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Journal of Chromatography A, 1018 (2003) 129–136
JOURNAL OF CHROMATOGRAPHY A
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Choice of procedures for preparative chromatography☆

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Received 1 November 2002; received in revised form 23 June 2003; accepted 2 July 2003

Abstract

Quantitative criteria, necessary and sufficient, were developed basing on the analysis of asymtotic solutions of border equations in elution sorption dynamics for realization of selectivity inversion for components in a chromatographic system due to the effect of kinetic selectivity. Analysis is suggested for experiments, where the new approach to realization of chromatographic processes using effect of kinetic selectivity allows optimization of preparative separation of biologically active substances. The approach suggested implies shortening of the experiment duration for separation processes, which can be crucial in most systems where components to be isolated are labile, or the process economics suffers considerably due to mobile phase, or energy consumption.

Keywords: Preparative chromatography; Selectivity; Dynamic sorption; Optimization

1. Introduction

It is clear that development of pharmaceutical industries and biotechnology generates the need for more and more powerful preparative purification methods. When purifying a target biologically active substance by preparative liquid chromatography, the objective is to choose conditions and materials that maximize the difference between the migration of this substance and all others in the sample. On a large scale, an essential aim is to maximize yield and minimize cost [1–3].

The economical task of preparative chromatography implies finding the conditions under which the required purification degree of the target substance is achieved with minimum expenditures (reagent and energy carrier consumption at all stages of the process including preparation of the starting matter for sorption and obtaining the ultimate commercial form) [4–7].

Critical aspect in optimization of preparative chromatographic process lies in achieving sharp boundaries between zones of components under separation. Traditionally, the general solution to this problem is being found in momentary inversion of component selectivity, when adsorption process changes to desorption due to using of appropriate eluant, or to

☆ Presented at the International Symposium on Preparative and Industrial Chromatography and Allied Techniques, Heidelberg, 6–9 October 2002.

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doi:10.1016/j.chroma.2003.07.011

frontal displacement. The selectivity inversion can be associated with fast change of conditions affecting the static sorptional equilibrium ("static selectivity inversion"): mobile phase composition, temperature, or other physico-chemical parameters.

In this case, the problem of solving the preparative chromatographic isolation of the target substance is to create the conditions under which sorption equilibrium has time to be established during the contact of the sorbate front with the sorbent. Under these conditions, the peak elution volumes of the components (V_i) and, therefore, the order in which the bands leave the column are determined by the equilibrium distribution constants (Γ_i) and do not depend on the velocity of the mobile phase:

$$V_i = (1 + \phi\Gamma_i)V_0 \tag{1}$$

where V_0 and ϕ are the dead volume and phase ratio of the column, respectively. Thus, the chromatographist achieves the required difference between the distribution constant of the target substance and the corresponding values of undesirable impurities.

The economic expediency of preparative processes requires the maximal increase in the rate of the mobile phase flow through the chromatographic bed in the experiment. Under these conditions, it is possible to maintain the regular sorption mode only by unproportionally decreasing the rate of the migration of the required band (by increasing the selectivity Γ_i). This is undesirable when working with labile sorbates.

In this paper, we shall show theoretically and experimentally the existence of the range of dynamic sorption modes, whereby not only the distance between the peaks, but also the order of elution of the substances from the column upon increasing the mobile phase velocity are determined by the combination of equilibrium and kinetic factors (effect of kinetic selectivity of sorption) [8–10]. Realization of this effect provides one more way to control chromatographic processes.

2. Theory

The asymptotic solutions of the elution dynamics adsorption in the case of intradiffusion limitation of the ith component of the system can be presented by the following equation [11]:

- at $0 < F_0[1 - (1 + \phi)\omega^{-1}t^{-1}] < 0.13\lambda_i$

$$C_i = C_0 \exp(\lambda_i)\frac{\operatorname{erfc}(\lambda_i\sqrt{F_0}[1 - (1 + \phi)\omega^{-1}t^{-1}])}{2} \tag{2}$$

