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Computer simulation of ion chromatography separation: an algorithm enabling continuous monitoring of anion distribution on an ion-exchange chromatography column

Marjana Novič*, Jure Zupan, Milko Novič

National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

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Abstract

A computer algorithm for the calculation of ion chromatography separation is presented. It is based on the calculation of equilibrium concentrations of present analyte in discrete column segments. The continuous column is treated as a number of discrete cells or segments where the equilibration process between the stationary phase and the eluent is simulated. The ion-exchange equilibration process is supposed to be instantaneous and quantitative. The continuous flow of the eluent is rendered by discrete transfers. The size of each transfer of the eluent corresponds to a portion of the volume contained in one column segment. The equilibrium calculations in all column segments are repeated for each transfer of the eluent, through all the stages of the chromatographic process. The distribution of the analytes between the stationary phase and the eluent can be monitored at any step and in any column segment which means that the described algorithm provides the spatial and time concentration profiles. The simulated chromatogram is acquired as a time–concentration profile in the last column segment. The obtained chromatograms are in good agreement with the experimental ones. The distribution of ions between the stationary phase and the eluent in the early stages of the ion chromatographic process can thus be studied with confidence.

Keywords: Computer algorithm; On-column distribution; Anions

1. Introduction

Several different approaches have been developed and exploited for the simulation of ion chromatography (IC) separation, mostly based on a defined relationship between the retention factor k and eluent concentration [1–5]. Some attempts were made on a dynamic approach simulation [6–9] in which both spatial and time concentration profiles are calculated. The simulation of an ion chromatogram is a valuable tool in the research and application of IC methods in analytical chemistry for at least two reasons. First, it helps to understand the processes going on during the sample flow through the analytical column, and second, it serves for optimization of the experimental conditions in IC separation. Since IC is one of the sub-branches in a broad area of chromatographic methods, the basis for the simulation algorithm can be obtained from the theoretical principles of widely used and elaborated liquid chromatography [10].

The algorithm for computer simulation of IC

*Corresponding author. National Institute of Chemistry, Hajdrihova 19, P.O. Box 660, SI-1001 Ljubljana, Slovenia. Tel.: +386-1-4760-253; fax: +386-1-4259-244.

E-mail address: marjana.novic@ki.si (M. Novič).

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separation presented in this work is based on the plate theory [11], which offers a simple but efficient description of the chromatographic process. The continuous column is treated as a number of discrete segments of equal length (theoretical plates) where an instantaneous equilibration process is simulated. The equilibration process of ions between the eluent and the stationary phase has to be dealt specifically for ion chromatography, but the discretization of the continuum process is the same as in liquid chromatography [10,12]. Similarly to the Craig distribution model [13,14], the basis of the algorithm proposed here is the discontinuous plate model. However, in contrast to the Craig distribution model, the proposed algorithm produces a final time–concentration profile calculated from all individual equilibrium stages through all spatial and time coordinates. Therefore, not only the retention times but also peak shapes that could be significantly asymmetrical are obtained. Because of the dynamic calculation approach, the calculated distribution of the anions between the stationary phase and the eluent can be monitored through the whole chromatographic process and for different experimental conditions. This means it is possible to follow up the process from the initial steps of the sample injection to the final steps when all the analytes are eluted from the column. Calculated concentration profiles can be dissected into contributions from different sources and stages of the separation process, according to the intermediate results of the simulation model. Different phenomena in IC, for example various matrix effects [15–17] can be thus studied in detail.

1.1. Theoretical basis of the model

The proposed model is a dynamic calculation approach estimating the ion-exchange equilibrium condition of all the analyte and eluent ions present in the chromatographic column. The chromatographic column is divided into N segments, later on associated with spatial coordinate z. The calculations are done on discrete column segments containing defined portions of volume of stationary phase and eluent, V_s/N and V_m/N, respectively. The eluent is assumed to progress in discrete steps, corresponding to time coordinate p. In each time step a portion of the eluent contained in each zth segment is transferred to the next segment $z+1$.

The basic ion-exchange equilibrium which has to be determined in each column segment for all analyte ions is defined by Eq. (1):

$$\sqrt[y]{K_E^{A^i}} = \frac{^{eq}(A_i^{x_i-}) \cdot \{^{eq}[E^{y-}]\}^{x/y}}{^{eq}[A_i^{x_i-}] \cdot \{^{eq}(E^{y-})\}^{x/y}} \quad i = 1 \ldots n \qquad (1)$$

where the following definitions are used:

$K_E^{A^i}$ is the selectivity constant of the ion-exchange reaction.

$^{eq}(A_i)$ is the equilibrium concentration of the ith analyte A_i in the stationary phase.

$^{eq}[A_i]$ is the equilibrium concentration of the ith analyte A_i in the eluent.

$^{eq}(E)$ is the equilibrium concentration of the eluent component E in the stationary phase.

$^{eq}[E]$ is the equilibrium concentration of the eluent component E in the eluent.

x_i is the absolute value of the analyte ion's charge.

y is the absolute value of the eluent ion's charge.

() denotes the expression of concentrations in the stationary phase.

[] denotes the expression of concentrations in the eluent.

The equilibrium between the stationary phase and the eluent is supposed to be achieved instantaneously, taking into account only the ion-exchange reaction. The equilibrium is distorted after each transfer of the eluent; the equilibrium concentrations of the ions in the eluent calculated in one column segment are transferred to the next segment, which contains different amount of ions in the stationary phase. Once a hypothetical steady-state condition after the transfer has been achieved, equilibrium concentrations have to be recalculated using Eq. (1), considering that total amount of ions in both phases have changed. Because four unknown quantities, i.e., the eluent component ions and the analyte ions concentrations in two phases, are related by Eq. (1), an additional three independent equations are needed for their resolution. Three conditions described by new equations that enable one to obtain an analytical solution of Eq. (1) have to be fulfilled.

