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Modelling of elution–extrusion counter-current chromatography using perfect replacement approach

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ABSTRACT

Basing on the perfect replacement approach the equilibrium cell model is developed to describe the separation process in elution–extrusion counter-current chromatography (EECCC). As is known, EECCC consists of three steps: classical elution, sweeping elution, and extrusion. The perfect replacement approach means that during sweeping elution step, the mobile phase contained in the column moves and interacts with the "old" stationary phase in the same mode as during the classical elution step; the "new" and "old" stationary phases do not mix; and after the contacting with the mobile phase the concentration of solutes in the "old" stationary phase remains constant and this stationary phase volume is pushed ahead to the exit of the column. Equations are presented allowing the simulation of the chromatogram of solutes eluted from the column with the mobile phase during the elution period and the chromatogram of solutes pushed out of the column with the stationary phase during the extrusion period of EECCC. These equations can help to choose the optimal conditions for conducting elution–extrusion counter-current chromatography.

1. Introduction

One of the specific features of counter-current chromatography (CCC) is the stationary phase mobility [1–9]. Taking advantage of the mobility of the stationary phase in the CCC columns, elution–extrusion counter-current chromatography (EECCC) combines classical chromatographic elution with stationary-phase extrusion to save large amounts of solvents and considerably reduce the time of experiments in cases where the retention volumes of solutes are too high [8,9]. EECCC consists of three steps: the first step is a classical elution, the second – sweeping elution, and the third – extrusion [9]. After the switch volume V_{CM} of mobile phase has eluted from the column, the solvent reservoir of the liquid pump system is switched from mobile phase to stationary phase, and sweep elution begins, during which only mobile phase leaves the column. The sweep elution step is completed after the volume $V_{ss} = V_{CM} + V_M$ of mobile phase has eluted from the column (here V_M is the mobile phase volume contained in the column during classical elution). During extrusion step the volume of stationary phase equal to V_s (the stationary phase volume contained in the column during classical elution) with all the solutes remaining in it is pushed out of the column. Thus, the separation of solutes in EECCC occurs in two ways: the solutes with lower retention volumes are separated by elution with the mobile phase (during the first and second steps) and the solutes with higher retention volumes are separated inside the column before leaving it and pushed out of the column with the stationary phase (during the extrusion step). Thus, the full recovery of all solutes contained in a sample can be achieved.

The EECCC method takes particular advantage of the fact that inside a CCC column solutes are moving in the form of narrower bands than they are eluting with the mobile phase.

Theoretical analysis of the three steps of the EECCC providing equations for retention volumes, peak widths, resolution factors, and distribution constants is carried out in [9]. The theory of EECCC is based on what can be called as perfect replacement approach: During the sweeping elution period, the mobile phase contained in the column moves and interacts with the "old" stationary phase in the same mode as during the classical elution period. The "new" stationary phase replaces the mobile phase in plug-flow mode.

The aim of this work is to extend the theoretical treatment to develop mathematical description of the chromatogram of solutes eluted with the mobile phase during the classical and sweeping elution periods and the chromatogram of solutes pushed out of the column with the stationary phase during extrusion period of EECCC.

2. Transport and separation of solutes inside a chromatographic column

We will use the equilibrium cell model to describe the transport and separation of solutes inside a chromatographic column. This model takes into account (in the number of cells) the com-

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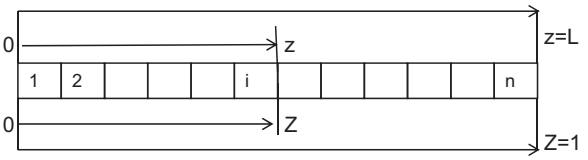

Fig. 1. Schematic diagram of the staged chromatographic column.

bined effect of axial mixing and interphase mass transfer and in mathematical description is identical to the plate theory of chromatography [10–14].

Consider a chromatographic column consisting of n ideally mixed equilibrium cells, i.e. the total length of the column L is divided into n stages (Fig. 1), in each of which an equilibrium concentration distribution between two liquid phases is reached. After the sample, containing Q amount of a solute, has been injected into the first cell, the residence time distribution of the solute in this chain of equilibrium cells, can be described as follows:

$$X(i,t) = \frac{x(i,t)}{\bar{x}} = \frac{n^i}{(i-1)!} p^i t^{i-1} \exp(-npt) \quad (1)$$