- at $F_0[1 - (1 + \phi)\omega^{-1}t^{-1}] > 0.13\lambda_i$

$$C_i = C_i^{(0)} \exp(-2\lambda_i)1 + \int_0^{2\sqrt{\lambda_i[1-(1-\phi)\omega^{-1}t^{-1}-0.13\lambda_i]}} \times \exp(-\xi/8\lambda_i)I_1(\xi)\,\mathrm{d}\xi \tag{3}$$

where $C_i^{(0)}$ is the concentration of the ith sorbate at the column inlet; $F_0 = \bar{D}_i t h^{-2}$ the Fourier number; $\omega = uh^{-1} = vV_g$ the reduced velocity of the mobile phase; t the experiment time; $\bar{D}_i$ the diffusion coefficient of the ith sorbate into the sorbent grain; h and V_g the height and geometric volume of the column, respectively; u and v the linear and volume velocities of the mobile phase feed, respectively; d the diameter of swollen grains of sorbent; $I_1(\xi)$ the first-order Bessel function of an imaginary argument; and λ_i is the dimensionless criterion for the process regularity.

$$\lambda_i = \frac{12\phi}{1 + \phi}\Gamma_i\bar{D}_i d^{-2}\omega^{-1} \tag{4}$$

The range $\lambda_i > 4$ corresponds to the regular sorption mode when the peak elution volume of the ith component is determined by Eq. (1).

Much less trivial seems to be the way of optimizing preparative chromatographic separations by using the differences in the sorption kinetics of the sorbate components. We shall discuss the possibility of separations in the range of irregular dynamic modes ($\lambda_i < 1$).

In this case, the relative peak elution volume, concentration profile, and the order of elution of chromatographic peaks are determined not only by the equilibrium coefficients of the distribution of the substances between the mobile and stationary phases, but also by the completeness of sorption during the time of the contact of the sorbate front with the sorbent bed.

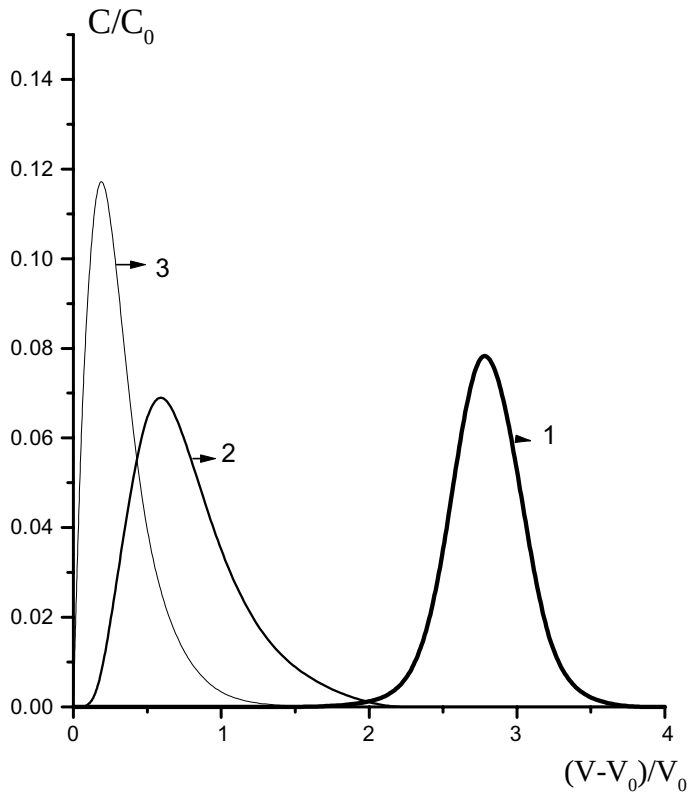

Fig. 1. Elution curves of single-component chromatography. C/C_0 is the relative concentration of the sorbate at the column outlet; $(V-V_0)/V_0$ is the reduced volume of the mobile phase passed through the column. $\Gamma = 1.2$, $\bar{D} = 5.0 \times 10^{-8}\,\mathrm{cm^2\,s^{-1}}$, $\phi = 2.3$, $d = 10^{-2}\,\mathrm{cm}$. (1) $\omega = 13.4 \times 10^{-4}\,\mathrm{s^{-1}}$, (2) $\omega = 230.4 \times 10^{-4}\,\mathrm{s^{-1}}$, (3) $\omega = 693.3 \times 10^{-4}\,\mathrm{s^{-1}}$.