Condition 1: The total amount of species in both phases before and after the equilibration in the intermediate state between two consecutive transfers of the eluent must remain constant:

$$\alpha^{\text{init}}(A_i^{x_i-}) + {}^{\text{init}}[A_i^{x_i-}] = \alpha^{\text{eq}}(A_i^{x_i-}) + {}^{\text{eq}}[A_i^{x_i-}] \quad (2)$$

The stationary phase concentrations are multiplied by α, the ratio of the volumes of stationary phase and eluent (V_s/V_m), in order to obtain additive terms proportional to the molar quantities of present ions. The superscript ''init'' denotes the initial concentrations acquired in both phases of the column segment z after the transition of the eluent, before the equilibrium is achieved. Initial concentrations ${}^{\text{init}}(A_i^{x_i-})$ and ${}^{\text{init}}[A_i^{x_i-}]$ in each step s are obtained from the calculations of a previous step $s-1$, while those of the first step ($s=1$) are introduced by the experimental conditions (concentration of the eluent ions and concentrations of the analyte ions in the sample).

$${}^{\text{eq}}(A_i^{x_i-})^{s-1,z} = {}^{\text{init}}(A_i^{x_i-})^{s,z} \quad (2a)$$

Eq. (2a) gives the expression for the stationary phase concentrations of the ith analyte ion in two consecutive steps, $s-1$ and s. The initial concentrations in the sth step are known as equilibrium concentrations of previous step ($s-1$). There is no transfer through the stationary phase, therefore index z is equal on both sides of Eq. (2a).

In general, the transfer of an analyte is defined by the mass balance equation:

$$f_1^{\text{eq}}[A_i^{x_i-}]^{s-1,z-1} + {}^{\text{eq}}[A_i^{x_i-}]^{s-1,z} - f_2^{\text{eq}}[A_i^{x_i-}]^{s-1,z} = {}^{\text{init}}[A_i^{x_i-}]^{s,z} \quad (2b)$$

with parameters f_1 and f_2 defining the fraction of the amount of ions transferred in two consecutive steps. Eq. (2b) becomes equal to Eq. (2c), if both parameters, f_1 and f_2 are equal to one:

$${}^{\text{eq}}[A_i^{x_i-}]^{s-1,z-1} = {}^{\text{init}}[A_i^{x_i-}]^{s,z} \quad (2c)$$

This means that a 100% amount of the analyte $A_i^{x_i-}$ contained in the eluent in the segment $z-1$ is transferred to the next segment z. Thus the indices $z-1$ and z on the left and right sides of Eq. (2c), respectively, reflect the spatial movement of the ion $A_i^{x_i-}$ in the eluent.

Condition 2: The relationship between the *initial* and the *equilibrium* concentrations of the eluent components ions in the stationary phase and in the eluent should be expressed in the same way as for the analyte ions:

$$\alpha^{\text{init}}(E^{y-}) + {}^{\text{init}}[E^{y-}] = \alpha^{\text{eq}}(E^{y-}) + {}^{\text{eq}}[E^{y-}] \quad (3)$$

$${}^{\text{eq}}(E^{y-})^{s-1,z} = {}^{\text{init}}(E^{y-})^{s,z} \quad (3a)$$

$${}^{\text{eq}}[E^{y-}]^{s-1,z-1} = {}^{\text{init}}[E^{y-}]^{s,z} \quad (3b)$$

Condition 3: The electroneutrality of the stationary phase has to be fulfilled. It means that all the ion-exchange sites of the stationary phase must be occupied either by the analyte or by the eluent ions. Since the ion-exchange retention (Q) of a given volume of the stationary phase is known or can be experimentally determined, the following expression correlates the concentrations in the stationary phase:

$$\frac{1}{y}\cdot\frac{Q}{V_m} = \alpha^{\text{eq}}(E^{y-}) + \sum_i \frac{x_i}{y}\cdot\alpha^{\text{eq}}(A_i^{x_i-}) \quad (4)$$

x and y being the absolute charges of the analyte and eluent ions, respectively.

Eqs. (1)–(4) completely describe the system in one column segment. In our procedure they are applied to every column segment for each transfer (step of the eluent movement s). At each step s the unknown quantities are the equilibrium concentrations, while the initial concentrations (superscript ''init'') are obtained in former steps $s-1$ according to the mass balance Eqs. (2a)–(2c) and (3a) and (3b) as described above.

