Journal of Chromatography A, 1218 (2011) 6135–6143

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Controlled-cycle pulsed liquid–liquid chromatography. A modified version of Craig's counter-current distribution

Artak E. Kostanyan*, Andrei A. Voshkin, Nikolai V. Kodin

Kurnakov Institute of General & Inorganic Chemistry, Russian Academy of Sciences, Leninsky Prospekt 31, Moscow 119991, Russia

ARTICLE INFO

Article history:
Available online 4 January 2011

Keywords:
Counter-current chromatography
Craig distribution
Liquid chromatography
Pulsed extraction columns
Controlled-cycle operation
Modelling

ABSTRACT

A new liquid–liquid chromatography technique developed from a combination of controlled-cycle operation and a pulsed-mixing technique is suggested and validated. The controlled-cycle pulsed liquid–liquid chromatography (CPLC) system operates without involving a centrifuge and consists, of a series of multistage units, and a method for imparting pulsation motion to the liquids inside the units (the pulsation cycle). This chromatography technique can be considered as an improved continuous form of Craig's counter-current distribution method, or, alternatively, as a form of droplet chromatography with the cycling mode of operation. The theoretical model has been designed to account for the effects of the basic parameters influencing the CPLC operation. The theoretical model's suitability was proved by direct comparison between the experimental and model responses. The CPLC devices containing 1, 2, 4 and 5 multistage columns (each column was divided into 26 stages) have been designed, fabricated and tested; experiments were conducted to test the chromatographic behavior of organic (monocarboxylic) and mineral acids. The mass transfer rate in the stages depends on the nature of both—phase and sample systems: the highest values were achieved in experiments with acetic acid by using the octane/water biphasic system, where an equilibrium concentration distribution between stationary and mobile phases in the stages was attained. The results obtained demonstrated the potential of the new technique for preparative and industrial scale separations.

1. Introduction

The development of support-free liquid–liquid chromatography methods called counter-current chromatography (CCC), having started many decades ago [1–5], has led to a number of new chromatography techniques for the separation and purification of natural products and synthetic substances on analytical, preparative and pilot scales [6–18]. Originating from Craig's counter-current distribution method [19], most of these techniques use a centrifugal force field to retain the stationary liquid phase inside the column. Several CCC methods were developed, that did not use any centrifuge: droplet CCC, rotation locular CCC and gyration locular CCC [3,4].

The CCC columns can be considered as extraction columns with an extremely high length to the diameter ratio, operating under non-steady state conditions [20]. The fundamental principle of separation is the same in both the chromatographic and the extraction columns. It is based on differential partitioning of individual components between two immiscible solvent phases. The distribution of components between the phases and along the extraction and chromatographic columns is governed by two phenomena: interphase mass transfer and longitudinal dispersion of components caused by axial mixing in the phases.

In the development of highly efficient techniques for preparative and industrial separations it is vital to combine both the high separation efficiency and the low fabrication cost of the apparatus. To meet these requirements of industrial practice in the field of CCC techniques we have suggested and evaluated two approaches: the application of the technique of controlled-cycle operation to counter-current chromatography [21–24] and the application of a periodic pulsation to supply the additional energy necessary to intensify the interphase mass-transfer [25–27]. Both approaches have long been known and used to increase the separation efficiency of distillation and extraction columns [28–38].

In previous work [27], a support-free liquid chromatography technique based on the application of a periodic pulsation to force the mobile phase through the stationary phase in a coiled column was described. Since the peak resolution was poor, a system of pulsed mixing and controlled cycle is proposed to improve the separation efficiency. In this paper, we present such a controlled-cycle pulsed liquid chromatography system. For a better evaluation and

* Corresponding author. Fax: +7 495 9554834.
E-mail address: kost@igic.ras.ru (A.E. Kostanyan).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.12.103

understanding of the basics of the new method a brief review of the theory follows.

1.1. Theory of support-free liquid–liquid chromatography

One of the specific features of support-free liquid chromatography is that the mathematical description of the separation process is less complex compared to the other forms of chromatography due to the lack of geometrical complexities of the packing materials. The complications of flow, diffusion, and mass transfer processes in the packing caused by adsorptive and capillary forces are not present in support-free liquid chromatography. The migration rates and the spreading of individual chromatographic peaks depend only on the partition coefficient, the rate of interphase mass transfer and the degree of axial mixing in the two liquid phases. The combined effect of the longitudinal mixing in the phases and the mass transfer between them on the partitioning behavior of a solute can be described on the basis of continuous (diffusion) or discrete (cell) models [20,39–43]. For low degrees of axial mixing (which is the case in chromatography) the calculations by both—continuous and cell models, lead to identical results.

1.2. Consider two operation modes

1.2.1. Continuous mode of operation

The cell model for continuous operation of a chromatographic column represents a chain of perfectly mixed, equally-sized cells. The thorough analysis of the model equations for linear chromatography was carried out in [41]: the model equations were solved in the Laplace domain and peaks were calculated by numerical inversion of the transfer function. The analytical solution of the model equations, obtained in [43], may be presented in the following dimensionless form:

$$X = \left[\lambda\frac{(\gamma - r_1)}{(r_2 - r_1)}\right]^n \exp(-nr_1 t)\sum_{j=0}^{n-1}\frac{t^j}{j!}n^{j+2}B_j \tag{1}$$

with

$$\lambda = \frac{1}{1-S_f};\quad \nu = \frac{T_c}{1-S_f};\quad \gamma = \frac{T_c}{K_D S_f};\quad r_1 = 0.5(\omega - \sqrt{\omega^2 - 4\lambda\gamma});$$

$$r_2 = 0.5(\omega + \sqrt{\omega^2 - 4\lambda\gamma});\quad \omega = \lambda + \nu + \gamma;$$

$$B_j = \sum_{k=0}^{n-1-j}\frac{(-1)^{n-1-j-k}(2n-2-j-k)!}{k!(n-k)!(n-1-j-k)!(\gamma - r_1)^k(r_2 - r_1)^{n-1-j-k}}$$

where $k' = K_D S_f/(1-S_f)$ is the retention factor (the ratio of amounts of solute in the stationary and mobile phases under equilibrium conditions), K_D is the partition coefficient, S_f is the fractional volume of the stationary phase; n is the number of perfectly mixed cells (a measure of the degree of the axial mixing in the column); $T_c = a_c k_x V_c/(Fn)$ is the number of mass transfer units in a cell (a measure of the interphase mass transfer rate), a_c is the interphase contact area per unit volume of the contacting liquids, F is the volumetric flow rate of the mobile phase, k_x is the overall mass transfer coefficient, V_c is the column volume; $t = \tau F/V_c$ and $X = x/\bar{x}$ are the dimensionless time and concentration, respectively, τ is time, $\bar{x} = Q/V_c$ is the mean concentration in the column, Q is the amount of the compound in the sample.