2.1. Influence of the mobile phase velocity on the concentration profile and peak elution volume

On the basis of Eqs. (2) and (3), let us consider the changes in the concentration profile and peak elution volume when there is an increase in the mobile phase velocity in a certain sorption system (Fig. 1). The symmetrical curve 1 close to the Gaussian distribution shape corresponds to low feed velocities of the mobile phase ω ($\lambda_i > 4$, curve 1). The location of the maximum on the curve is determined by Eq. (1). The increase in ω leads to an emerging asymmetry of the elution profiles: their leading edges become steeper, and the trailing edges become more diffuse (curves 2 and 3). Evidently, a limiting velocity of the mobile phase exists, at which the peak elution volume becomes equal to the dead volume of the column.

Let us estimate the value of the mobile phase limiting rate. In irregular dynamic chromatographic modes, the relative peak elution volume of the *i*th component is related to Γ_i, by the following equation:

$$\tilde{V}_i = \left(1 + \frac{\phi \Gamma_i \lambda_i}{2}\right) V_0 = \left(1 + \frac{6\phi^2}{1+\phi} \Gamma_i^2 \bar{D}_i d^{-2} \omega^{-1}\right) V_0 \quad \text{at} \quad 0 < \lambda_i < 1 \tag{5}$$

where $\tilde{V}_i$ is the peak elution volume of the *i*th component in the irregular sorption mode [12,13].

Substituting Eq. (4) into Eq. (5) shows that $\tilde{V}_i / V_0 \to 1$ at:

$$\omega \gg \frac{6\phi^2}{1+\phi} \Gamma_i^2 \bar{D}_i d^{-2} \tag{6}$$

This inequality has practical sense, because it determines the range of dynamic modes, when the target substance can be isolated in the "breakthrough" of the chromatographic column.

2.2. The order of the chromatographic bands column leaving

Let us show that the order in which the chromatographic bands leave the column in the irregular sorption modes depends on the mobile phase feed velocity.

Suppose that, in the regular sorption mode in a two-component system, $\Gamma_1 < \Gamma_2$ and, therefore, $V_1 < V_2$. Then three cases can be considered, when the dynamic mode is irregular at least with respect to one of the components.

(i) Sorption of the first component occurs in the irregular mode ($\lambda_1 < 1$), and sorption of the second one—in the regular mode ($\lambda_2 > 4$). Then, $\tilde{V}_1 < V_1 < V_2$ and, therefore, the order in which the bands leave the column cannot be changed at any ω values.

(ii) The sorption of the first component occurs in the regular (Eq. (1)), and that of the second one, in the irregular dynamic modes. To this case the following range of reduced velocities ω corresponds:

$$\frac{12\phi}{1+\phi}\Gamma_2\bar{D}_2 d^{-2} < \omega < \frac{3\phi}{1+\phi}\Gamma_1\bar{D}_1 d^{-2} \tag{7}$$

Inversion of the elution order of the chromatographic bands will be observed under the sufficient condition:

$$\omega > \frac{6\phi}{1+\phi}\frac{\Gamma_2^2}{\Gamma_1}\bar{D}_2 d^{-2} \tag{8}$$

The necessary condition for the existence of the physically realizable range of dynamic modes (8) is determined by the ratio of distribution constants of the components:

- at $\Gamma_2/\Gamma_1 > 2$

$$\bar{D}_1 > 2\frac{\Gamma_2^2}{\Gamma_1^2}\bar{D}_2 \tag{9}$$

- at $\Gamma_2/\Gamma_1 \leq 2$

$$\bar{D}_1 > 4\frac{\Gamma_2}{\Gamma_1}\bar{D}_2 \tag{10}$$

(iii) Both components are sorbed in the irregular mode, if:

$$\omega > \frac{12\phi}{1+\phi}\Gamma_1\bar{D}_1 d^{-2} \quad \text{at} \quad \frac{\Gamma_1\bar{D}_1}{\Gamma_2\bar{D}_2} > 1 \tag{11}$$

$$\omega > \frac{12\phi}{1+\phi}\Gamma_2\bar{D}_2 d^{-2} \quad \text{at} \quad \frac{\Gamma_1\bar{D}_1}{\Gamma_2\bar{D}_2} :< 1 \tag{12}$$