For one analyte ($i=1$) an analytical solution of the above system of four equations can be found. The unknown quantities are the equilibrium concentrations ${}^{\text{eq}}[A^{x-}]$, ${}^{\text{eq}}(A^{x-})$, ${}^{\text{eq}}[E^{x-}]$, and ${}^{\text{eq}}(E^{x-})$. The column retention Q is a constant given for each ion-exchange column, while the dynamic selectivity constant K_E^A is obtained from fitting the retention equations to experimental chromatogram. Every combination of the analyte/eluent charge ratio gives a different solution of the system. In the simple case of the charge ratio $y/x=1$, one obtains a quadratic equation with the following solution for ${}^{\text{eq}}[A^{x-}]$:

$${}^{\text{eq}}[A^{x-}] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (5)$$

$$a = (K_E^A - 1) \quad (5a)$$

$$b = (K_E^A - 1)\cdot Q + (2 - K_E^A)\cdot\left\{\alpha^{init}(A^{x-}) + {}^{init}[A^{x-}]\right\} + \alpha^{init}(E^{y-}) + {}^{init}[E^{y-}] \quad (5b)$$

$$c = \left\{\alpha^{init}(A^{x-}) + {}^{init}[A^{x-}]\right\} \cdot\left\{Q - \alpha^{init}(E^{y-}) - {}^{init}[E^{y-}] - \alpha^{init}(A^{x-}) - {}^{init}[A^{x-}]\right\} \quad (5c)$$

Once the concentration of the analyte in the eluent has been obtained, other three unknowns can be easily calculated from Eqs. (2), (3) and (4). The described system (Eqs. (1)–(4)) which can be solved analytically for one analyte ion ($i=1$), is proposed to be solved for n ions ($i=1\ldots n$) iteratively. In the case of contemporary presence of more than one ion in the sample, the number of equations is increased:

For n ions Eqs. (1) and (2) must be repeated n times;

Eq. (3) does not depend on n;

In Eq. (4) for each new analyte ion one contribution is added to the total sum.

During the search for a solution of one individual ion i, the concentrations of all others have to be known ($j=1\ldots n,\ j\neq i$). The analytically expressed concentration of the ith ion in the eluent is again a solution of a quadratic equation, however, the coefficients b and c include also all the concentrations of other present ions in the eluent (see Eqs. (6)–(6c)). With other words, to determine the concentration of the ith analyte in the eluent, $[A_i^{x_i-}]$, one has to know the concentrations of all others, $[A_j^{x_j-}]$, $j=1\ldots n,\ j\neq i$:

$${}^{eq}[A_i^{x_i-}] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (6)$$

$$a = (K_E^{A_i} - 1) \quad (6a)$$

$$b = (K_E^{A_i} - 1)\cdot Q + (2 - K_E^{A_i}) \cdot\left\{\alpha^{init}(A_i^{x_i-}) + {}^{init}[A_i^{x_i-}]\right\} + \alpha^{init}(E^{y-}) + {}^{init}[E^{y-}] - (K_E^{A_i} - 1)\cdot\sum_{j=1, j\neq i}^{n}\left\{\alpha^{init}(A_j^{x_i-}) + {}^{init}[A_j^{x_i-}] - {}^{eq}[A_j^{x_i-}]\right\} \quad (6b)$$

$$c = \left\{\alpha^{init}(A_i^{x_i-}) + {}^{init}[A_i^{x_i-}]\right\} \cdot\left\{Q - \alpha^{init}(E^{y-}) - {}^{init}[E^{y-}] - \alpha^{init}(A_i^{x_i-}) - {}^{init}[A_i^{x_i-}] - \sum_{j=1, j\neq i}^{n}(\alpha^{init}(A_j^{x_j-}) + {}^{init}[A_j^{x_j-}] - {}^{eq}[A_j^{x_j-}])\right\} \quad (6c)$$

In the iteration procedure, for the initial guess of the concentrations in the eluent $-[A_j^{x_j-}]$, $j=1\ldots n$, $j\neq i$, the values obtained from previous step are taken. Once an $[A_i^{x_i-}]$ for the ith ion is calculated from Eq. (6), it becomes an initial guess in the calculation of the next, $i+1$ first ion. In one iteration cycle the concentrations of all n ions are determined. The next cycle starts with the new initial guesses and the procedure is terminated when the difference between the concentrations of the ith ion calculated in two consecutive cycles does not exceed a predefined threshold value. The steps at the beginning of the chromatographic process in which different ions are not yet separated, are computationally the most intensive because many cycles of the iterative procedure are needed to achieve convergence of the equilibrium concentrations of present ions.

2. Experimental

2.1. Reagents and standard solutions

All the reagents used in this study were of analytical-reagent grade (Merck, Darmstadt, Germany). Only the synthetic samples containing nitrite and nitrate in water were analysed. The stock solutions of NO_2^- and NO_3^- (1.000 g l^{-1}) were prepared by dissolving appropriate amounts of $NaNO_2$ and $NaNO_3$ (dried at 103°C) in water. The stock chloride eluent was prepared by 2.925 g of NaCl in 1 l of water. Working eluent solution was prepared by on-line dilution of stock chloride-based eluent with deionised water (18 MΩ cm^{-1}; Millipore, Bedford, MA, USA) using a quaternary analytical pump.

2.2. Apparatus

For all measurements in the study, the Dionex

4000i system (Sunnyvale, CA, USA) equipped with the Dionex IonPac AG4A-SC (50×4 mm) guard column and the IonPac AS4A-SC (250×4 mm) separation column was used. The chromatograms were obtained using the UV–Vis spectrophotometer Spectra-Physics SpectraSYSTEM (Fremont, CA, USA). The sample-loop volume was 50 μl. The eluent flow-rate was 2 ml min^{-1}.

The data for further evaluation were obtained by exporting the appropriate chromatograms as ASCII files which were further handled using Microcal Origin (Microcal Software, USA) software package.

2.3. Computational

The program for simulation of IC separation is written in FORTRAN 95 for Windows and runs on a personal computer with a Pentium II processor. To simulate a chromatogram of a sample of two ions with retention times around 2 min at flow-rate 2 ml min^{-1}, computational times of 10, 34, or 130 s are needed if the column (AS4) is assumed to be parceled into 1000, 2000, or 4000 segments (theoretical plates), respectively. The calculation time for equal number of column segments increases linearly with increasing retention times.

