the stationary phase, w is the mass of the stationary phase, V_m is the volume of the eluent phase, and $[E^{y-}{}_m]$ is the concentration of eluent E^{y-} in the mobile phase.

Typically eq 1 is simplified to

$$\log k'_A = C - (x/y)\log[E^{y-}{}_m] \quad (2)$$

where, C is constant for a given eluent composition and stationary phase. If experimental measurements are performed to determine the slope and intercept of a plot of eq 2 (e.g., by measuring retention data at two eluent concentrations), eq 2 becomes

$$\log k'_A = C_1 + C_2\log[E^{y-}] \quad (3)$$

where C_1 and C_2 are experimentally determined constants. This approach has been shown to give good predictability of analyte retention under conditions where the eluent contains only one competing ion (e.g., hydroxide). However, successful application to eluents with two or more competing ions requires a different approach. Eluents comprising carbonate and bicarbonate species also contain hydroxide as a competing species due to the high pH of the mobile phase. If the carbonate-to-bicarbonate molar ratio is as high as 90%, the theoretical pH is 11.06, giving a hydroxide concentration as high as 1.1 mM. It should be noted that, in the presence of carbonate and bicarbonate, the effects of hydroxide on retention are relatively minor and appear as a small change in slope of the plot of eq 3 rather than a departure from linearity. An empirical approach to determining the slope of eq 3 would therefore include any effects of hydroxide. For these reasons, the effects of hydroxide on retention are ignored. For eluents composed of two competing species, data for the system must be acquired using a suitable experimental design. This consists of the eluent compositions defined by a grid, itself defined by variations in one dimension of the mole fraction (R) of the eluent species of higher selectivity coefficient and in the other dimension by the total concentration of the two eluent species (E_T) as shown in Figure 1.

Next, retention data are acquired using the eluent compositions designated by points A−D in Figure 1 and eq 3 is applied for points A and B (to give eq 4) and for points C and D (to give eq 5):

$$\log k' = C_{12} + C_{22}\log E_T \quad \text{(for high } R\text{)} \quad (4)$$

$$\log k' = C_{11} + C_{21}\log E_T \quad \text{(for low } R\text{)} \quad (5)$$

These equations can be used to calculate retention times for eluent compositions falling along the horizontal lines in Figure 1. To calculate retention times for the vertical lines in Figure 1 (i.e., for intermediate R values) eq 6 can be used:

$$\log k' = D_1 + D_2\log[E^{y-}] \quad (6)$$

where $[E^{y-}]$ is the concentration of the eluent species having the highest ion-exchange selectivity coefficient, which can be calculated from R as follows:

$$[E^{y-}] = E_T.R \quad (7)$$

Retention factors can be calculated for any combination of E_T and R values by first applying eqs 4 and 5 to determine retention factors at the desired value of E_T and low and high values of R, followed by solving for D_1 and D_2 using eqs 6 and 7. Once D_1 and D_2 are known for a given E_T value, retention factors for any value of R can be calculated for that E_T value using eq 6. It is necessary to solve for D_1 and D_2 separately for each value of E_T.

Virtual Column 2 Software. The LSSM-EA has been incorporated into a new software program called Virtual Column 2,

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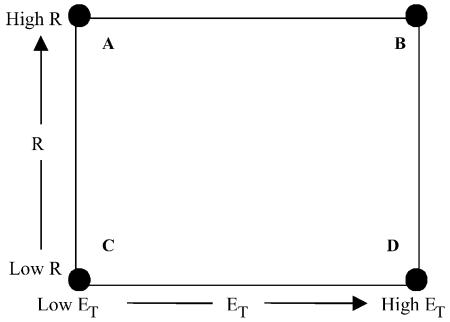

Figure 1. Experimental design for data acquisition to solve the LSSM-EA for eluents with two competing ions. Experiments are conducted at eluent compositions designated by points A−D.