$$Y(i,t) = \frac{y(i,t)}{\bar{x}} = K_D X(i,t) \quad (2)$$

$$p = \frac{1}{1 - S_f + S_f K_D}$$

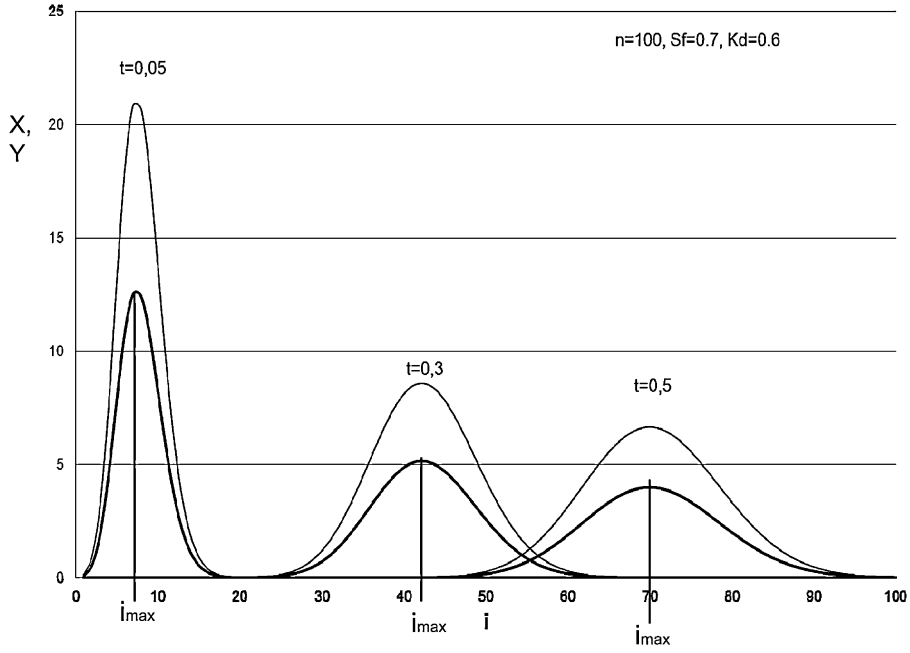

Fig. 2. Peak movement of the solute $K_D = 0.6$ inside the column consisting of 100 equilibrium cells ($n = 100$): bold line – stationary phase; $S_f = 0.7$.

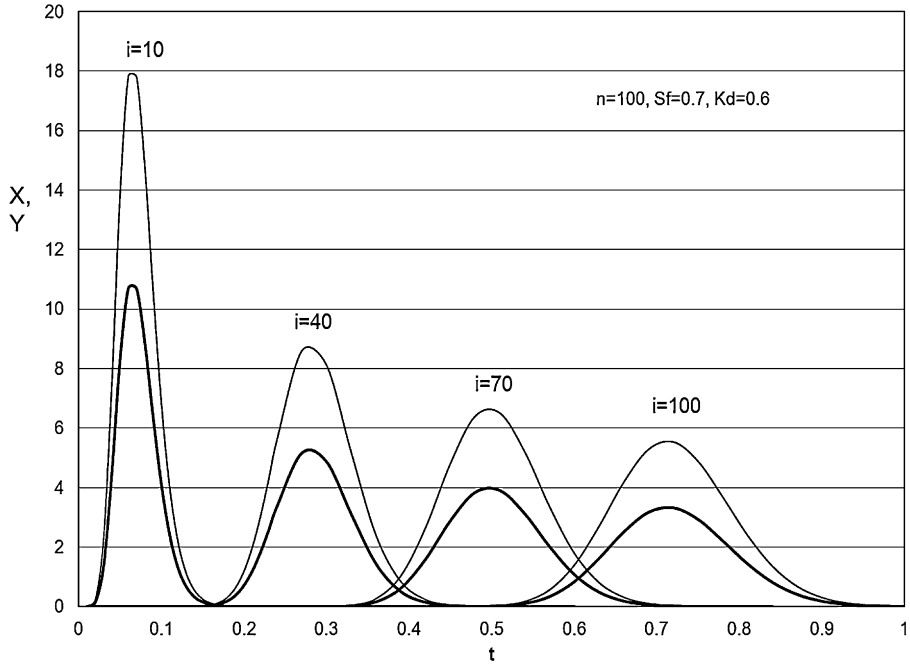

Fig. 3. Time distribution of the solute $K_D = 0.6$ in different cells of the column consisting of 100 equilibrium cells: bold line – stationary phase; $S_f = 0.7$.

where $i = 1, 2, \ldots, n$ is the current number of equilibrium cells, characterizing the dimensionless distance from the inlet of the column; n is the total number of equilibrium cells characterizing the length of the column in dimensionless unites; S_f is the fractional volume of the stationary phase; $t = \tau F/V_c$ and $X = x/\bar{x}$ are the dimensionless time and concentration, respectively, F is the volumetric flow rate of mobile phase and τ is time; $\bar{x} = Q/V_c$ is mean concentration in the column; Q is the amount of the compound in the sample; V_c is the column volume; x is solute concentration in the mobile phase, y – in the stationary phase.