In this case, as follows from Eq. (5) the inversion of the selectivity ($\tilde{V}_1 \succ \tilde{V}_2$) can occur at any ω values if the following relationship is realized:

$$\bar{D}_1 > \frac{\Gamma_2^2}{\Gamma_1^2}\bar{D}_2 \tag{13}$$

Thus, in the irregular dynamic sorption modes, processes very unusual in the chromatography can be realized, when the component with a lower distribution constant appears at the column outlet after the second one. This inversion of the elution order of the chromatographic bands is due to the effect referred to as "kinetic selectivity" of sorption. The effect arises in dynamic systems at certain combinations of equilibrium and kinetic parameters of sorption (see Eqs. (9), (10) and (13)). In the above case (ii), the component sorbed in the regular mode is characterized by a higher kinetic selectivity. In case (iii), the sorption selectivity inversion is observed when both components are sorbed in the irregular modes. These situations are demonstrated in Fig. 2: the increase in the mobile phase feed rate ω is accompanied by a decrease in the relative peak elution volume (V_1/V_0) of sorbate 1 upon transition to the irregular mode so that it becomes lower than the corresponding values of components 2 and 3. In other words, the elution peak of substance 1, on one hand, and those of sorbates 2 and 3, on the other hand, replace each other at the column outlet (the sorption modes of 2 and 3 at the inversion moment turn out to be regular and irregular, respectively).

3. Experimental

In this paper, we report a number of experiments demonstrating the practical possibilities of kinetically selective chromatography. We consider separation in two-component systems, when at least one of the substances is separated in the irregular mode (the *i*th peak elution volume $\tilde{V}_i$, is a linear function of reciprocal

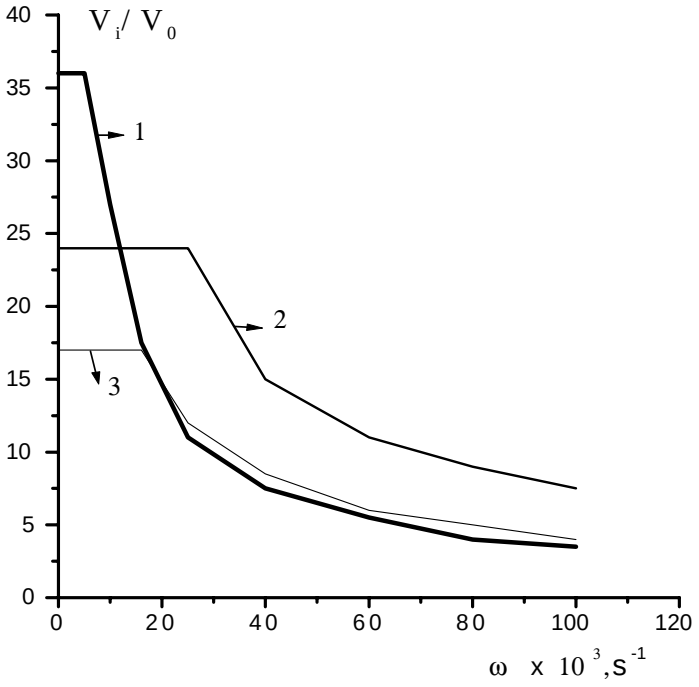

Fig. 2. Inversion of the order of the peak elution in the three-component chromatographic system. ω is the reduced feed rate of the mobile phase (s^{-1}), V_i/V_0 is relative peak elution volume (peak maximum position of the ith component). $\phi = 2.3$, $d = 10^{-1}$ cm. (1) $\Gamma_1 = 15.0$, $\bar{D}_1 = 1.1 \times 10^{-7}$ cm^2 s^{-1}, (2) $\Gamma_2 = 10.0$, $\bar{D}_2 = 6.0 \times 10^{-7}$ cm^2 s^{-1}, (3) $\Gamma_3 = 7.1$, $\bar{D}_3 = 5.6 \times 10^{-7}$ cm^2 s^{-1}.

velocity rate of mobile phase ω, Eq. (5)). The numbering of the components correspond to the order of elution of the peaks from the column in the regular chromatographic mode, i.e. $V_1 < V_2$.

In the experiments discussed below, distribution constants and internal diffusion coefficients were determined using linear regions on the sorption isotherms and kinetic curves, respectively (limited volume technique) [14]. The intradiffusion nature of sorption kinetics limiting is confirmed by the data obtained in phase contact interruption experiments and by the linearity of the relationship between sorbent filling degree and square root of the sorption time required to reach filling degrees of 0.3–0.4.