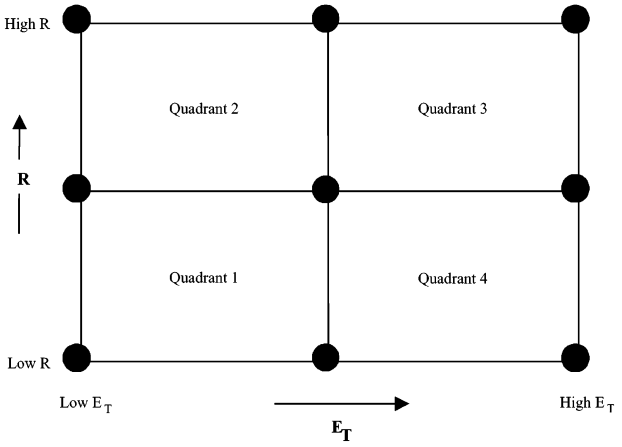

Figure 2. Experimental design for Virtual Column 2. Black circles indicate eluent compositions at which retention data are determined experimentally.

which can be used for the simulation and optimization of retention times in IC. This software is available in two forms. The first is a training version of the software, which can be downloaded without cost from www.virtualcolumn.com. The second is a demonstration version of the software, which is distributed free as part of the Dionex Corp. (Sunnyvale, CA) "Consumables/Instrumentation, Manuals and Literature v14" compact disk. Virtual Column 2 functions using embedded retention data that have been acquired using the correct experimental design and are stored in a "column database". Each database holds sufficient information to solve the LSSM-EA for each analyte using a particular type of eluent and stationary phase. The training version includes two such databases, a Dionex AS4A-SC column with a carbonate−bicarbonate eluent and a Dionex AS11 column with a hydroxide eluent. The demonstration version of Virtual Column 2 includes six databases: Dionex AS4A-SC, AS9-HC, and AS14A columns with carbonate−bicarbonate eluents and the Dionex AS10, AS15, and AS16 columns with hydroxide eluents. All calculations in this paper were performed using the demonstration version of Virtual Column 2.

Column databases hold information about the column (identity, retention time of an unretained species, and number of theoretical plates) the eluent (identity, limiting concentrations used to define the search area over which optimization and simulations can be performed, and pK_a values), and retention data for each analyte measured at specific eluent compositions. For single-species eluent systems, three retention data points are used, one at each end of the search area and one at the midpoint between them. For dual-species eluent systems, retention data were obtained at nine eluent compositions, as shown in Figure 2. These eluent compositions divided the search area into four quadrants, and retention data within each quadrant are obtained by solving eqs 4−7 in the manner described earlier. This yields values for C_{11}, C_{21}, C_{12}, and C_{22} for each quadrant and thence values for D_1 and D_2 for any given E_T value, leading to retention data for a desired combination of R and E_T. This approach was preferable to calculation of retention data using only the four extreme points in the search area since greater accuracy was obtained by reducing the degree of interpolation between known data points.

EXPERIMENTAL SECTION

Statistical analyses of the performance of the various retention models were carried out using experimental retention data acquired using the IC system described below. All calculations were performed using Microsoft Excel 2000 on a PC running Windows 2000 Professional.

Reagents and Solutions. Stock solutions of 50 mM sodium carbonate (Ajax, Auburn, Australia) and sodium bicarbonate (Prolabo, Paris, France) were prepared from analytical grade salts and diluted as required with ultrapure Milli-Q water (Millipore, Bedford, MA) using the Dionex GS50 gradient serial pump to produce the desired eluent conditions.

Anion standards were prepared by dissolution of analytical grade sodium salts in Milli-Q water. Perchlorate, iodide, thiocyanate, chlorite, ethanesulfonate, propanesulfonate, butanesulfonate, and pentanesulfonate were obtained from Aldrich (Milwaukee, WI); bromate, chlorate, tungstate, chromate, thiosulfate, sulfate, and succinate were from BDH (Kilsyth, Australia); iodate, phosphate, nitrate, nitrite, formate, phthalate, selenate, molybdate, and chloride were from Ajax; bromide, methanesulfonate, hexanesulfonate, and octanesulfonate were from Sigma (St. Louis, MO); oxalate, tartrate, and sulfite were from Mallinckrodt (Paris, France); and acetate and fluoride were from Prolabo. The concentrations of the anions varied from 1 to 150 mg/L.

With the AS14A column, analyte retention data were obtained at three total eluent concentrations ($E_T = [CO_3^{2-}] + [HCO_3^-]$) of 4, 12, and 20 mM and three carbonate-to-bicarbonate mole ratios ($[CO_3^{2-}] = 50$, 70 and 90%) giving nine different eluent compositions. For the AS9-HC column, three total eluent concentrations of 8, 14, and 20 mM, together with three mole ratios of 60, 80, and 100% carbonate, were chosen. Retention data for the Dionex AS4A-SC column were taken from previously published work[3−5] using total eluent concentrations of 2, 4, and 6 mM and mole ratios of 10, 50, and 90% carbonate.