If the partition equilibrium in each cell is reached, the model reduces to the equilibrium cell model, which is usually considered as the model of theoretical plates:

$$X = \frac{n^n}{(n-1)!}\left(\frac{\tau}{\bar{\tau}}\right)^n\frac{V_c}{\tau F}\exp(-n\tau/\bar{\tau}) \tag{2}$$

where $\bar{\tau} = (1 - S_f + S_f K_D)V_c/F$ is the mean residence (retention) time of a solute in the column.

The following relationship between the non-equilibrium and equilibrium models can be established:

$$n_e = \frac{nT_c(1+k')^2}{T_c(1+k')^2 + 2k'^2} \tag{3}$$

where n_e is the effective number of theoretical plates (a measure of column efficiency in terms of the equilibrium model).

Replacing n in Eq. (2) with n_e provides:

$$X = \frac{n_e^{n_e}}{(n_e-1)!}\left(\frac{\tau}{\bar{\tau}}\right)^{n_e}\frac{V_c}{\tau F}\exp(-n_e\tau/\bar{\tau}) \tag{4}$$

For $n_e \geq 30$ chromatographic peaks calculated using Eqs. (1) and (4) become identical.

1.2.2. Cycling mode of operation

The concept of cycling mode we have first used [44,45] in the comparative analysis of CCC and Craig's counter-current distribution (CCD) processes. Sutherland and Folter have proposed the application of CCD as eluting counter-current distribution model for mathematical description of CCC [46–48]. While this approach from our point of view is not the best way to describe the CCC processes, it should be noted that the idea of the controlled-cycle chromatography emerged through the analysis of the works of the afore-mentioned authors. Actually, the eluting CCD model describes the cycling mode of operation of a cascade of equilibrium stages, which is the way the Craig's apparatus would operate, if the CCD process could be extended beyond the number of transfers equal to the chain of CCD stages. In these circumstances, the concentrations in the portions of the mobile phase leaving the Craig's apparatus would be [44]:

$$X = \frac{n_{es}}{(1-S_f)}\frac{(n_{es}+i-1)!\lambda^{n_{es}}\gamma^i}{(n_{es}-1)!i!}\quad i = 0,\ 1,\ 2,\ 3,\quad \ldots$$

with

$$\lambda = \frac{1}{(1+k')},\quad \gamma = \frac{k'}{(1+k')} \tag{5}$$

where n_{es} is the number of equilibrium stages in the Craig's apparatus; i is the number of the portions of the mobile phase leaving the apparatus (the number of transfers): $i = 0$ corresponds to the solvent front elution.

The application of the controlled-cycle method to the processes of column chromatography in general [22], and to the centrifugal partition chromatography [21], in particular, was recently suggested and discussed. Considering a cascade of equilibrium stages, it was shown that the controlled-cycle technique can provide greater efficiencies (measured with the number of theoretical plates) than conventional operation of a chromatographic column. The following relationship between the efficiencies of continuous and controlled-cycle chromatography processes can be established:

$$n_{cyc} = n\frac{1+k'}{k'} \tag{6}$$

where n and n_{cyc} are the numbers of theoretical plates reached in the continuous and cycling modes of a chromatographic column operation, respectively.

2. Description of the general principle and operation of the controlled-cycle pulsed liquid–liquid chromatography (CPLC)

The fundamental principle of the CPLC is the combination of two techniques:

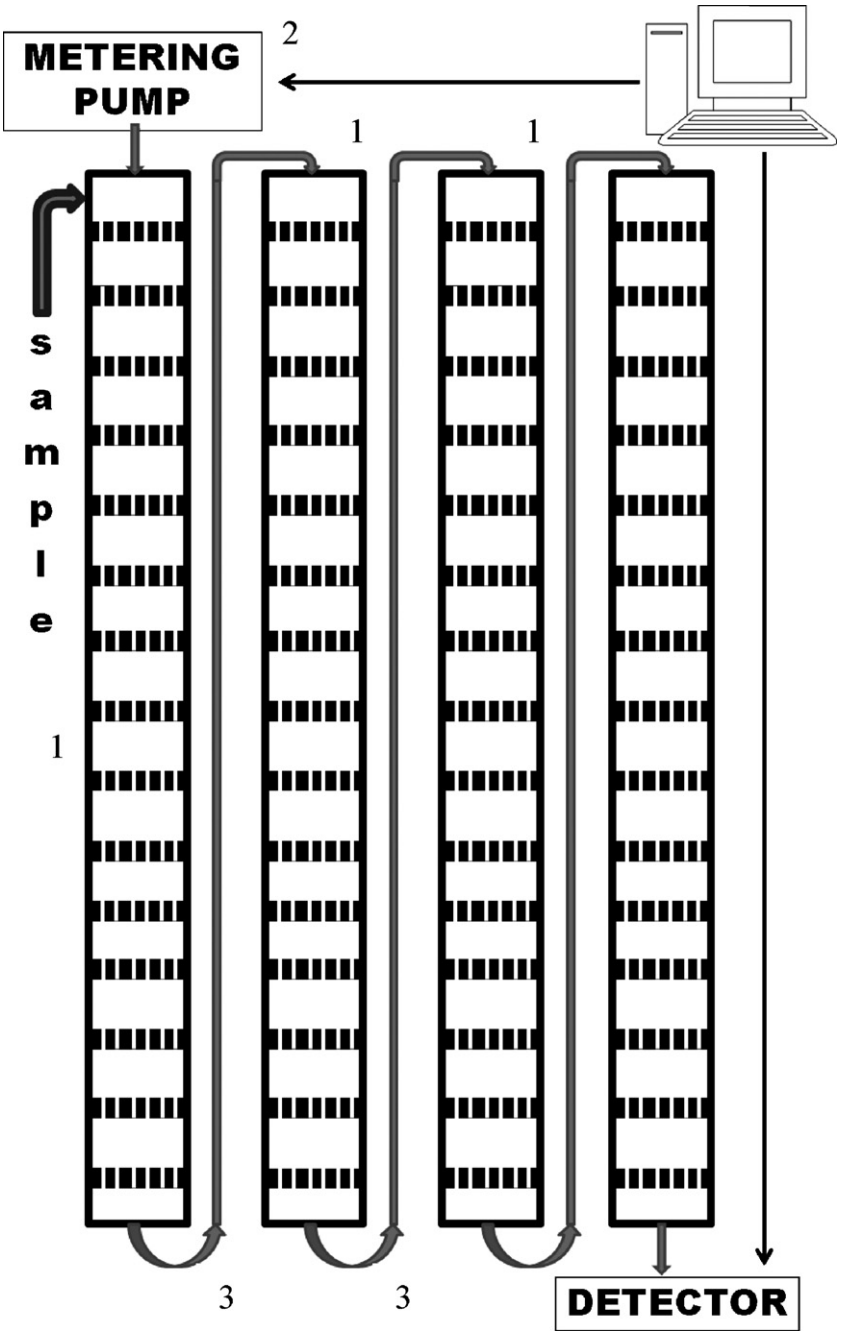

Fig. 1. Simplified diagram of the arrangement of a controlled-cycle pulsed liquid–liquid chromatography (CPLC) system: (1) multistage columns; (2) pulsation-cycling flow system; (3) transfer tubes.