The following sorbents were used: carboxylic sorbents BDAM synthesized in the Institute of Macromolecular Compounds (St. Petersburg, Russia), are products of radical copolymerization of acrylic, methacrylic acid and ethyleneglycol dimethacrylate [15]; SKN (non-ionic carbon sorbent); KB-4P-2 (carboxylic ion-exchanger on the basis of methacrylic acid and divinylbenzene); KU-23 (macroporous sulfonated polystyrene–divinylbenzene copolymer) [16].

4. Results

4.1. Inversion of the peak elution order: component 1 is sorbed in the regular mode, and component 2 in the irregular dynamic mode

Fig. 3 shows chromatographic separation of two major components of bee venom: (1) polypeptide melittin (M) and (2) phospholipase A_2 (PLA_2). It can be seen that, when the mobile phase velocity rate increases, the PLA_2 peak shifts toward small relative hold-up volumes reaching to the dead volume of the column. This is accompanied by selectivity inversion in the point $\omega = 6\phi(1+\phi)^{-1}\Gamma_2^2\Gamma_1^{-1}\bar{D}_2 d^{-2}$ (in our case, $\omega = 0.29 \times 10^{-3}$ s^{-1}); that is, component elution

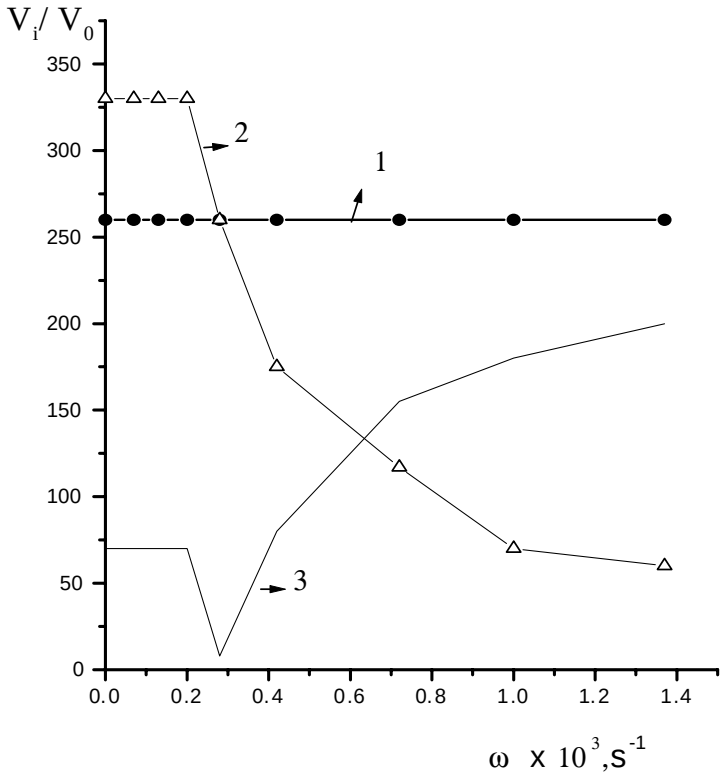

Fig. 3. Selectivity inversion in separation melittin (1) and phosholipase A_2 (2) on a BDAM–90–9 carboxylic cation-exchange resin upon increasing the mobile phase feed rate ω. Values of relative peak elution volumes V_i/V_0 and distances between the fronts $\Delta V/V_0$ are presented (curve 3). Column parameters: 1.5 cm × 1.5 cm, $d = 0.1$ cm, $\phi = 2.3$; sorption parameters: $\Gamma_1 = 112$, $\Gamma_2 = 142$, $\bar{D}_1 = 3.6 \times 10^{-7}$ cm^2 s^{-1}, $\bar{D}_2 = 3.9 \times 10^{-9}$ cm^2 s^{-1}.

order changes, and phospholipase first emerges at the column outlet. It should be noted that, in this example, the sufficient condition of inversion formulated is obeyed.

$$\bar{D}_1 > 4\frac{\Gamma_2}{\Gamma_1}$$

Fig. 3 also shows an optimization curve for the separation of two peaks (absolute magnitude of the distance between the peaks reduced to column geometry, $\Delta V/V_0$). The optimization was made based on the eluant feed rate. Under the described conditions, when melittin sorption is regular, an increase in the mobile phase velocity results in the improvement of the peak resolution and a decrease in the purification cycle time: optimization of the process is obvious.