3. Results and discussion

3.1. The algorithm and model parameters

The flowcharts of the algorithm for the simulation of a step-wise progression of ion chromatography separation and the calculation of the equilibrium concentrations are shown in Fig. 1 and in Fig. 2, respectively. The program simulates the propagation of ions through a grid in which one axis is proportional to the time coordinate (time axis, i.e., transfer steps), while the other to the spatial coordinate (spatial axis, i.e., column segments). Thus the final result of such a calculation is a set of concentration values written in a matrix form with $n_s^{end} \times N$ elements (n_s^{end} is the final number of step-wise movements of the eluent through the chromatographic column and N is the number of column segments). Each row of the matrix corresponds to one step progress of the eluent through the chromatographic column; the number of steps n_s^{end} should be large enough to enable the elution of the most strongly retained anion.

In the proposed simulation program the migration of the analyte ions through the analytical column is decomposed into two contributions (named horizontal and vertical), which are supposed to be separated. The horizontal movement is direct transfer of ions by the flow of the eluent (Eqs. (2b), (2c) and (3b)). During the horizontal movement the contact between the two phases is artificially disabled. Horizontal movement is supposed to be performed instantaneously. After each step an artificial pause is inserted in which the contact between the two phases is restored so that the vertical movement (equilibration) is enabled (Eq. (1)). The duration of such a pause is set to the value t_0/N, where t_0 is experimentally obtained void volume elution time (flow-rate 2 ml min^{-1}) and N is the number of column segments. Such an adaptation of the time steps is necessary to enable the direct comparison of the calculated and experimentally obtained chromatograms.

The model parameters that have to be provided at the beginning of the simulation procedure are column retention Q, column void volume V_m, the eluent ions concentration [E], and the concentrations of the individual ions in the sample [A_i]. The number of theoretical column segments N and the approximate dynamic selectivity coefficients K_A^E have to be set as the initial guess. They are later adjusted to achieve the best fit with the experimental chromatograms. Only one experimental chromatogram is necessary to determine the selectivity coefficients of the complete set of anions of which separation is modelled by the proposed algorithm. By tuning the dynamic selectivity coefficient the retention times of the individual anions are accommodated to the optimal value, while by changing the number of the theoretical column segments the baseline peak width is adapted to the one obtained in the real experiment (see Fig. 3). In order to obtain the appropriate detector response factors, maximal peak height of the calculated peak is divided by the maximal value of the experimental peak. In this way the ordinate units (sum of molar concentrations of eluted ions) become comparable to the experimentally measured AU values.

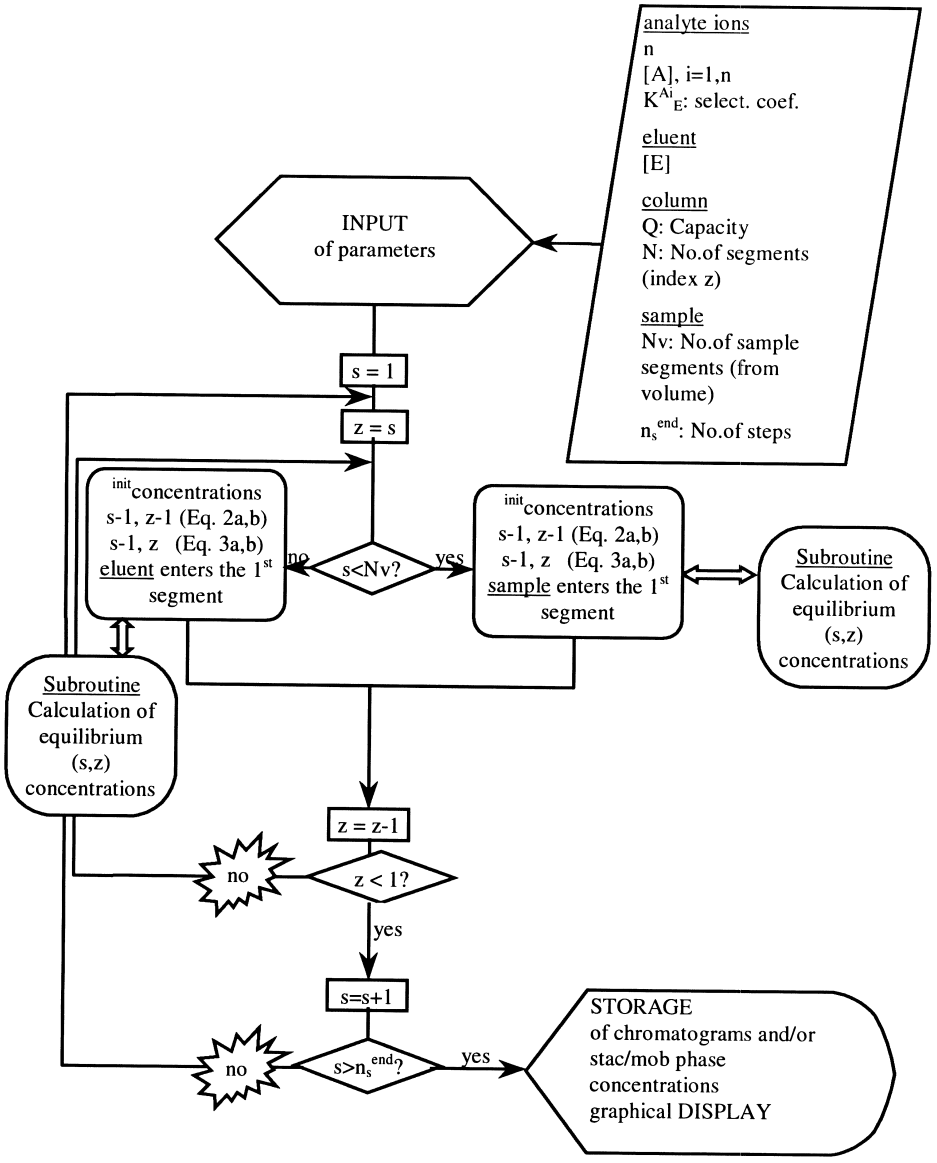

Fig. 1. The flowchart of the computer program for the simulation of the ion chromatography separation.