(1) pulsed-mixing technique—to supply the additional energy necessary: (a) to force the mobile phase through the stationary one and (b) to intensify the interphase mass-transfer (by increasing the interphase area and phase mixing);
(2) controlled-cycle performance—to increase the separation efficiency by decreasing the axial mixing in the phases.

Several variants of the implementation of this principle have been developed [49]: one of them is schematically shown in Fig. 1.

A CPLC apparatus fundamentally consists, in all embodiments, of a series of multistage units 1 connected in the form of a coil, and a pulsation-cycling flow system 2 for imparting pulsation motion to entire contents of the apparatus by regulated discrete supply of the mobile phase as repetitive pulses to the apparatus (Fig. 1). Significant features of the chromatographic systems being considered are:

(1) each unit contains a cascade of chambers (stages);
(2) the pulsation-cycling flow system provides both, the discrete phase supply to the apparatus and pulsation motion to the liquids in it.

In the embodiments shown in Fig. 1 each unit presents a column divided into stages by equally spaced horizontal perforated plates. The pulsation-cycling flow system presents a piston pump with adjustable piston stroke length and PC regulated frequency and piston velocity.

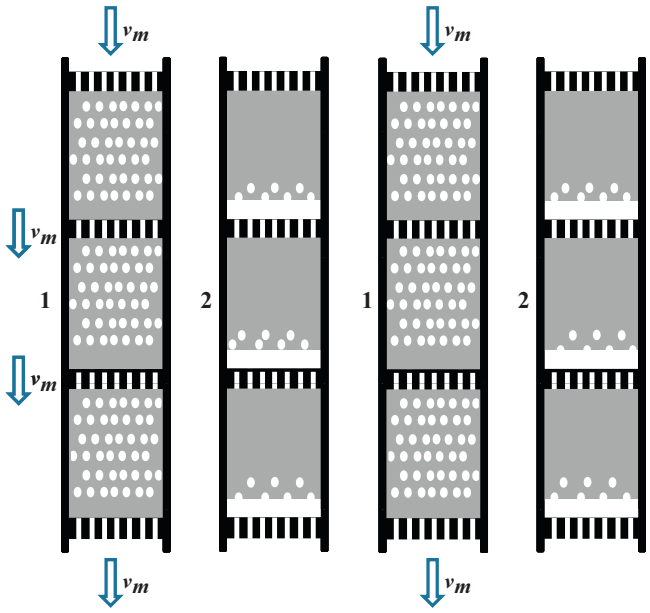

Fig. 2. The general principle of the CPLC operation: (1) flow (contact) periods; (2) settling periods.

Fig. 2 demonstrates the general principle of the CPLC operation after a hydrodynamic equilibrium is established. An operating cycle consists of two individually timed parts:

(1) flow (contact) period: a specified portion of mobile phase v_m is forced into the top of the first column at a predetermined rate. An equal portion of mobile phase v_m is displaced downward through each stage of the first column and via the transfer tubes 3—through each stage of all the other columns, being dispersed in the stationary phase in each stage of the apparatus. During this period, an equal portion of mobile phase v_m is discharged out of the bottom of the last column;
(2) nonflow (settling) period: the droplets of the mobile phase coalesce in the stages forming layers of the heavy mobile phase at the bottom of each stage.

This sequence of periods is repeated for each cycle. The duration of the first period is generally short. During this period droplet dispersion is created in each stage. The kinetic energy required for effective contacting is supplied externally by the pulsation-cycling flow system 2. The duration of the settling periods following each flow period must be sufficient to allow complete phase separation.

From the above description, it follows that the new chromatography technique can be considered as a form of droplet chromatography with the cycling mode of operation (i.e. intermittent flow) and additional kinetic energy supply (the pulsation), or, alternatively, as an improved continuous form of Craig's countercurrent distribution.

3. Theory

We consider linear chromatographic processes and, since the CPLC, is actually an improved version of Craig's counter-current distribution method, we will use the eluting CCD model approach proposed by Sutherland and Folter to describe the CPLC separation process. Based upon the operation of the CPLC system just described and confirmed by visual observations, the following propositions of the CPLC theory can be postulated:

(1) the individual stages in the units are perfectly mixed, equally-sized cells operating with a specified sequence of mobile phase transfers and phase separations;
(2) the mobile phase is fed to the apparatus in separate portions. The volume of each portion v_m is equal to the mobile phase volume in each stage of the units;
(3) a portion of mobile phase on each stage moves stepwise to the adjacent stage and there is no mixing of mobile phase during the flow period (that is, the mobile phase transfers as plug flow);
(4) the mass transfer rate between the dispersed mobile phase and the continuous stationary phase in each stage is, like in evaluating of Eq. (1), expressed by the flux $k_x\alpha_c V_c(x - y/K_D)/n_s$

(here x and y are concentrations in mobile and stationary phases, respectively).

Under these assumptions, and using the dependences from previous works [45,50], the expression for concentrations of a solute in the eluted portions of mobile phase can be established in the following form:

$$X^i_{n_s} = \frac{[1 + k' \exp(-K)]\, X^{i-1}_{n_s-1} + [1 - \exp(-K)]}{1 + k'} \times \left[\sum_{j=n_s-2}^{i-2} X^j_{n_s-1} - \sum_{j=n_s-1}^{i-1} X^j_{n_s} \right] \quad n = 2,\ 3,\ 4,\ 5, \ldots \quad i = n,\ n+1, \quad n+2, \quad n+3, \quad n+4, \ldots \tag{7}$$

where the concentration in eluting solvent front portion for current stage i is:

$$X^{i-1}_i = \frac{n_s}{1 - S_f} \left[\frac{1 + k' \exp(-K)}{1 + k'} \right]^i, \quad i = 1,\ 2,\ 3,\ \ldots,\ n_s$$

$$K = \frac{(1 + k')T_s}{k'}, \quad T_s = \frac{k_x a_c V_c}{n_s F}$$

And for the first stage, the concentrations in the portions following solvent front elution are:

$$X^i_1 = \frac{k' n_s}{1 - S_f} \left[\frac{1 - \exp(-K)}{1 + k'} \right]^2 \left[\frac{k' + \exp(-K)}{1 + k'} \right]^{i-1}, \quad i = 1,\ 2,\ 3,\ 4, \ldots$$

In Eq. (7) the superscripts refer to the numbers of eluted portions of mobile phase, whereas the subscripts denote the stage numbers.