Using the relationship:

$$t = \frac{\tau F}{V_c} = \frac{V}{V_c}$$

Eq. (1) can be rewritten in terms of volume:

$$X(i, V) = \frac{n^i p^i}{(i-1)!}\left(\frac{V}{V_c}\right)^i \frac{V_c}{V}\ \exp(-npV/V_c) \tag{3}$$

Eqs. (1)–(3) describe the broadening of a solute band moving inside a chromatographic column. Fig. 2 and Fig. 3 show an example (calculated by Eqs. (1) and (2)) of such a movement of the solute $K_D = 0.6$ in the column consisting of 100 cells ($n = 100$).

The position of the peak maximum (band position) inside the column can be established from Eq. (1) or Eq. (3). The factorial in these equations, when the number of cells is not too small, may be approximated by the exponential function:

$$(i-1)! \approx \sqrt{2\pi(i-1)}\frac{(i-1)^{i-1}}{\exp(i-1)} \tag{4}$$

If we substitute $(i-1)!$ by using Eq. (4) and i by using the relationship between the cell number and the dimensionless coordinate along the flow tube $Z = z/L$ (Fig. 1):

$$i = \frac{nz}{L} = nZ \tag{5}$$

it then follows that

$$X(i, t) = X(Z, t) = \frac{e^{-(1+npt)}}{\sqrt{2\pi t}}(npte)^{nZ}(nZ-1)^{0.5-nZ} \tag{6}$$

The position of the peak maximum can be found as follows:

$$\frac{dX(Z, t)}{dZ} = 0 \quad i_{\max} \approx npt \tag{7}$$

In Fig. 2 the peak positions calculated by using Eq. (7) are marked.

Eqs. (1)–(3) describe the movement of a single peak inside a chromatographic column. The expression for the theoretical chromatogram of a sample containing j components can be derived in general form as follows:

$$X(i, t) = \frac{x(i, t)}{\bar{x}} = \frac{n^i}{(i-1)!}t^{i-1}[q_1 p_1^i \exp(-np_1 t) + q_2 p_2^i \exp(-np_2 t) + \cdots + (1 - q_1 - q_2 - \ldots q_{m-1})p_m^i \exp(-np_m t)] \tag{8}$$

$$p_1 = \frac{1}{1 - S_f + S_f K_{D1}}, \quad p_2 = \frac{1}{1 - S_f + S_f K_{D2}}, \ldots\ldots$$

$$p_m = \frac{1}{1 - S_f + S_f K_{Dm}}; \quad q_1 = \frac{Q_1}{Q}, \quad q_2 = \frac{Q_2}{Q}, \ldots\ldots q_m = \frac{Q_m}{Q}$$

where K_{D1}, K_{D2}, K_{Dm} are the distribution constants of the components $1, 2, \ldots, m$; $Q_1, Q_2, \ldots, Q_m$ are the amounts of components in the sample; $Q = Q_1 + Q_2 + \cdots + Q_m$ is the total amount of components in the sample.

Eq. (8) describes the travel of a chromatogram of a mixture along a column. Fig. 4 demonstrates the separation of the two component mixture during such a travel along the column consisting of 100 cells ($n = 100$).