4.2. Optimization without inversion: component 1 is sorbed in the irregular mode, and component 2 in the regular dynamic one

An example of purification of the enzyme hyaluronidase (G, component 1) from ballast proteins from bovine testes (BP, component 2) on a modified SKN sorbent, when the sufficient condition of inversion is not realized, illustrates, nevertheless, the possibility of optimizing the separation process (Fig. 4). In the region of mobile phase velocity rates where the peak elution volume still remains constant, the peak of the enzyme to be purified approaches the column dead volume, and the distance between the peaks increases until the sorption of the second component becomes irregular. Thus, the optimization curve passes through a maximum, and further

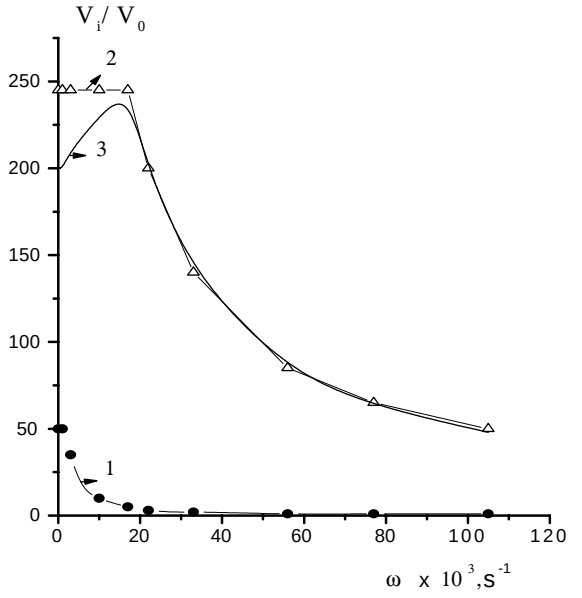

Fig. 4. Optimization with the use of irregularity of sorption of the first component for chromatographic separation of hyaluronidase (1) and ballast proteins (2) from cattle testes on an SKN modified sorbent. Designations: see Fig. 1. Column parameters: 0.7 cm × 1.0 cm, $d = 0.1$ cm, $\phi = 2.3$; sorption parameters: $\Gamma_1 = 80.5$, $\Gamma_2 = 105$, $\bar{D}_1 = 1.8 \times 10^{-8}$ cm^2 s^{-1}; $\bar{D}_2 = 4.3 \times 10^{-7}$ cm^2 s^{-1}.

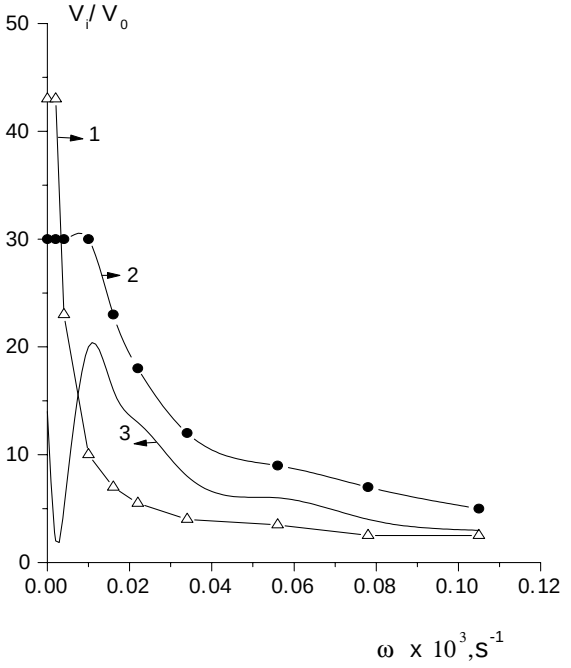

Fig. 5. Kinetically selective separation of eremomycin (1) and adriamycin (2) on a KB-4P-2 resin. Designations: see Fig. 1. Column parameters: 0.7 cm × 1.0 cm, $d = 0.06$ cm, $\phi = 2.3$; sorption parameters: $\Gamma_1 = 12.4$, $\Gamma_2 = 18.2$, $\bar{D}_1 = 9.0 \times 10^{-7}$ cm^2 s^{-1}; $\bar{D}_2 = 1.15 \times 10^{-8}$ cm^2 s^{-1}.

increase in the eluant rate only deteriorates peak resolution.

4.3. Inversion of peak elution order: both components are sorbed in the irregular dynamic mode

Separation of antibiotics: (1) eremomycin (EM) and (2) adriamycin (AM) on a KB-4P-2 cation-exchange resin (Fig. 5) looks analogously to the first example.