In normalized form the model has four parameters: the partition coefficient K_D, the fractional volume of the stationary phase S_f, the number of mass transfer units in a stage (the stage efficiency) T_s, and the number of actual stages n_s.

As follows from the previous section, the retained volume of the stationary phase in a CPLC system is determined by the volume of the mobile phase portions v_m fed to the apparatus:

$$v_m = \frac{V_m}{n_s} = \frac{(1 - S_f)V_c}{n_s} \tag{8}$$

$$S_f = 1 - \frac{n_s v_m}{V_c}$$

Thus, for a solute with a given partition coefficient the only parameter to be determined experimentally is the mass transfer one. When the concentration equilibrium between the phases is reached in each stage, the partitioning behavior and the chromatographic peak profiles of solutes can be predicted from the partition coefficients, which can be determined by nonchromatographic means.

Several useful equations expressing the relationship between the CPLC and conventional continuous liquid chromatography can be derived using Eq. (8):

Table 1
The results of experiments and calculations (the duration of a cycle τ_c = 15c).

No.	Phase system	Sample	F (ml/min)	F_{in} (ml/c)	S_f	K_D	N_b	$N_{h/2}$	T_s
1	Octane/water	Acetic acid	1.1	1.8	0.76	0.15	70	71	3
2		Propionic acid	1.1	1.8	0.76	0.17	46	50	0.8
3		Butyric acid	1.1	1.8	0.76	0.24	26	28	0.4
4		Valeric acid	1.1	1.8	0.76	0.51	19	17	0.6
5		Acetic acid	1.3	2.3	0.70	0.15	82	88	2
6		Propionic acid	1.3	2.3	0.70	0.17	50	53	0.47
7		Butyric acid	1.3	2.3	0.70	0.28	28	25	0.36
8		Valeric acid	1.3	2.3	0.70	0.58	19	17	0.55
9	Octane + 10%	Acetic acid	1.3	1.44	0.70	0.25	20	21	0.29
10	octanol/water	Acetic acid	1.3	2.3	0.70	0.26	26	25	0.37
11		Acetic acid	1.3	2.88	0.70	0.26	27	26	0.38

By equating the eluted volumes:

$$V = \tau F = i v_m = \frac{i(1 - S_f)V_c}{n_s} \tag{9}$$

This gives

$$\frac{\tau F}{V_c} = t = \frac{i(1 - S_f)}{n_s} \tag{10}$$

and

$$\frac{i}{n_s} = \frac{V}{V_m} \tag{11}$$

The height of the layers built up in the stages is determined by the volume of the mobile phase portions fed to the apparatus. The question arises, of course, how the system will behave with low interfacial tension phase systems (the heavy mobile phase may drip through the perforated holes at zero flow). On the balance of gravity and interfacial tension forces the relationship between the diameter of holes (d_h), the height of the layer (h_l) and the surface tension (γ) can be established:

$$\Delta\rho h_l g \pi \frac{d_h^2}{4} = \pi d_h \gamma, \quad d_h = \frac{4\gamma}{\Delta\rho h_l g}$$

where $\Delta\rho$ is the density difference between the two phases, and g is the gravitational acceleration. To estimate the threshold size of the holes, we select three liquid systems with low interfacial tension: (1) Ethyl acetate/water (1:1): γ = 6.8 dyn/cm, ρ_1 = 0.92 g/cm^3, ρ_2 = .99 g/cm^3; (2) *n*-Butanol/1 M NaCl (1:1): γ = 5 dyn/cm, ρ_1 = 0.84 g/cm^3, ρ_2 = 1.04 g/cm^3; 3 *n*-Butanol/acetic acid/1 M NaCl (4:1:5), γ = 1 dyn/cm, ρ_1 = 0.88 g/cm^3, ρ_2 = 1.05 g/cm^3 [3].

4. Experimental

4.1. Apparatus

The CPLC system shown in Fig. 1 was designed and fabricated in our institute. Experiments were carried out with the CPLC systems containing 1, 2, 4 and 5 units. Each unit presented a column divided into 26 stages by equally spaced horizontal perforated plates at 35 mm intervals (stage volume – 1.1 ml). The columns were of 6.4 mm internal diameter FEP tubing (Cole-Parmer, USA); the perforated plates were fabricated from PTFE sheet, 3 mm thick and contained 13 0.25 mm diameter holes. The pulsation-cycling device was a purpose-built piston pump with adjustable piston stroke length and PC regulated frequency and piston velocity. The injection velocity of the mobile phase F_f can be varied by varying either the volume of the mobile phase portions v_m (determined by the piston stroke length) or piston velocity of the pulsation-cycling flow system. The PC control system allowed independent setting of the desired values of frequency and velocity of piston movement. The chromatograms were fixed by the flow-through conductivity electrode ET908 (eDAQ) connected to the PC via a conductivity meter E 7-22 (Taiwan). The peristaltic pump Heidolph PD 5101 (Germany) was used to fill the apparatus with stationary phase.

4.2. Phase and sample systems

To evaluate the performance of the CPLC device, the chromatographic behavior of monocarboxylic (acetic, propionic, butyric, valeric) and mineral (HCl, HBr, HNO_3 and $HClO_4$) acids was studied using for the monocarboxylic acids – octane/water, octane + 5% octanol/water, octane + 10% octanol/water and for the mineral acids – 0.025 M dialkylphosphate of quaternary ammonium base (QAB) in octane/0.1 M H_2SO_4 biphasic systems.

4.3. Experimental procedures

Experiments were carried out as follows: the CPLC device was first filled with the lighter stationary organic phase, and then the specified portions of mobile phase were introduced into the top stage of the first column by the pulsation-cycling device. After the system has reached equilibrium, the piston movement of the pump was stopped and the sample was injected in the first (top) stage of the first column, then the piston movement was resumed. The output response was continuously monitored by the flow-through conductivity electrode connected to the PC via a conductivity meter. All experiments were performed at room temperature.