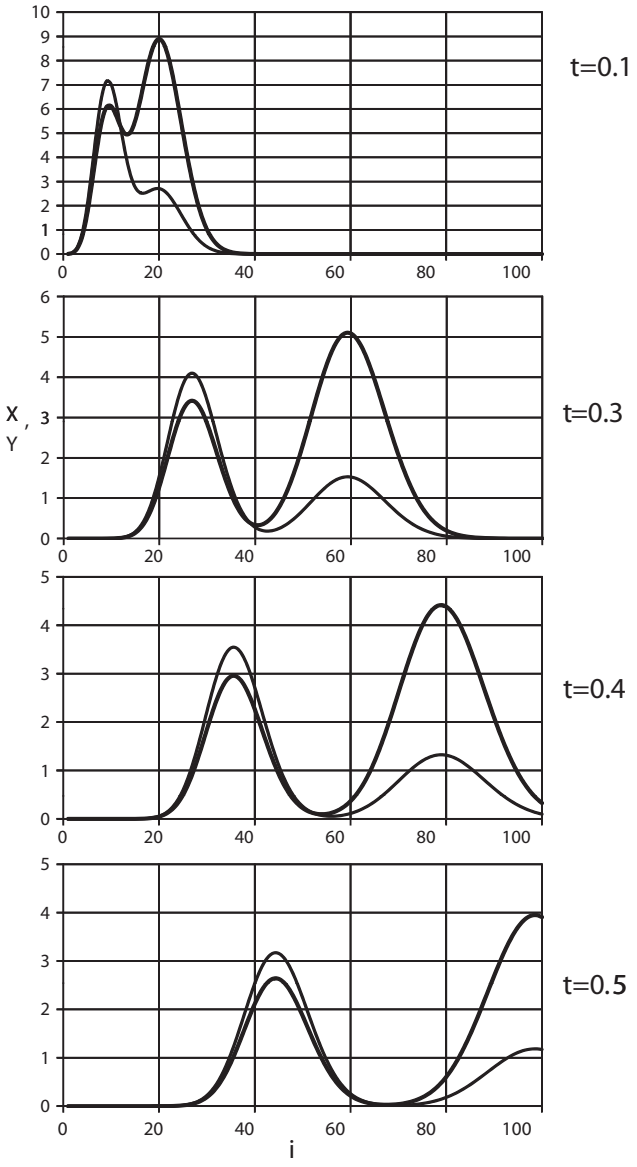

Fig. 4. Shape change of the chromatogram of the mixture of solutes $K_{D1} = 0.3$ and $K_{D2} = 1.2$ along the column consisting of 100 equilibrium cells: bold line – stationary phase; $S_f = 0.7$, $q = 0.5$.

3. Transport and separation of solutes in the elution–extrusion counter-current chromatography

For the efficient conducting of the elution–extrusion counter-current chromatography it is desirable to know how the main factors influence the separation of a given mixture of compounds. To simulate the elution–extrusion counter-current chromatography we will base on the perfect replacement cell model. Let us specify assumptions of the model.

After the switch volume V_{CM} of mobile phase has eluted from the column, sweep elution begins, during which only mobile phase leaves the column. The mobile phase moves and interacts with the "old" stationary phase in the same mode as before. The "new" stationary phase moves and replaces the mobile phase in plug-flow mode that means: (i) the "new" and "old" stationary phases do not mix; (ii) after the contacting with the mobile phase the concentration of solutes in the "old" stationary phase in a cell remains

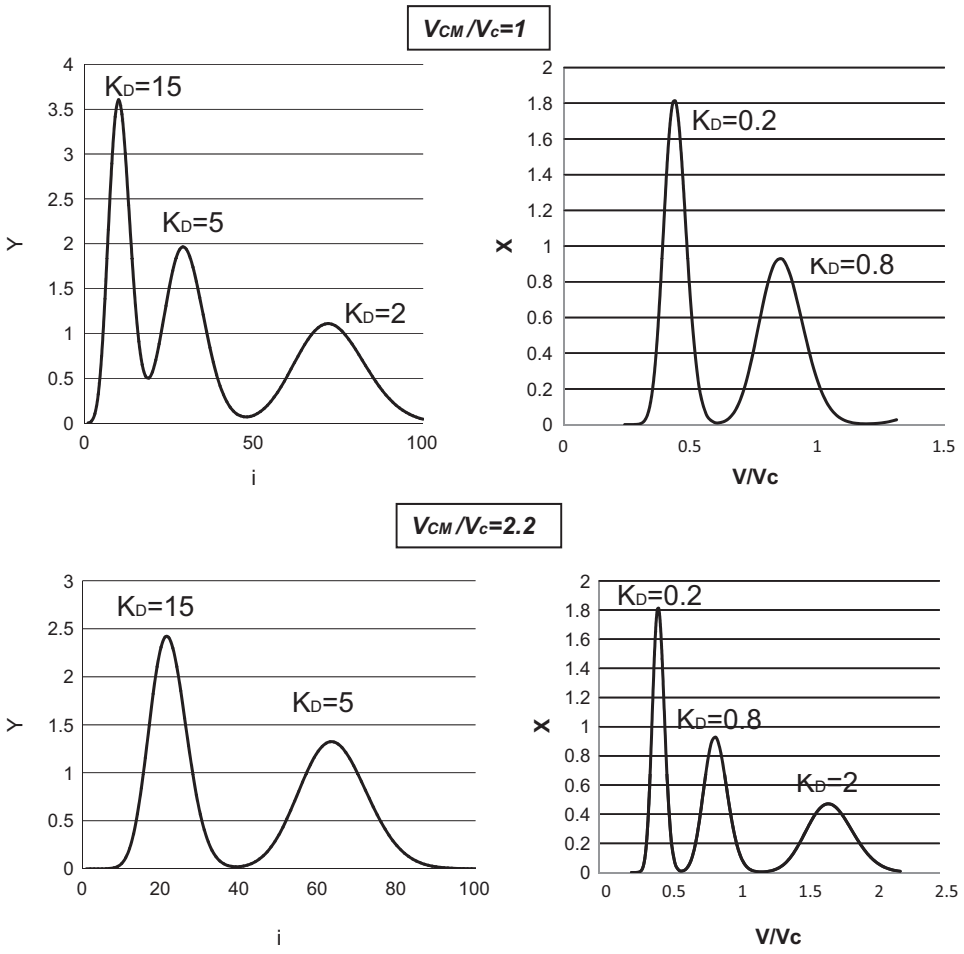

Fig. 5. Simulated chromatograms of the component mixture K_D = 0.2, 0.8, 2, 5, and 15 eluted from the column with the mobile phase (right side) and pushed out with the stationary phase during extrusion period: n = 100, S_f = 0.7, $q_1 = q_2 = q_3 = q_4 = q_5$ = 0.2.