Here, the sufficient condition of inversion in the case of two irregular modes, $\bar{D}_1 > (\Gamma_2^2/\Gamma_1^2)D_2$, is obeyed. Due to the difference in the kinetic selectivities of sorption, the EM peak, retained in the column in the regular mode with lower selectivity, leaves the column after AM. The optimization curve has a well-defined maximum affording much better separation of the components as compared with low elution rates.

4.4. Optimization without inversion: both components are sorbed in the irregular dynamic mode

Sorbing both components in the irregular mode, as shown by an example of separation of: (l) ribonuclease and (2) DNase from bovine pancreas (Fig. 6), can also result in an increase in the separation efficiency, although the sufficient condition for peak inversion is not obeyed (the optimization curve has a maximum). In this case, obviously, the low stability of the components under separation limits the time of the chromatographic experiment, and completely regular sorption dynamics is not achievable. However, because of a relatively low diffusion rate of the first component, with an increase in the eluant feed rate, the ribonuclease peak reaches the column interstitial volume more rapidly than the DNase peak. As a result, the mobile phase rate can be selected to optimize the peak resolution within a short time.

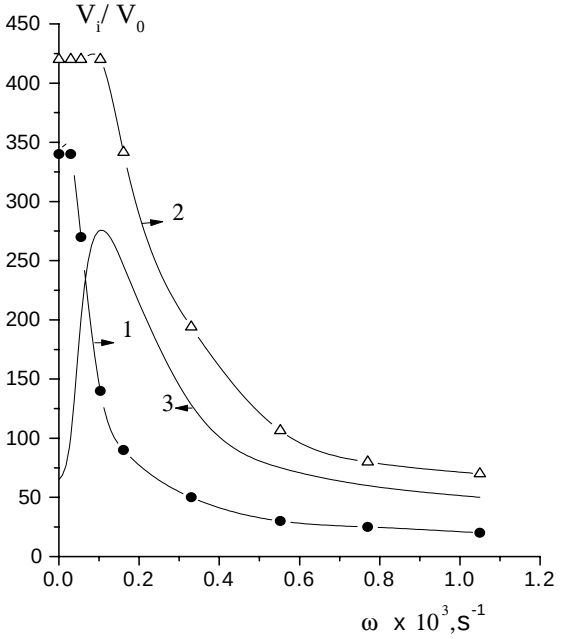

Fig. 6. Optimization due to different degrees of sorption irregularity of ribonuclease (1) and DNase (2) on a KU-23 cation-exchange resin. Designations: see Fig. 1. Column parameters as in Fig. 4; sorption parameters: $\Gamma_1 = 147$, $\Gamma_2 = 182$, $\bar{D}_1 = 0.7 \times 10^{-9}\,\mathrm{cm^2\,s^{-1}}$, $\bar{D}_2 = 1.8 \times 10^{-9}\,\mathrm{cm^2\,s^{-1}}$.

5. Conclusions

In this paper, we presented theoretical basis and practical realization of the effect of kinetic selectivity of sorption in regulating the chromatographic separation processes. It was shown that, in cases of concrete sets of equilibrium and kinetic characteristics of components to be separated, the ranges where irregular sorption dynamic modes persist can be determined. In these regions, the distance between the components on the chromatogram remarkably increases, and the experiment time is considerably shorter as compared with the traditional principle of maximum difference in distribution constants in terms of the regular chromatographic process. The order of components at the column outlet is directed by dynamic regime of adsorption, the latter being determined at given mobile phase flow rate by combination of equilibrium (distribution coefficient, Γ_i) and kinetic (internal diffusion coefficient, $\bar{D}_1$) factors. This phenomenon of "kinetic selectivity inversion", observed experimentally on a series of objects when mobile phase flow rates exceeded certain limits, can be described in terms of criterial theory of adsorption regularity, when one, or both components under separation are adsorbed in irregular, non-stationary mode.

Finally, note the fundamental importance of the obtained results. While the traditional approaches are based on the dependence of the substances to be separated on the mobile phase composition, we used the effect of kinetic selectivity of sorption–inversion of the elution order of the chromatographic bands due to the irregularity of the dynamic modes. The suggested principle of realization of chromatographic processes is applicable in all the known modifications of column chromatography.

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