The partition coefficient (K_D) values (defined as the concentration in the stationary phase divided by the concentration in the mobile phase), were calculated from experimental data by using peak maximum retention volume V_R or time and S_f.

5. Results and discussion

5.1. Visual observations

Visual observations (the shells of the columns were transparent) verified the actual physical picture of the CPLC operation described in Section 3: in flow (contact) periods the heavy mobile phase was dispersed in the stationary phase in each stage of the apparatus. The size of droplets depends on the nature of phase and sample systems used: the presence of surfactants (acetic acid, dialkylphosphate of quaternary ammonium base) lowers the surface tension, which leads to the formation of small droplets. It was noted that the size of droplets decreases with increasing injection rate (F_f). In addition, there was a delay of movement of portions of the mobile phase in a system of 5 columns and a decrease in the degree of dispersion of droplets in the last columns. This is due to the flexibility of the material from which the columns were made. During the settling periods, the droplets coalesce in the stages forming layers of the heavy mobile phase on the perforated plates. The results of

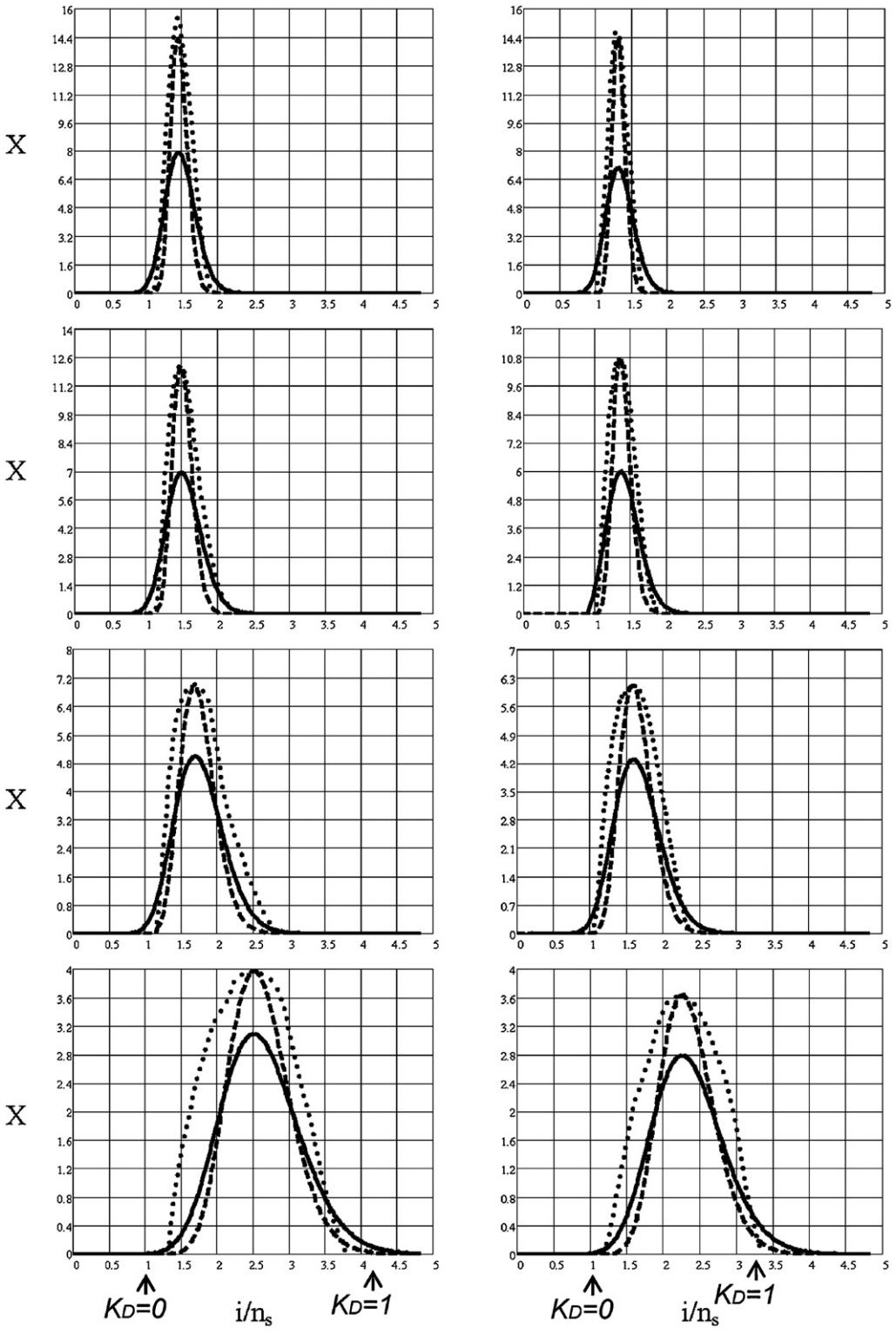

Fig. 3. Comparison of experimental peaks (dotted line) with peaks calculated by Eq. (1): solid line; and Eq. (7): dashed line. Number assigned to each diagram corresponds to that of experiment in Table 1. The concentrations on the vertical axis are expressed in non-dimensional form by using the total amount of the sample. The units on the horizontal axis (i/n_s) represent the number of mobile phase volumes V_m contained in the apparatus that have passed through it (the number of solvent front passages).

calculation for the height of the layer $h_l = 0.5$ cm: (1) $d_h = 0.79$ cm; (2) $d_h = 0.2$ cm; (3) $d_h = 0.05$ cm, demonstrate that the geometry of the plates will not limit the operation of a CPLC apparatus by using phase systems with lower interfacial tension.

It is important to note, that the operation of the CPLC system can be stopped and resumed without interfering with the separation process.

The fractional volume of the stationary phase S_f was estimated by measuring the layers of the phases in the stages.

5.2. Stage efficiency and model validation

The results of experiments and calculations with the two columns CPLC system are given in Table 1 and in Fig. 3.