Instrumentation. The chromatographic instrumentation consisted of a Dionex DX-600 ion chromatograph comprising AS50 automated sampler, AS50 thermal compartment, EG40 eluent generator, CD25 conductivity detector, and GS50 gradient serial pump. Dionex AS14A 3 mm and AS9-HC anion separator columns with appropriate guard columns were used with a Dionex ASRS-Ultra self-regenerating anion suppressor housed in the AS50 thermal compartment. A 25-μL injection loop was used throughout. All samples were analyzed in duplicate with a flow rate of 1.0 mL/min.

Table 1. Mean and Standard Deviation of the Percentage Differences between Predicted (Virtual Column 2) and Experimentally Determined Retention Times for 33 Analytes on a Dionex AS4A-SC Anion Separator Column Used with a Carbonate−bicarbonate Eluent

	total [eluent] (mM)							
	3.0	5.0	2.0	3.0	4.0	5.0	6.0	3.0
eluent molar ratio (%)	10	10	40	40	40	40	40	50
mean error(%)	−0.50	1.85	1.77	0.94	0.72	1.46	2.16	0.50
standard deviation (%)	0.72	1.52	3.32	3.53	3.53	3.59	3.59	3.43

	total [eluent] (mM)							
	5.0	2.0	3.0	4.0	5.0	6.0	3.0	5.0
eluent molar ratio (%)	50	60	60	60	60	60	90	90
mean error (%)	0.22	0.50	−0.86	−1.06	−1.29	−0.66	−1.15	−0.53
standard deviation (%)	3.65	2.96	2.97	3.10	2.88	2.96	0.79	0.57

Method for Solving the Dual-Eluent Species LSSM-EA. Four data points were required to solve this model. These data points were located at the corner points of each of the quadrants shown the experimental design given in Figure 2. Values for C_{21} and C_{11} were obtained using eqs 9 and 10. Values for k'_1 correspond to retention factors acquired using eluents with a low ratio and low total eluent concentration, while values for k'_2 correspond to retention factors acquired using eluents with low ratio and high eluent concentration. E_{T1} and E_{T2} correspond to low and high total eluent concentrations, respectively. For example, for the AS9-HC column, k'_1 would be the retention factor of the analyte using an eluent with 60% carbonate and a total eluent concentration (E_T) of 8.0 mM, while k'_2 would be the retention factor of the same analyte using an eluent with 60% carbonate and a total eluent concentration of 20.0 mM.

$$C_{21} = \frac{\log(k'_1/k'_2)}{\log(E_{T1}/E_{T2})} \quad (8)$$

$$C_{11} = \log(k'_1) - C_{21}\log(E_{T1}) \quad (9)$$

Values for C_{22} and C_{12} were obtained from eqs 10 and 11. Values for k' and E_T with subscripts of 3 and 4 were for high R/low E_T and high R/high E_T, respectively.

$$C_{22} = \frac{\log(k'_3/k'_4)}{\log(E_{T3}/E_{T4})} \quad (10)$$

$$C_{12} = \log(k'_3) - C_{22}\log(E_{T3}) \quad (11)$$

Values for D_1 and D_2 could be obtained only for a specific value of E_T and were obtained using eqs 12 and 13. Values for k_A' and k_B' were calculated using eqs 4 and 5, respectively. Values for $[E_A^{y-}]$ and $[E_B^{y-}]$ were calculated using eq 7 by employing the specified E_T and values for both low and high R, respectively.