As discussed already, the number of column segments (N) influences the peak shape, mainly the baseline peak-width. The results of two segmentations, $N=2000$ and $N=4000$, are shown in Fig. 3. The latter choice (thin dashed line) gives a better match between the calculated and experimental peaks regarding baseline widths. The units of the detector response are indicated on the right, while the calculated molar concentration on the left axis of Fig. 3.

3.2. On-column monitoring of the separation procedure

The chromatography procedure is basically di-

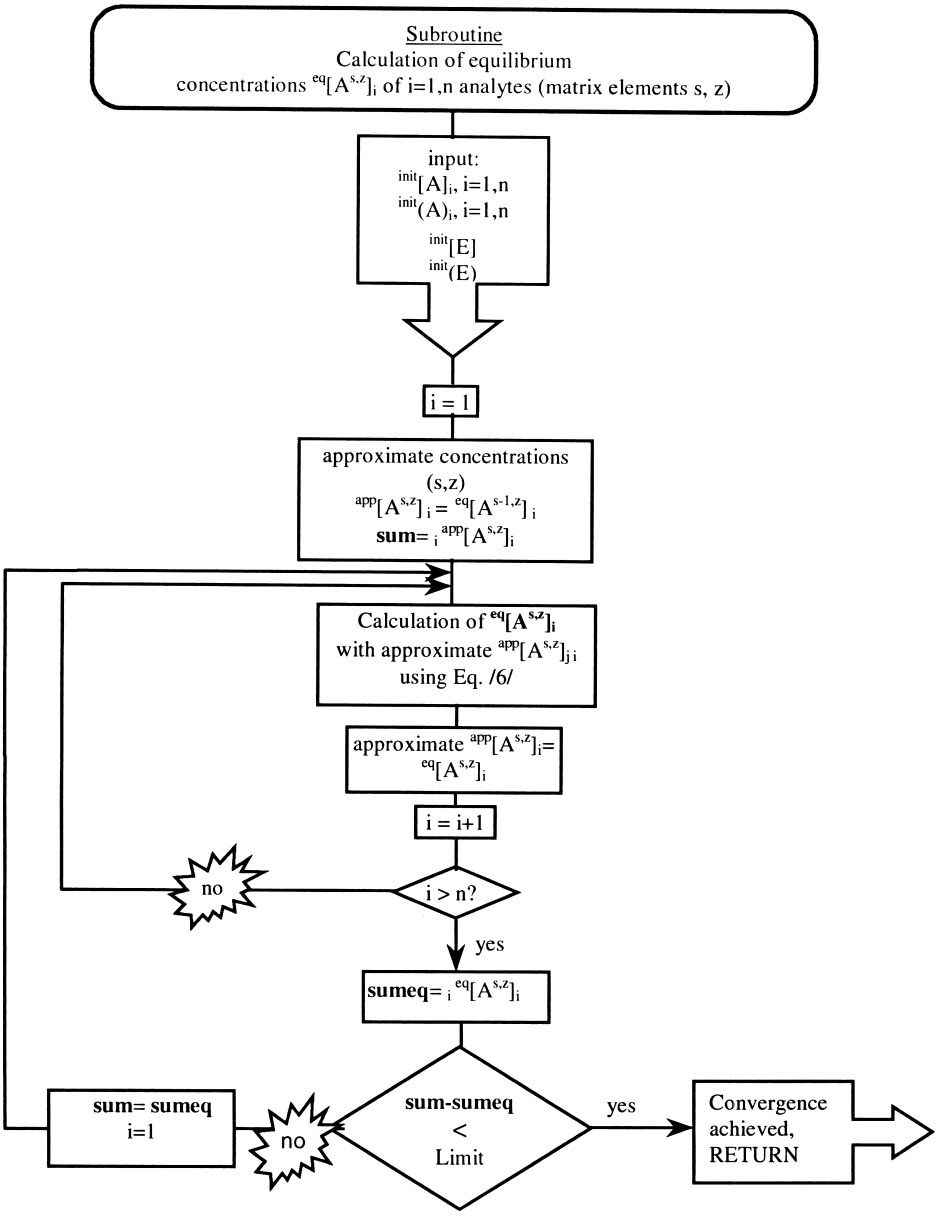

Fig. 2. The flowchart of the subroutine calculating equilibrium concentrations.

vided into two most important phases: the initial extraction of the analytes from the sample plug on the stationary phase and the separation/elution of the analytes by the eluent. The initial extraction is fast and performed in first few column segments if the sample contains no complex matrix. The details of the described steps are demonstrated in the simulation example shown in Fig. 4.