The number of mass transfer units in a stage (in a cell, or in a column) $T_s = (k_x a_c V_c)/(n_s F)$ is commonly used as a kinetic parameter (a measure of the interphase mass transfer rate) in extraction and other separation processes. We can use it to evaluate the effectiveness of mass transfer in stages (the stage efficiency). For cyclic operation the volumetric flow rate of the mobile phase F in expression of T_s is the average flow rate per cycle:

$$F = \frac{F_f \tau_f}{\tau_c} = \frac{v_m}{\tau_c} \tag{12}$$

where F_f is the mobile phase flow rate during the flow periods (injection velocity), τ_f is the duration of a flow period and τ_c is the duration of a cycle. The stage efficiency T_s depends on the nature of both—phase and sample systems and on the magnitude of injection velocity, which in turn, is determined by the magnitudes of the volume of the mobile phase portions v_m and the duration of the flow periods $F_f = v_m/\tau_f$. In theory, equilibrium distribution of a solute between the phases in the stages is attained at $T_s \to \infty$. In fact, the equilibrium distribution is achieved at some relatively low values of T_s (see below). The stage efficiency T_s was estimated by fitting experimental data with theoretical peaks calculated by Eq. (7). The highest values of T_s were achieved in experiments with acetic acid (experiments 1 and 5 of Table 1), where an equilibrium concentration distribution between stationary and mobile phases appears to be attained: the theoretical chromatograms calculated by Eq. (5) (cycling operation of a cascade of equilibrium stages), and Eq. (7) (cycling operation of a cascade of non-equilibrium stages) with the values of $T_s = 2$ and 3, coincide and are very close to the experimental curves. This behavior of acetic acid can be explained by the fact that its interphase transfer is accompanied by the phenomenon of interfacial instability (interfacial turbulence): instability development intensifies considerably the mass transfer between liquid phases.

In Fig. 3 the experimental peaks are compared with peaks calculated by Eqs. (1) – continuous mode of operation of a cascade of non-equilibrium stages, and (7) using the experimental values of K_D, S_f and T_s. The units on the horizontal axis (i/n) represent the number of mobile phase volumes V_m contained in the apparatus that have passed through it (the number of solvent front passages). For a better understanding of Fig. 3 let us rewrite the fundamental equation of partition chromatography

$$V_R = V_m + K_D V_S$$

in terms of V/V_m

$$\frac{V_R}{V_m} = 1 + K_D \frac{S_f}{1 - S_f} \tag{13}$$

As can be seen, peaks in Fig. 3 are located strictly in accordance with equation (13). The value $K_D = 0$ corresponds to the x-axis point $i/n_s = 1$, the value $K_D = 1$—to the point $i/n_s = 1 + S_f/(1 - S_f)$.

The comparison of CPLC (Eq. (7)) and continuous techniques (Eq. (1)) was made using the relationships (10) and (11), i.e. the value of t in Eq. (1) was replaced by $i(1 - S_f)/n_s$. As can be seen, with the same parameters the continuous mode of operation (calculated by Eqs. (1)) would provide lower process efficiency. The results in Fig. 3 demonstrate in general a fair agreement between the theory and the experiment; however, there is a systematic deviation between the experimental and theoretical peaks: theoretical curves have narrower widths than the experimental ones. This can be explained by the partial mixing of separate portions of the mobile phase during the flow periods, which means that the assumption 3 of the theoretical model (Section 3) is not quite valid.

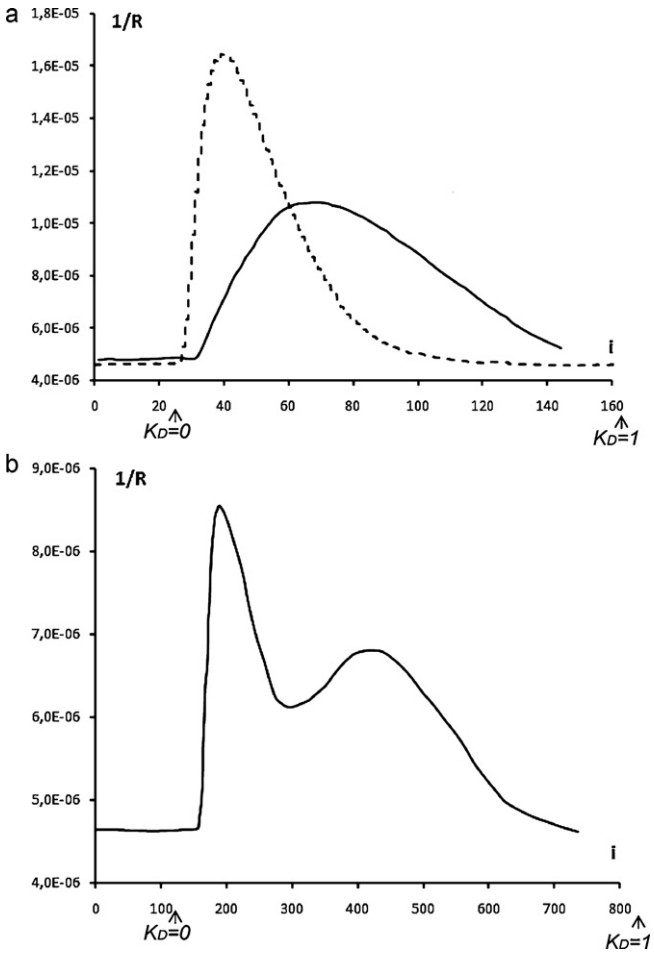

Fig. 4. The individual experimental peaks of acetic ($K_D = 0.25$) and propionic ($K_D = 0.45$) acids obtained in one column (a) and the chromatogram of their mixture obtained in 5 columns (b). Biphasic system: octane + 10% octanol/water; $S_f = 0.84$.

5.3. CPLC separation efficiency

The CPLC efficiency was estimated as the number of theoretical plates using the base width of the peaks (w_b) and the peak width at one-half the height of the peak ($w_{h/2}$). Both methods gave similar results (in Table 1, N_b is the number of theoretical plates calculated using w_b, and $N_{h/2} - w_{h/2}$). In experiments 1 and 5 of Table 1, the number of theoretical plates is greater than the number of actual stages. Previous works on the theoretical analysis of continuous and cyclic operations of the chromatographic device consisting of a chain of ideally mixed equilibrium stages [21,44] have shown that while in continuous mode the separation efficiency measured with the number of theoretical plates corresponds to the number of actual stages in the cyclic mode, it can be considerably higher. It must be noted that the cyclic operation of countercurrent extraction columns gives the theoretical separation power of $2n_s$ theoretical stages using n_s actual stages [30].