$$D_2 = \frac{\log(k'_A/k'_B)}{\log([E_A^{y-}]/[E_B^{y-}])} \quad (12)$$

$$D_1 = \log(k'_A) - D_2\log([E_A^{y-}]) \quad (13)$$

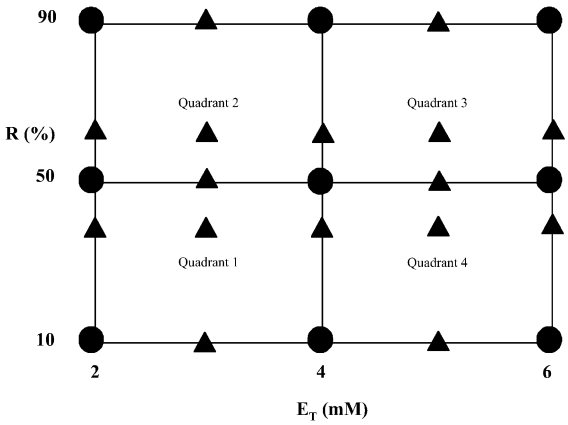

Figure 3. Eluent compositions (indicated by ▲) used to evaluate the performance of Virtual Column 2 for the AS4A-SC column and carbonate−bicarbonate eluents. The eluent compositions used to obtain primary retention data for solving the LSSM-EA are indicated by black circles.

Once values for D_1 and D_2 had been determined, the retention factor of an analyte using an eluent with a specified E_T value and any R value within the search area could be calculated using eq 6.

RESULTS AND DISCUSSION

Accuracy of Virtual Column 2. Retention data were calculated for all 33 analytes using Virtual Column 2 for the Dionex AS9-HC, AS14A, and AS4A-SC columns with eluent compositions that differed from the experimental points in the search area shown in Figure 2 used to solve the LSSM-EA. The predicted retention factors were then compared with those observed experimentally using the same eluent conditions. The results of this comparison are summarized in Table 1 for the AS4A-SC column, and Figure 3 shows the location of these eluent compositions in the search area. From Table 1 it can be seen that the agreement between predicted and observed retention factors was good, indicating that Virtual Column 2 could be used for reliable optimization and simulation of separations performed on this column. Similar results were obtained on the AS9-HC (average error of 0.50% and a standard deviation of 1.77% for the entire data set) and AS14A (average error of −2.82% and a standard deviation of 3.46% for the entire data set) columns using carbon-

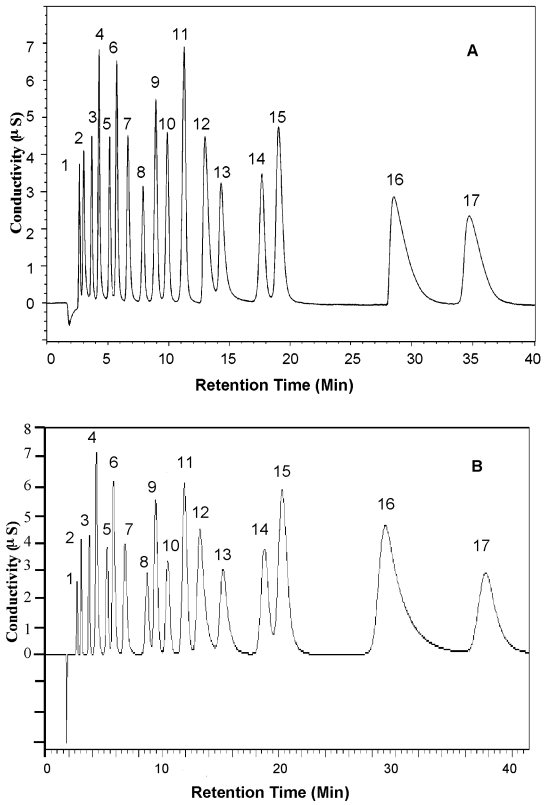

Figure 4. Comparison between (A) experimental and (B) Virtual Column 2 chromatograms for 17 analytes on the AS14A column, using an eluent of 10.08 mM total carbonate containing 72% CO_3^{2-}: 1, fluoride; 2, acetate; 3, chloride; 4, nitrite; 5, bromide; 6, nitrate; 7, chlorate; 8, phosphate; 9, tartrate; 10, sulfate; 11, oxalate; 12, iodide; 13, tungstate; 14, thiosulfate; 15, chromate; 16, thiocyanate; 17, phthalate. Analyte concentrations, 2−80 mg/L.