constant and this stationary phase volume is simply pushed ahead to the exit of the column.

At the switch point, the location of a solute that is still inside the column can be established using Eqs. (5) and (7):

$$z = Lpt_{CM} = L\frac{V_{CM}}{V_C(1 - S_f + K_D S_f)} = L\frac{V_{CM}}{V_M + K_D V_S} \tag{9}$$

Eq. (9) is identical to Eq. (4) in [9].

For the cell *1* eluted mobile phase volume needed to finish the sweep elution step is

$$V_1 = V_{CM} + v, \quad v = \frac{(1 - S_f)V_c}{n}$$

The volume v is equal to the "new" stationary phase volume fed to the column (to the cell *1*).

For this cell the sweep elution step finishes at the time:

$$t_1 = \frac{V_1}{V_C} = \frac{V_{CM}}{V_C} + \frac{1 - S_f}{n}$$

According to Eqs. (1) and (2), the solute concentration in the "old" stationary phase in the cell *1* at this time will be

$$Y_1 = K_D X_1 = K_D np \exp(-npt_1)$$

For the part of the column including i cells the "new" stationary phase volume needed to complete EECCC sweep elution step is equal to

$$iv = \frac{i(1 - S_f)V_c}{n}$$

The sweep elution step in this part of the column finishes when eluted mobile phase volume is

$$V_i = V_{CM} + iv = V_{CM} + \frac{i(1 - S_f)V_c}{n} \tag{10}$$

And in terms of time:

$$t_i = \frac{V_i}{V_C} = \frac{V_{CM}}{V_C} + \frac{i(1 - S_f)}{n} \tag{11}$$

The solute distribution in the "old" stationary phase along the part of the column, including the cells from *1* to *i*, at this time can be described by the equation:

$$Y(i) = K_D \frac{n^i p^i}{(i-1)!}\left(\frac{V_{CM}}{V_c} + \frac{i(1 - S_f)}{n}\right)^{i-1} \exp - \left[\frac{npV_{CM}}{V_c} + ip(1 - S_f)\right] \tag{12}$$

with i = 1, 2, . . ., n.

The stationary phase volume needed to complete EECCC sweep elution step is equal to V_M:

$$V_M = nv = (1 - S_f)V_c$$

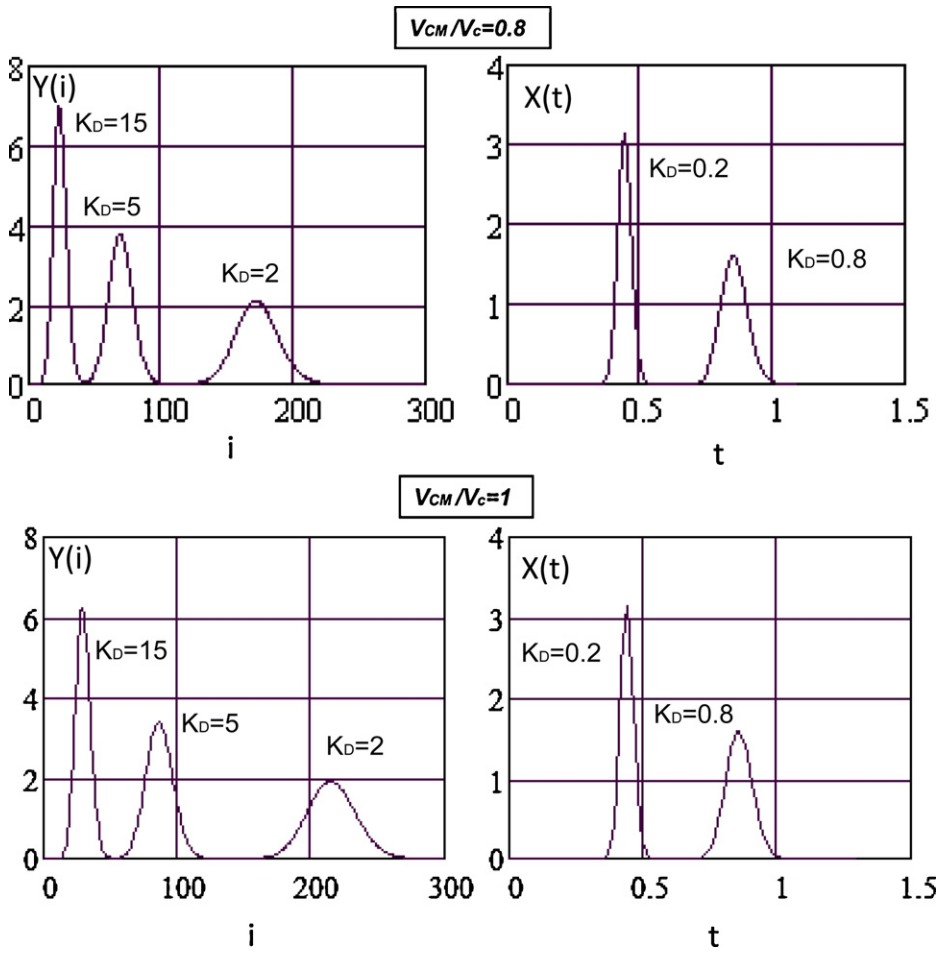

Fig. 6. Simulated chromatograms of the component mixture presented in Fig. 5 for the column consisting of 300 equilibrium cells ($n = 300$, $S_f = 0.7$).

By this time (the end of the sweep elution step) the mobile phase volume V_{ss} has passed through the CCC column:

$$V_{ss} = V_{CM} + V_M = V_{CM} + (1 - S_f)V_c \tag{13}$$

And in terms of time:

$$t_{ss} = \frac{V_{ss}}{V_C} = \frac{V_{CM}}{V_C} + 1 - S_f \tag{14}$$

The peak of a solute eluted by this time from the column can be described by the equation:

$$X(n) = \frac{(np)^n}{(n-1)!}\left(\frac{V}{V_c}\right)^{n-1} \exp\left(\frac{-npV}{V_c}\right) \tag{15}$$

with V changing from *0* to V_{ss}.

The chromatogram of all solutes eluted from the column by this time can be calculated by using the following equation:

$$X(n) = \frac{n^n}{(n-1)!}\left(\frac{V}{V_c}\right)^{n-1}\left[q_1 p_1^n \exp\left(\frac{-np_1V}{V_c}\right)\right.$$
$$\left.+q_2 p_2^n \exp\left(\frac{-np_2V}{V_c}\right)) + \ldots\ldots + q_m p_m^n \exp\left(\frac{-np_mV}{V_c}\right)\right] \tag{16}$$

with values of V changing from *0* to V_{ss}.

During extrusion step the volume $V_s = V_c - V_m$ of the "old" stationary phase containing all the solutes remaining in the column will be pushed out of the column. The shape of the single peak pushed out of the column will be described by Eq. (12) with $i = 1, 2, \ldots, n$.

The expression for the chromatogram of all solutes pushed out of the column with the "old" stationary phase during extrusion step can be written in the following form:

$$Y(i) = \frac{n^i}{(i-1)!}\left(\frac{V_{CM}}{V_c} + \frac{i(1-S_f)}{n}\right)^{i-1}\left[K_{D1}q_1 p_1^i \exp\left[-np_1\frac{V_{CM}}{V_c}\right.\right.$$
$$\left.-ip_1(1-S_f)\right] + K_{D2}q_2 p_2^i \exp\left[-np_2\frac{V_{CM}}{V_c} - ip_2(1-S_f)\right]$$
$$\left.+ \ldots + K_{Dm}q_m p_m^i \exp\left[-np_m\frac{V_{CM}}{V_c} - ip_m(1-S_f)\right]\right] \tag{17}$$

with $i = 1, 2, \ldots, n$ and $p_1, p_2, \ldots, p_m$ and $q_1, q_2, \ldots, q_m$ determined as in Eq. (8).

Using the relationship (4) Eqs. (16) and (17) can be transformed to

$$X(t) = \left(\frac{n}{n-1}\right)^n \frac{\sqrt{n-1}}{\sqrt{2\pi}} t^{n-1}[q_1 p_1^n \exp(n - 1 - np_1 t)$$
$$+ q_2 p_2^n \exp(n - 1 - np_2 t) + \ldots\ldots + q_m p_m^n \exp(n - 1 - np_m t)] \tag{18}$$