The calculations were done using the input parameters as follows: chloride eluent was 30 m*M* Cl^-, dynamic selectivity constants for nitrite (2.15) and nitrate (4.09), the number of column segments for

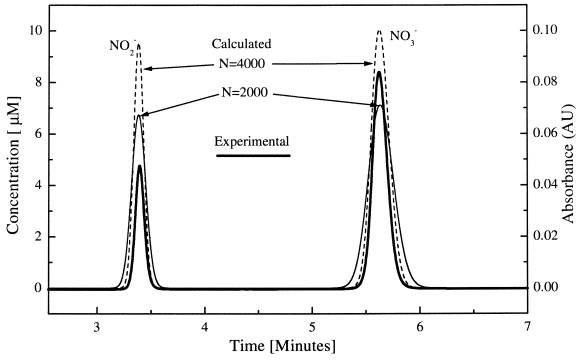

Fig. 3. Comparison of experimental (thick line) and two simulations of ion-exchange chromatograms of NO_2^- (2 mg l^{-1}) and NO_3^- (5 mg l^{-1}) using chloride-based eluent (8.6 m*M* Cl^-). The adjusted selectivity constants $K_{NO_2^-,\,Cl^-}$ and $K_{NO_3^-,\,Cl^-}$ have values of 2.15 and 4.09, respectively, while the number of theoretical segments were set to $N=2000$ (broader peaks) and $N=4000$ (narrower peaks). The total ion-exchange column retention was 20 μequiv. (AS4A-SC), the sample volume was 50 μl.

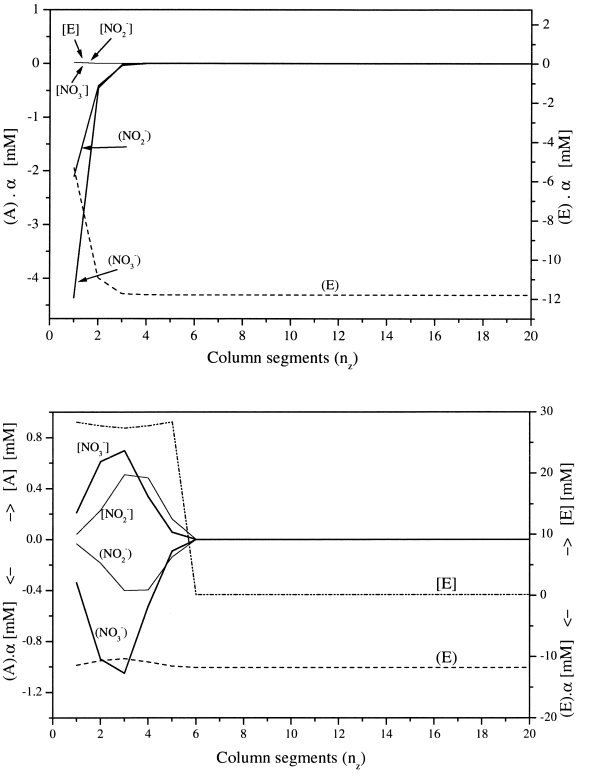

Fig. 4. The distribution of the eluent and analyte ions between the stationary phase and the eluent after 59 (a) and 65 (b) discontinuous eluent movements (time steps n_s) has been performed. The stationary phase concentrations are plotted as negative values. Other experimental conditions as in Fig. 3.

the calculations was $N=2000$ and the sample volume was 50 μl which corresponded to 59 column segments (column void volume was 1.7 ml).

During the first 59 step-wise eluent movements ($n_s=1\ldots59$), the water solution of the sample was introduced into the system (concentration of the eluent component ions is zero, no sample dispersion). After 59 steps, the analytes extracted by the stationary phase, were still covered by the matrix (water), see Fig. 4a. During the water sample introduction the concentrations of the eluent component ions as well as of the analytes in the eluent are close to zero. Because the anions concentrations are low compared to the column retention, the analytes ''stick'' to the stationary phase of the first few column segments until the eluent enters the column and the separation process is started. In Fig. 4b the situation on the column after 65 time steps when the eluent started to enter the column is shown. In the 60th time step the eluent began to flow into the system and in the 65th step reached the sixth column segment what is evident from plot (b). Already after six step-wise movements since the sample has been introduced into the column ($59<n_k<65$), the NO_2^- ions with lower selectivity constant begin to ''overtake'' the NO_3^- ions, as can be seen from Fig. 4b.

The proposed algorithm allows the on-column tracing of the distribution of the analytes between the stationary phase and the eluent at any time step and in any column segment. In Fig. 5 the distribution of NO_2^- and NO_3^- ions between the stationary phase and the eluent is shown for 65 (a), 100 (b), 150 (c), 200 (d), and 2000 (e) time steps (n_s). The calculated concentrations of ions in the eluent and in the stationary phase are plotted above (with positive sign), and below (with negative sign) the abscise, respectively. It can be seen that already in the 200th step (d) the two ions are practically separated. Fig. 5f shows the resulting chromatogram obtained after 6000 time steps. The time coordinate and the response factor of the absorption units are defined on the same way as described for the example shown in Fig. 3.