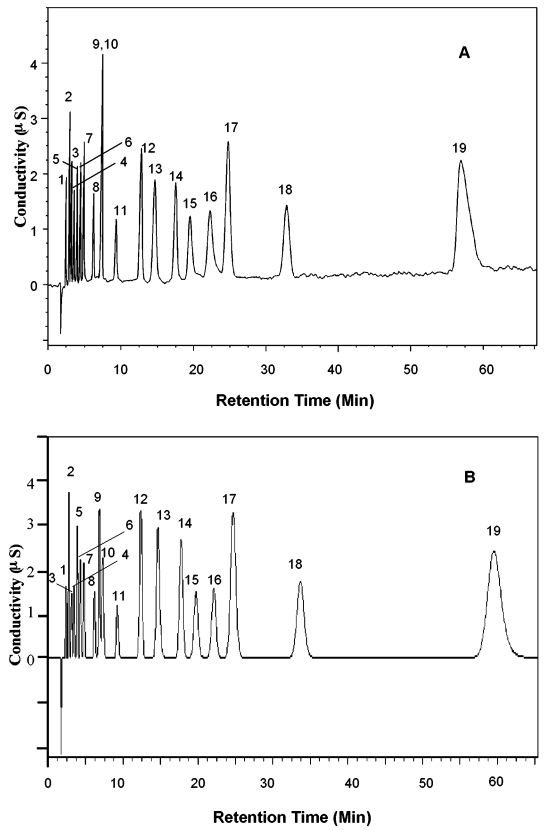

Figure 5. Comparison between (A) experimental and (B) Virtual Column 2 chromatograms for 19 analytes on the AS9-HC column, using an eluent of 16.64 mM total carbonate containing 95.2% CO_3^{2-}: 1, fluoride; 2, formate; 3, chlorite; 4, bromate; 5, chloride; 6, pentanesulfonate; 7, nitrite; 8, bromide; 9, phosphate; 10, nitrate; 11, sulfate; 12, oxalate; 13, octanesulfonate; 14, iodide; 15, tungstate; 16, molybdate; 17, chromate; 18, thiocyanate; 19, perchlorate. Analyte concentrations, 2−150 mg/L.

ate−bicarbonate eluents. Examination of the data for individual analytes revealed that the greatest difference between predicted and observed retention factors occurred for phosphate, which gave a maximum difference of 7.49%. This can be explained by the fact that the eluent pH changes occurring over a search area in which the carbonate mole ratio varied from 10 to 90% were sufficiently high to cause changes in the degree of protonation of phosphate. Such variations are not accommodated in the LSSM-EA, and errors can therefore be expected.

The accuracy of the software in predicting retention data can be further illustrated by the following two examples. First, the software was used to simulate the chromatogram obtainable with an AS14A column using an eluent comprising 10.08 mM total carbonate and a 72% mole ratio of CO_3^{2-}, and a comparison was made with an experimental chromatogram recorded using the same eluent. This eluent composition is well-removed from any of the experimental data points used to solve the LSSM-EA for this system. The experimental and simulated chromatograms are shown in Figure 4. Differences in retention times between the simulated and experimental chromatograms averaged 3.4%. It can be seen that the simulated chromatogram is remarkably close to the experimental chromatogram. This process was repeated for an AS9-HC column using an eluent comprising 16.64 mM total carbonate and a 95.2% mole ratio of CO_3^{2-}, and the results are shown in Figure 5. The average error was 2.0%. In each of these examples, phosphate showed the greatest error (7.5% in Figure 4 and 6.8% in Figure 5).

Finally, it should be stressed that agreement between experimental and predicted retention data does not imply conclusively that the retention theory is correct. Rather, this agreement demonstrates that the LSSM-EA model is a useful practical tool for simulation and optimization purposes.

Features of Virtual Column 2. Virtual Column 2 is a 32-bit Windows program designed to run on any computer with a Pentium class processor or higher. It has the capabilities of simulating all possible chromatograms within the search area defined by the embedded database and determining the optimal eluent composition using one of two available optimization criteria. All potential chromatograms in the search area are ranked according to the chosen criterion, and the results of this ranking are displayed as a response surface.

Virtual Column 2 operates in two modes, isocratic separations using eluents with a single competing ion and isocratic separations using eluents with two competing ions. In a single-eluent species isocratic separation, the only variable is the concentration of the eluent species. Thus, the search area is one dimensional and is bounded by the upper and lower limits of concentration of the eluent, while the response surface is a line graph of the optimization criterion versus eluent concentration. In dual-eluent species