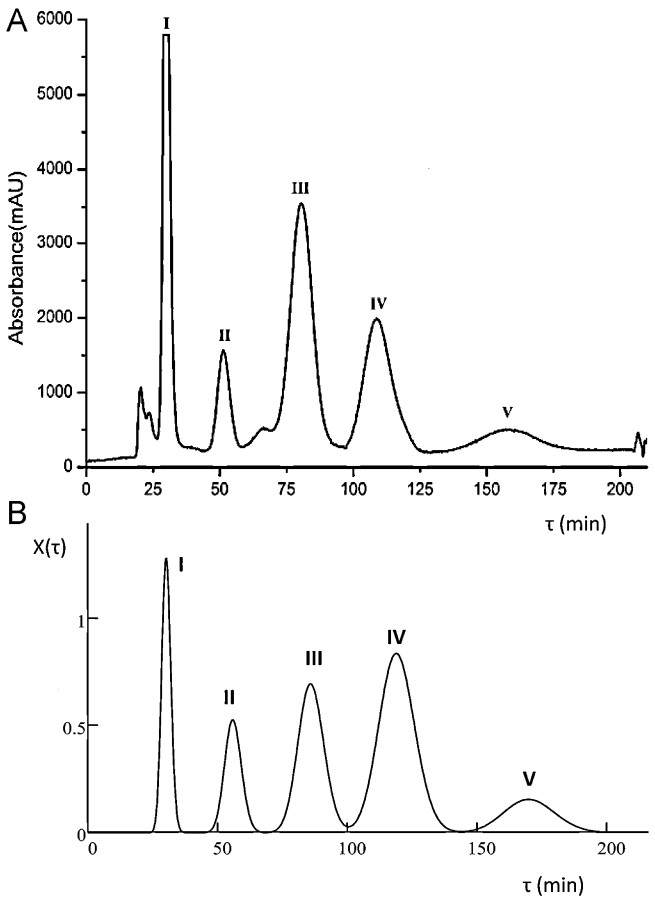

Fig. 7. Comparison of experimental (A) and theoretical (B) chromatograms for normal CCC. Experimental data used from literature [15]. Model parameters: $n = 304$; $V_c = 912.5$ ml; $F = 25$ ml/min; $S_f = 0.44$.

with values of t changing from 0 to t_{ss}:

$$Y_i = \left(\frac{n}{i-1}\right)^i \left[\frac{V_{CM}}{V_c} + i(1-S_f)/n\right]^{i-1} \frac{\sqrt{i-1}}{\sqrt{2\pi}} \times \left[K_{D1} q_1 p_1^i \exp\left(i - 1 - np_1 \frac{V_{CM}}{V_c} - ip_1(1-S_f)\right) + K_{D2} q_2 p_2^i \exp\left(i - 1 - np_2 \frac{V_{CM}}{V_c} - ip_2(1-S_f)\right) + \ldots\ldots + K_{Dm} q_m p_m^i \exp\left(i - 1 - np_m \frac{V_{CM}}{V_c} - ip_m(1-S_f)\right)\right] \quad (19)$$

with $i = 1, 2, \ldots, n$.

For values of $n > 100$ these equations are more convenient for PC-calculations.

Eqs. (15)–(19) allow the simulation of the chromatogram of solutes eluted from the column with the mobile phase during the elution period and the chromatogram of solutes pushed out of the column with the stationary phase during the extrusion period of EECCC. Figs. 5 and 6 demonstrate an example of such simulations for the sample consisting of equal quantities of 5 solutes ($K_{D1} = 0.2$, $K_{D2} = 0.8$, $K_{D3} = 2$, $K_{D4} = 5$, $K_{Di} = 15$). As can be seen, the separation process in EECCC is controlled by the value of V_{CM} and column efficiency n. Figs. 5 and 6 show that by choosing the value of V_{CM} two points should be taken into account: The value of V_{CM} must be possibly low to save solvent expenditure during the elution step and get narrow peaks during the extrusion step. On the other hand, the value of V_{CM} should be sufficient to provide separation of peaks of the solutes remaining in the column after the sweep elution step.