The separation efficiency of the CPLC can be improved by adding new columns to the system. In principle, a CPLC system like a droplet CCC system [4] may include hundreds of columns. In Figs. 4 and 5 the influence of stage numbers (the column length) on the separation of acids is shown. In Fig. 4 the individual experimental peaks of acetic and propionic acids obtained in one column and the chromatogram of their mixture obtained in 5 columns are shown. It should be noted that the resolution increase does not quite match the increase in column length, which can be attributed

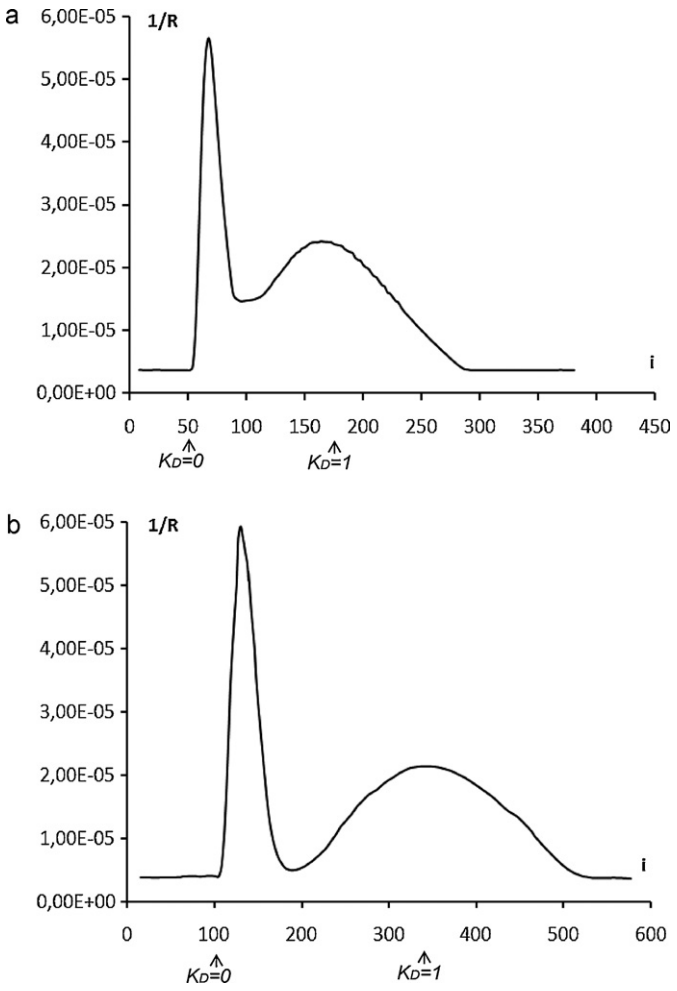

Fig. 5. The separation of acetic (K_D = 0.2) and butyric (K_D = 1) acids in 2 (a) and 4 (b) columns. Biphasic system: octane + 5% octanol/water; S_f = 0.72.

to the flexibility of column shells (see Section 5.1). In Fig. 5 the separation of acetic and butyric acids in 2 and 4 columns are compared. The units on the *x*-axis (i) represent the number of eluted portions of mobile phase (the number of transfers). From Eqs. (11) and (13) follows the relationship between the different units:

$$i_R = n_s \frac{V_R}{V_m} = n_s \left(1 + K_D \frac{S_f}{1 - S_f}\right)$$

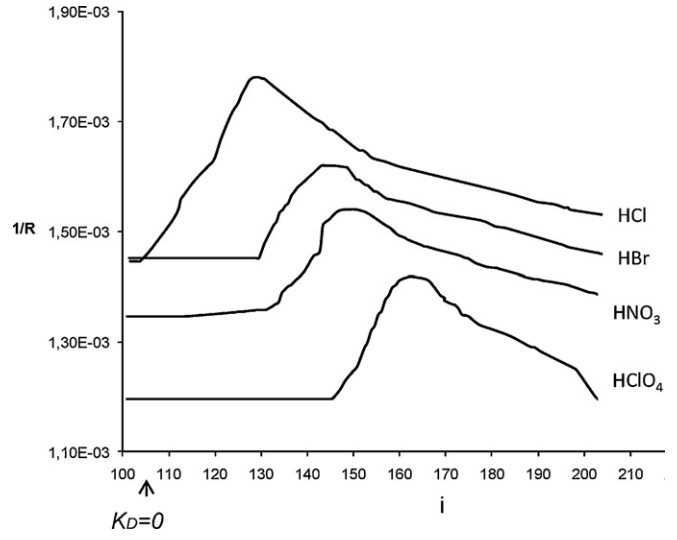

Fig. 6. The individual experimental peaks of mineral acids obtained in the 4 column CPLC device. Biphasic system: 0.025 M dialkylphosphate of quaternary ammonium base (QAB) in octane/0.1 M H_2SO_4; S_f = 0.84.

Fig. 6 demonstrates the chromatographic behavior of mineral acids in the 4 column CPLC system. As can be seen, extractivity in the extraction of mineral acids by binary extractant, dialkylphosphate of quaternary ammonium base (QAB), decreases in the series HBr > HCl > HNO_3 > $HClO_4$ that corresponds to the known series of extractivity for QAB salts. These chromatograms are non-Gaussian, because chemical reactions are the bases of the extraction processes.

The effectiveness of a CPLC device can also be increased by increasing the number of stages in the columns (by decreasing the height of stages).

6. Conclusion and future work

The controlled-cycle pulsed liquid–liquid chromatography (CPLC) devices containing 1, 2, 4 and 5 multistage columns made of FEP tubing have been designed, fabricated and tested. It was found that in a multi-column device with the flexible column shells intensity of contact between the phases decreases along the length of the system due to delay of mobile phase transfers. The theoretical model has been designed to account for the effects of the basic parameters (number of actual stages, partition coefficient, fractional volume of the stationary phase, and the interphase mass transfer rate) influencing the CPLC operation. The theoretical model's suitability was validated by direct comparison of the experimental and model responses.

The technique may be especially useful for large scale preparative and industrial chromatography, since the apparatus would be much simpler and less expensive than conventional centrifugal countercurrent chromatographic equipment.

There are several advantages of this method of separation: simplicity and fewer geometry and solvent system restrictions, the retained volume of the stationary phase is completely determined by the volume of the mobile phase portions fed to the apparatus; when the concentration equilibrium is reached in the stages, the chromatographic peak profiles of solutes can be predicted from the partition coefficients; and, once the hydrodynamic equilibrium is reached, the operation of the system can be stopped and resumed without interfering with the separation process.

Additional studies will be needed to improve the design of the stages, increase the number of stages in the columns and optimize the operational parameters. The columns are to be made of rigid (not flexible) materials. The dual-mode operation of this new technique has still to be investigated.

Acknowledgements

The authors are grateful to the Programme of the Presidium of Russian Academy of Sciences no. 8 entitled "Creating and improving methods of chemical analysis and study the structure of substances and materials" for the financial support of this work.

References

[1] W.D. Conway, Countercurrent Chromatography: Apparatus Theory and Applications, VCH, New York, 1990.
[2] Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography, Wiley-Interscience, New York, 1996.
[3] J.M. Menet, D. Thiebaut (Eds.), Countercurrent Chromatography (Chromatographic Science Series, vol. 82), Marcel Dekker, New York, Basel, 1999.
[4] Y. Ito, Sep. Purif. Rev. 34 (2005) 131.
[5] Y. Ito, J. Chromatogr. A 1065 (2005) 145.