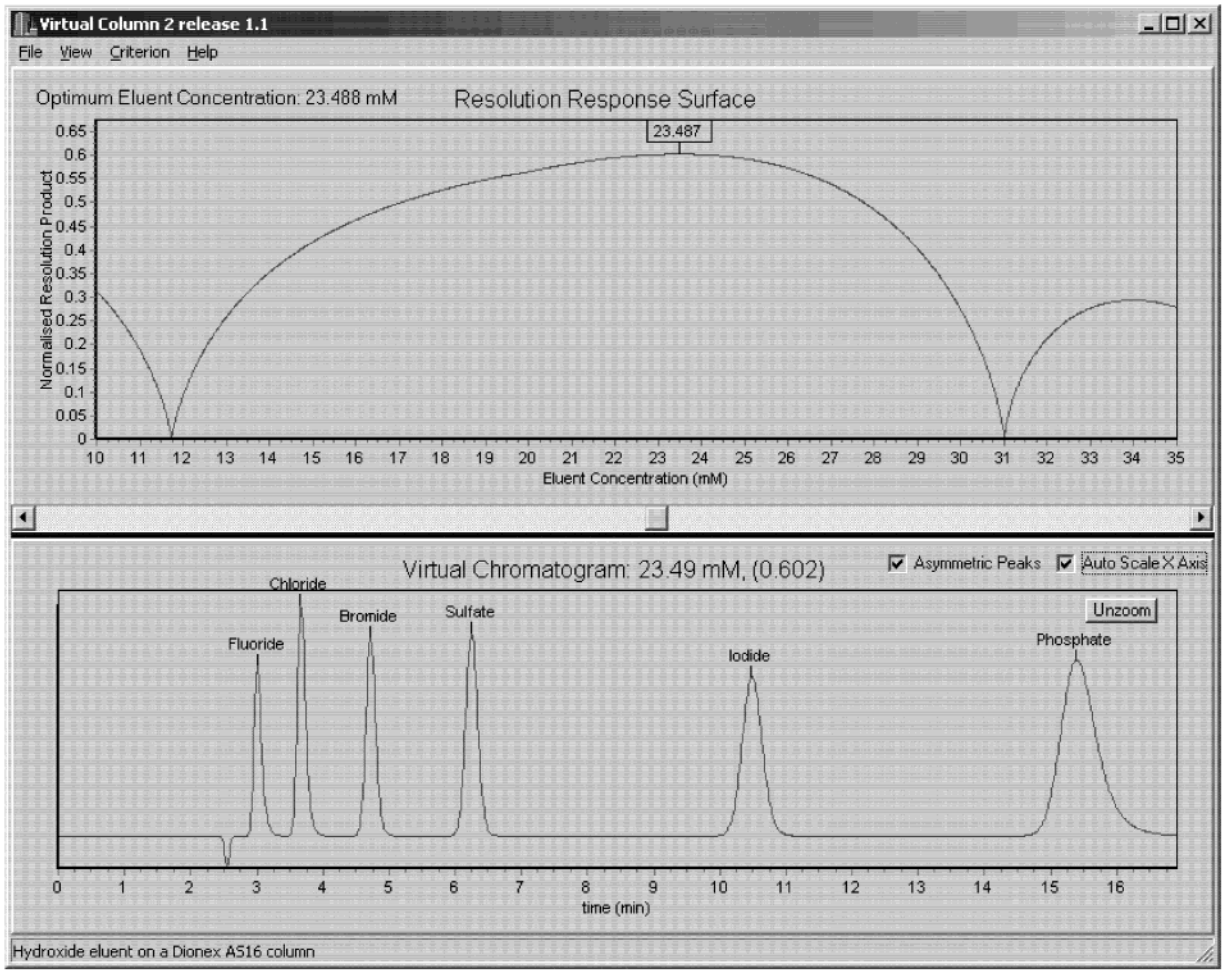

Figure 6. Virtual Column 2 output display (virtual chromatogram and resolution response surface) for a set of six analytes eluted from a Dionex AS16 column with an eluent containing a single competing ion (hydroxide).

isocratic separations, there are two variables, the total eluent concentration and the eluent molar ratio. The search area is therefore two dimensional and takes the form shown in Figure 1, while the response surface is a contour plot or a three-dimensional surface showing the variation of the optimization criterion over the search area.