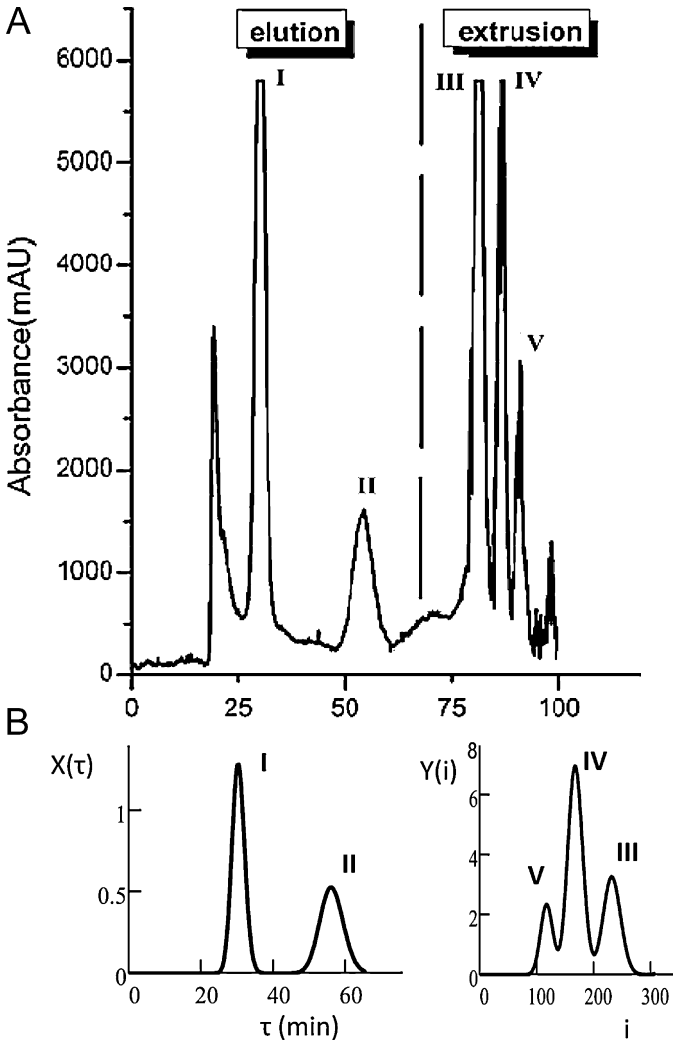

Fig. 8. Comparison of experimental (A) and theoretical (B) chromatograms for EECCC. Experimental data used from literature [15]. Model parameters: $n = 304$; $V_c = 912.5$ ml; $F = 25$ ml/min; $S_f = 0.44$; the time point of extrusion $\tau_{ss} = 65$ min; $t_{ss} = 1.78$ and $V_{CM}/V_c = 1.22$.

An increase in column efficiency n decreases the optimal value of V_{CM}.

Before the calculation of theoretical chromatograms column efficiency in cell number must be experimentally determined using one of the compounds of the mixture to be separated.

4. Comparison of model and experimental data

The model was compared with recently published experimental results [15]. In [15], the crude extract of *Dendrobium chrysototxum* Lindl. containing five target compounds (I – $K_D = 0.609$, II – 2.214, III – 4.078, IV – 6.139, V – 9.319) was separated using normal CCC (Fig. 7) and EECCC (Fig. 8) methods.

In Figs. 7 and 8 the experimental and theoretical chromatograms are compared. Model parameters obtained from [15] are: experimental column efficiency $n = 304$ (average value for the five compounds: 309, 279, 315, 300, 316); coil volume $V_c = 912.5$ ml; flow rate $F = 25$ ml/min; retention of the stationary phase $S_f = 0.44$; the time point of extrusion $\tau_{ss} = 65$ min; $t_{ss} = 1.78$ and $V_{CM}/V_c = 1.22$.

The theoretical chromatogram for the normal CCC was calculated by using Eq. (18) with $t = F\tau/V_c$. The theoretical chromatograms for the EECCC were calculated by using Eqs. (17) and (18) with $t = F\tau/V_c$ and τ changing from 0 to τ_{ss}.

The concentrations in theoretical chromatograms are expressed in non-dimensional form by using the total amount of the sample, $Q = Q_1 + Q_2 + Q_3 + Q_4 + Q_5 = 413$ mg. In the [15] the following amounts of five compounds were obtained from the sample of 1.2 g crude extract: $Q_1 = 63$ mg, $Q_2 = 48$ mg, $Q_3 = 97$ mg, $Q_4 = 162$ mg and $Q_5 = 43$ mg.

The theoretical curves in Fig. 8 represent the chromatogram of the compounds I and II eluted from the column with the mobile phase during the elution period and the distribution of the compounds III, IV and V inside the column, which will be pushed out of the column (first the compound III, then IV and V) with the stationary phase during the extrusion period of EECCC.

The agreement between theory and experiment appears to be acceptable.

5. Conclusion

The separation process in EECCC is described on the basis of equilibrium cell model using the perfect replacement approach. Equations are developed allowing the simulation of the chromatogram of solutes eluted from the column with the mobile phase during the elution period and the chromatogram of solutes pushed out of the column with the stationary phase during the extrusion period of EECCC, provided that the distribution constants of the solutes and the column efficiency are known. These equations can help to choose the optimal value of V_{CM} for conducting elution–extrusion counter-current chromatography. In determining of the optimal value of V_{CM} two effects should be considered: The low value of V_{CM} reduces solvent consumption and provides narrow peaks during the extrusion step, at the same time the value of V_{CM} should be sufficiently high to provide separation of peaks during the sweep elution step.

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