[6] T. Tanimura, J.J. Pisano, Y. Ito, R.L. Bowman, Science 169 (1970) 54.
[7] P. Hewitson, S. Ignatova, H. Yeb, L. Chen, I. Sutherland, J. Chromatogr. A 1216 (2009) 4187.
[8] I. Sutherland, P. Hewitson, S. Ignatova, J. Chromatogr. A 1216 (2009) 4201.
[9] H. Oka, K. Harada, M. Suzuki, H. Nakazawa, Y. Ito, J. Chromatogr. 482 (1989) 197.
[10] Y.W. Lee, J. Chromatogr. 538 (1991) 37.
[11] F. Oka, H. Oka, Y. Ito, J. Chromatogr. 538 (1991) 99.
[12] F.-Q. Yang, T.-Y. Zhang, B.-X. Mo, L.-J. Yang, Y.-Q. Gao, Y. Ito, J. Liq, Chromatogr. Rel. Technol. 21 (1998) 209.
[13] T.-Y. Zhang, in: A. Berthod (Ed.), Countercurrent Chromatography, The Support-Free Liquid Stationary Phase (Comprehensive Analytical Chemistry, vol. 38), Elsevier, Amsterdam, 2002, 201.
[14] T.-Y. Zhang, X. Cao, X. Han, J. Liq, Chromatogr. Rel. Technol. 26 (2003) 1565.
[15] G.F. Pauli, R.J. Case, T. Inui, Y.H. Wang, S.Y. Cho, N.H. Fischer, S.G. Franzblau, Life Sci. 78 (2005) 485.
[16] J.B. Friesen, G.F. Pauli, Anal. Chem. 79 (2007) 2320.
[17] A. Berthod, J.B. Friesen, T. Inui, G.F. Pauli, Anal. Chem. 79 (2007) 3371.
[18] N. Rubio, S. Ignatova, C. Minguillón, I.A. Sutherland, J. Chromatogr. A 1216 (2009) 8505.
[19] L.C. Craig, O. Post, Anal. Chem. 21 (1949) 500.
[20] A.E. Kostanyan, A. Berthod, S.N. Ignatova, T.A. Maryutina, B.Ya. Spivakov, I.A. Sutherland, J. Chromatogr. A 1040 (2004) 63.
[21] A.E. Kostanyan, J. Chromatogr. A 1211 (2008) 55.
[22] A.E. Kostanyan, Theor. Found. Chem. Eng. 42 (5) (2008) 538.
[23] A.E. Kostanyan, A.A. Voshkin, P.A. Pjatovskij, Russian Federation Patent RU 2,342,970 (2009).
[24] A.E. Kostanyan, A.A. Voshkin, Russian Federation Patent RU 2,342,971 (2009).
[25] A.E. Kostanyan, Russian Federation Patent RU 2,304,453 (2007).
[26] A.E. Kostanyan, A.A. Voshkin, Theor. Found. Chem. Eng. 43 (5) (2009) 729.
[27] A.E. Kostanyan, A.A. Voshkin, J. Chromatogr. A 1216 (2009) 7761.
[28] M.R. Cannon, Ind. Eng. Chem. 53 (1961) 629.
[29] R.G. Robinson, A. Engel, J. Ind. Eng. Chem. 59 (1967) 22.
[30] P.A. Belter, S.M. Speaker, Ind. Eng. Chem. Process Des. Develop. 6 (1967) 36.
[31] J.A. Gerster, H.M. Scull, A.I.Ch.E.J 16 (1970) 108.
[32] B.I. Konobeev, M.A. Fandeev, G.R. Arutyunyan, P.S. Nazarov, Theor. Found. Chem. Eng. 10 (2) (1976) 173.
[33] B.I. Konobeev, G.R. Arutyunyan, P.S. Nazarov, Theor. Found. Chem. Eng. 11 (4) (1977) 405.
[34] Yu.A. Komissarov, L.S. Gordeyev, D.P. Vent, Scientific Bases of Distillation Processes, Chimia, Moscow, 2004.
[35] S.M. Karpacheva, L.S. Raginskii, L.P. Khorkhorina, Zh. Prikl. Khim. 59 (1986) 1955.
[36] D.H. Longsdail, J.A. Jenkins, K.S. Robinson, Process Metall. 7B (1992) 1343.
[37] W. Fournier, D. Hugelmann, G. Dalverny, D. Bernard, P. Miquel, A. Leudet, Process Metall. 7A (1992) 747.
[38] K. Aravamudan, M.H.I. Baird, Ind. Eng. Chem. Res. 38 (1999) 1596.
[39] J.C. Giddings, Dynamics of Chromatography—Principles and Theory, Marcel Dekker, New York, 1965.
[40] A.S. Said, Theory and Mathematics of Chromatography, Huthig, New York, 1981.
[41] J. Villermaux, in: A.E. Rodrigues, D. Tondeur (Eds.), Percolation Proceses: Theory and Applications, Sijthoff and Noordhoof, New York, 1981, p. 83.
[42] J.A. Jonsson (Ed.), Chromatographic Theory and Basic Principles (Chromatographic Science Series, vol. 38), Marcel Dekker, New York, Basel, 1987.
[43] A.E. Kostanyan, Theor. Found. Chem. Eng. 40 (6) (2006) 587.
[44] A.E. Kostanyan, V.V. Belova, A.I. Kholkin, J. Chromatogr. A 1151 (2007) 142.
[45] A.E. Kostanyan, A.A. Voshkin, J. Chromatogr. A 1151 (2007) 126.
[46] I.A. Sutherland, in: J. Cazes (Ed.), Encyclopaedia of Chromatography, Marcel Dekker, New York, 2001, p. 734.
[47] I.A. Sutherland, J. de Folter, P. Wood, J. Liq. Chromatogr. 9–10 (2003) 1449.
[48] J. de Folter, I.A. Sutherland, J. Chromatogr. A 1216 (2009) 4218.
[49] A.E. Kostanyan, A.A. Voshkin, V.V. Belova, A.I. Kholkin, Russian Federation Patent RU 2,403,949 (2010).
[50] A.E. Kostanyan, I.A. Sutherland, S.N. Ignatova, T.A. Marutina, in: Proceedings of the 15th International Congress of Chemical and Process Engineering, CHISA 2002, Prague, 25–29 August, Process Engineering Publ., Prague, 2002, p. 20.