The software allows the user to customize the separation to suit a desired optimization or simulation. Any number of the analytes included in the databases can be chosen, up to a maximum of 33 analytes for some databases. The relative peak areas of the analytes can be varied to closely resemble the composition of a desired sample. The asymmetry of the eluted peak can be altered to match the actual performance of a column. The peak asymmetry exerts a large influence on resolution,[17,18] and Virtual Column 2 uses an exponentially modified Gaussian function to describe the peak shape, which is considered in the calculation of resolution and in the drawing of peaks in simulated chromatograms. The number of theoretical plates applicable for the column under use can also be varied, and changes to this parameter affect the width of the peaks displayed and, therefore, have a direct impact on both the resolution calculations and the virtual chromatogram. Finally, since differences in the configuration of various systems can cause large variations in the void time, Virtual Column 2 permits the value for the void time to be varied. Default values are included for all these variables.

Operation of Virtual Column 2. When the program starts, the user is presented with a "wizard" screen. All variables required by Virtual Column 2 are entered using this wizard in a series of steps. Step one of the wizard involves selection of the database. Each database is presented as a combination of an eluent and column, e.g., carbonate−bicarbonate on a Dionex AS4A-SC column. All databases available to Virtual Column 2 are displayed, but only one can be selected at any time. Step two involves the selection of analytes to be included in the separation to be simulated or optimized. Any of the analytes available in a database can be selected. Step three is the designation of peak areas to analytes. Each analyte selected in step two has a default peak area that can be altered to reflect the expected peak area on a particular system. Step four is the assignment of peak asymmetry values. Asymmetry is defined as the ratio of the width of the trailing half of the peak divided by the width of the leading half of the peak. The software will accept peak asymmetry values in the range 1−10. Step five involves entering a value for the void time and the number of theoretical plates. Adjustment of the void time allows the effects of eluent flow rate to be matched to a particular system. This step also allows the user to select one of two

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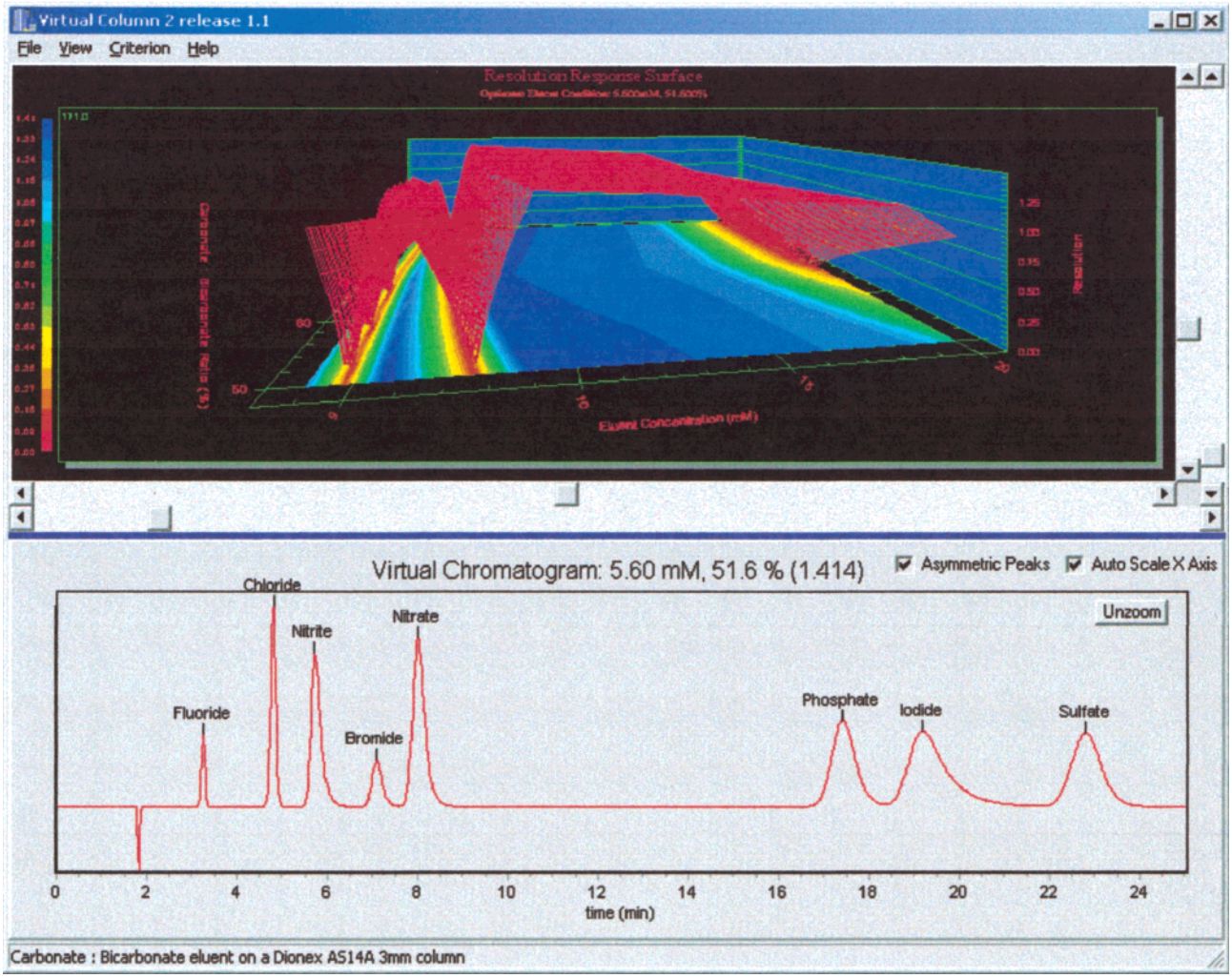