From Fig. 5a–e it is evident that with increasing

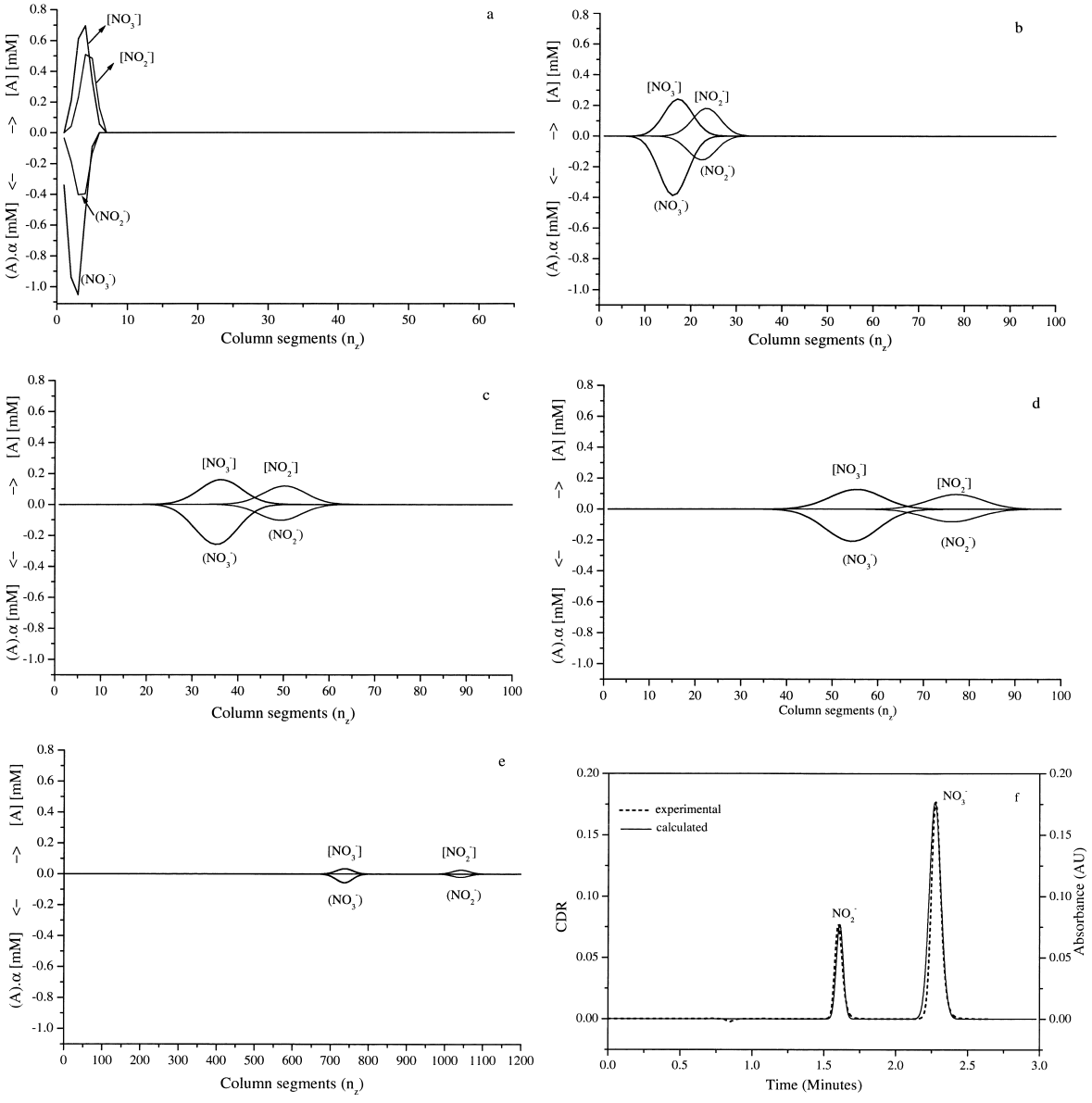

Fig. 5. Simulation of on-column ion chromatography separation process of 2 mg l^{-1} NO_2^- and 5 mg l^{-1} NO_3^- with chloride-based eluent (30 m*M* Cl^-). Plots (a) to (e) represent the progress of the separation after 65 (a), 100 (b), 150 (c), 200 (d), and 2000 (e) steps n_s. In (f) the comparison of the experimentally obtained and calculated chromatogram is presented. The calculated detector response (CDR) is obtained by multiplication of the calculated concentrations by the response factors for individual ions. The calculated chromatogram was extracted as a set of 6000 elements ($n_s = 1 \ldots 6000$) calculated in the 2000th column segment n_z. All other experimental conditions as in Fig. 3.

number of steps the peak heights are decreasing while the peak widths are enlarging. In agreement with the theoretical background, the peaks are becoming lower and broader, at constant peak areas.

3.3. The influence of the eluent ions concentrations on the retention times

The simulated chromatograms show a correct

dependence of the retention times on the eluent ions concentrations. To compare the relationships of the log k (retention factors determined experimentally or from calculated chromatograms) and log [E], a set of nine chromatograms of a two component sample, 2 mg l^{-1} NO_2^- + 5 mg l^{-1} NO_3^-, using nine different eluent ions concentrations was recorded. With the simulation program nine chromatograms for the set of nine different eluent concentration values were calculated. In Fig. 6a the experimental and calculated log k versus log [E] are shown. The eluent (Cl^-) concentrations were in the range from 3 to 150 mM. All the calculations were performed with $N=2000$ column segments, while the largest number of necessary time steps was 45 000 in the case of the weakest eluent (3 mM Cl^-). The selectivity constants used for all calculations were 2.15 and 4.09 for NO_2^- and NO_3^- ions, respectively, and were determined from one the experimental chromatogram shown in Fig. 3. The chosen number of column segments, $N=2000$, is not the best choice for all eluent ions concentrations; with this number of column segments the best match with the experimental peak shapes is obtained for the eluent ions of concentration of 30 mM in which the retention time for NO_3^- ions is 2.3 min what requires 41 s of CPU time. For higher eluent ions concentrations the peaks simulated with $N=2000$ were too narrow, while for lower eluent ions concentrations they were too broad. However, for the study of the influence of the eluent ions concentrations on the retention times only (not peak shapes) the simulations were done with the same number of column segments.