Figure 7. Virtual Column 2 output display (virtual chromatogram and resolution response surface) for a set of eight analytes eluted from a Dionex AS14A column with an eluent containing two competing ions (carbonate and bicarbonate). The response surface shows both a contour plot and a three-dimensional surface showing the variation of the optimization criterion (*r*) over the search area.

optimization criteria, minimum resolution ($R_{s,min}$) or normalized resolution product (r). $R_{s,min}$ quantifies the resolution (R_s) value of the most poorly separated pair of peaks in the chromatogram, whereas r is maximized when all peaks in the chromatogram are evenly spaced over the chromatogram.[19] The optimization criterion can be altered after completion of the wizard, but all other variables cannot be altered once the wizard operation is complete.

Next, Virtual Column 2 calculates retention times for the selected analytes across the entire range of eluent compositions in the search area. For each eluent composition, the retention times are analyzed and a value for the optimization criterion of the entire chromatogram is calculated. These results are then displayed as a response surface in the top half of the screen. In the lower half of the screen, a virtual chromatogram is displayed using the eluent conditions selected as the optimum in the search area. Figure 6 shows this display for an eluent with a single competing ion (in this case, hydroxide) while Figure 7 shows the display for an eluent with two competing ions (in this case, carbonate–bicarbonate). Virtual chromatograms for any other eluent composition in the search area can be obtained by moving the slider bars in the upper window of Figure 6 or 7 or by clicking the mouse anywhere in the search area. The chromatograms are updated instantly.

There are a number of further features available to the user. These include the ability to optimize for the fastest chromatogram giving a selected value of $R_{s,min}$, enlarging the display of a region of the virtual chromatogram in order to scrutinize separation, the eluent composition calculator, a useful tool for providing assistance in preparing eluents, changing the optimization criterion, or displaying the optimization criterion value for the chosen set of analytes for all databases in the software. This latter feature permits the user to determine which eluent/column combination provides the best separation of the chosen mixture.

Uses of Virtual Column 2. The foremost use for Virtual Column 2 is practical method development. There are always new and challenging separations confronting today's chemists, and Virtual Column 2 offers a rapid and inexpensive way of developing a new practical method. Before columns are purchased and time is invested in method development, Virtual Column 2 can provide accurate information about how a particular column and eluent combination will behave with a particular set of analytes. The ability of Virtual Column 2 to provide immediate feedback of the

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effect of changing the eluent and stationary-phase conditions also makes it ideal for education and training purposes.[20] A further use of Virtual Column 2 is the refinement of existing analytical methods to permit them to be adapted to different samples or to increase the speed of separation. Finally, Virtual Column 2 can be used to suggest starting conditions from which conventional trial-and-error optimizations can be conducted.

CONCLUSIONS

Virtual Column 2 provides a rapid and reliable tool for the prediction of retention times in IC, even with eluents containing two competing ions. Chromatograms obtainable at any eluent composition over the stipulated search area can be simulated with a high degree of accuracy and these can be used to identify the optimal separation using either the minimum resolution or normalized resolution product optimization criteria. Finally, Virtual Column 2 provides sufficient flexibility to enable it to be adapted to analytes of differing concentrations and variable peak asymmetry and to columns with varying plate counts and void times.

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