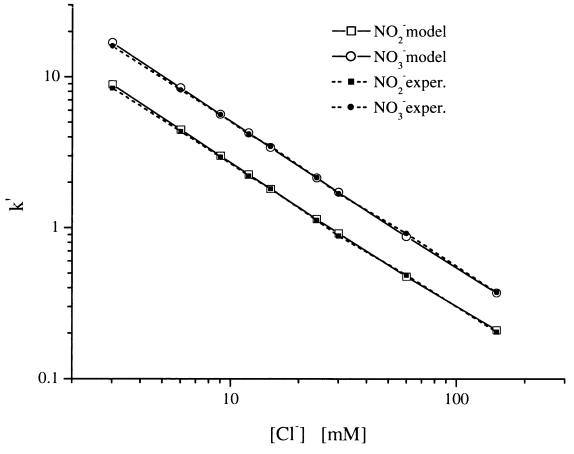

Fig. 6. (a) The comparison of the influence of the eluent concentration [E] on the retention factor k $[(t-t_0)/t_0]$ obtained experimentally (open symbols) and from calculated chromatograms (filled symbols). The chromatograms from which the experimental k values were extracted were obtained using the sample containing NO_2^- (2 mg l^{-1}) and NO_3^- (5 mg l^{-1}) and chloride-based eluent with concentrations 36, 9, 12, 15, 24, 30, 60, and 150 mM. The same concentration values were also used for the calculation of the nine chromatograms from which the "model" k were extracted. 2000 column segments were used for simulations, the other parameters were as in Fig. 3.

The number of column segments affects the peak widths of the calculated chromatograms. Smaller N–s would yield broader peaks and vice versa. Very large N–s give rise to the peaks that are too narrow compared to the experimental ones, however, with increasing N the peak widths show an exponential decay towards the limiting value larger than zero. To obtain the limiting peak widths of simulated chromatograms, the calculations using column dissections with N up to 70 000 were tried and the calculation time prolonged to several hours. It is anticipated that using very large number of column segments, the errors induced by the discretization of the chromatographic process of the proposed model are minimized. Additional mechanisms should be included to simulate the correct peak shapes.

4. Conclusions

The computer program based on the presented algorithm offers the possibility to calculate the distribution of ions between the stationary phase and the eluent at any stage of the separation process. The distribution of the analytes between the stationary phase and the eluent can be monitored at any time and in any column segment. Theoretical chromatograms can be calculated for different column lengths and different eluent and analyte concentrations. The calculated chromatograms are in good agreement with the experimental ones. A good correlation between the experimental and the simulated chromatograms found for the nitrite/nitrate ions and chloride eluent confirms the reliability of the calculated time and spatial profiles in the column. The approach of finding the analytical solution of the system of equations defining the equilibrium provides a very fast algorithm for the simulation pro-

gram that can be of great help in the routine work as well.

Different phenomena in ion chromatography can be studied by implementation of the proposed algorithm: the peak asymmetry at usual and at high concentrations, the self-elution mechanism, the influence of matrix on the ion separation process, etc. Sample-induced micro-gradient elution and on-column change of the eluent and are two challenging applications that could be studied in details; the research in this direction is in progress. Simulation of gradient elution can be achieved by minor changes in the algorithm and preliminary results are encouraging.

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References

[1] J.E. Madden, Critical Comparison of Retention Models for Use in Computer Aided Interpretive Optimisation of Separation in Anion Chromatography, Ph.D. Thesis, University of Tasmania, 1999.
[2] J.E. Madden, P.R. Haddad, J. Chromatogr. A 850 (1999) 29.
[3] J. Havel, J.E. Madden, P.R. Haddad, Chromatographia 49 (1999) 481.
[4] K. Ando, A. Yamamoto, M. Nishimura, K. Hayakawa, J. Chromatogr. A 789 (1997) 43.
[5] A. Yamamoto, K. Hayakawa, A. Matsunaga, E. Mizukami, M. Miyazaki, J. Chromatogr. 627 (1992) 17.
[6] H. Sato, Anal. Chem 62 (1990) 1567.
[7] H. Sato, H. Watanabe, React. Polym. 17 (1992) 1.
[8] M. Novič, B. Čeh, B. Pihlar, IICS'98 – International Ion Chromatography Symposium'98, Osaka, 28 September–1 October 1998, final program.
[9] B. Čeh, M. Novič, IICS'98 – International Ion Chromatography Symposium'98, Osaka, 28 September–1 October 1998, final program (42).
[10] G. Guiochon, S. Golshan-Shirazi, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.
[11] H. Small, Ion Chromatography, Plenum Press, New York, 1989.
[12] B. Lin, S. Golshan-Shirazi, G. Guiochon, J. Phys. Chem. 93 (1989) 3363.
[13] L.C. Craig, J. Biol. Chem. 35 (1941) 1358.
[14] L.J. Craig, J. Biol. Chem. 155 (1944) 519.
[15] M. Novič, B. Divjak, B. Pihlar, V. Hudnik, J. Chromatogr. A 739 (1996) 35.
[16] M. Novič, B. Lečnik, V. Hudnik, B. Pihlar, J. Chromatogr. A 764 (1997) 249.
[17] M. Novič, B. Divjak, B. Pihlar, J. Chromatogr. A 827 (1998) 83.