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Simultaneous concentration and separation of target compounds from multicomponent mixtures by closed-loop recycling countercurrent chromatography

Artak Kostanyan*, Maria Martynova, Andrey Erastov, Vera Belova

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

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ABSTRACT

Closed-loop recycling countercurrent chromatography (CLR CCC) with multiple sample injection has been shown to provide simultaneous concentration and separation of target compounds from multicomponent mixtures. Previous analysis of CLR CCC with multiple sample injections has been limited to the ideal recycling model, which neglects the effects caused by the pump and connecting lines. In this study, an analysis of the process is carried out based on the non-ideal recycling model: recycling chromatograms at two points of the closed-loop – the inlet of the column (A) and the outlet of the column (B) – are considered. The sample is repeatedly introduced at the inlet of the column when the circulating peak of target compound passes point A. Analytical expressions are developed, allowing the design and simulation of different variants of simultaneous separation and concentration of target compounds from multicomponent mixtures. Examples of separation of target compounds from three and five-component mixtures are discussed. Experimental results are presented demonstrating a reasonable agreement between the theory and the experiment. Due to its ability to concentrate individual solutes, CRL CCC with multiple sample injections can become an efficient analytical method to determine minor components in complex mixtures.

1. Introduction

Distinctive features of support-free liquid–liquid chromatography, called counter-current chromatography (CCC), are the high volume fraction of stationary phase, which enhances the resolution, and the mobility of both phases, which allows the application of various schemes and modes of separation [1–43]. However, intrinsic limitations in the separation power of CCC restrict the scope of this method. As is well known, the separation power of CCC is much lower than that of HPLC. The efficiency of CCC separations can be improved by increasing the length of the column or applying operation modes simulating the lengthening of the column, such as multiple dual mode (MDM) CCC, and closed-loop recycling (CLR) CCC. In MDM CCC, the separation consists of a succession of two countercurrent steps and is carried out in series alternating between light and heavy phase flow: the sample moves forward and back with the phases inside the column until the peaks are separated [18–29]. In closed-loop recycling chromatography, the sample is cycled through the column until the desired separation is reached [30–43].

The performance of the MDM CCC and CLR CCC can be further improved by the application of multiple sample injections technique, which offers several new options to separate a mixture of compounds and concentrate target compounds. In contrast to the MDM CCC, the CLR CCC method is easier to implement and requires no dedicated equipment. A repeated sample injection allows obtaining the purified concentrated fraction containing a target compound. The application of multiple sample injections technique can help to overcome the difficulties associated with analyses of minor components in complex mixtures by CLR CCC. For example, when analyzing drugs, it is necessary to determine by-products down to an exceedingly low level of their content in the drug substance. In such cases, the separation of these components in a concentrated form is required.

In this article, we will discuss the theory of the CLR CCC as applied to separation and concentration of target compounds from multicomponent mixtures providing analytical expressions allowing the simulation and design of such separation processes.

For the mathematical description of the closed-loop recycling chromatography, two approaches have been used: 1. The ideal

* Corresponding author.
E-mail address: kost@igic.ras.ru (A. Kostanyan).

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recycling model, which assumes that the column outlet is directly connected to the column inlet, and the effects of extra-column dispersion are neglected [30–32,34,40,41]. 2. The non-ideal recycling model, which takes into account the extra-column dispersion caused by the recycling system (pump, connecting lines, valves) [31,32,40–42]. In our previous work [43], the ideal recycling model, which can be used when the volume of the recycling system does not exceed one percent of the column volume [40], was employed to simulate CLR CCC with multiple sample injections. In this study, we will take into account the effect of the recycling system and be based on the non-ideal recycling model to simulate the separation of target compounds from multicomponent mixtures by CLR CCC. It has previously been shown [41] that increasing the length of the recycling line in CLR CCC can improve the separation of the components.

2. Mathematical model of the closed-loop non-ideal recycling countercurrent chromatography with multiple sample injections

The principle of CLR CCC with multiple sample injections is schematically illustrated in Fig. 1, based on non-ideal recycling equilibrium-cell model. The model under consideration, takes into account both phenomena, the spreading of the injected solute in the chromatographic column – caused by the axial mixing in the phases and the mass transfer between them – in the form of the effective number of theoretical stages N (equilibrium cells) and the extra-column dispersion in the recycling system (pump, connecting lines, valves, etc., which allow for the recycling of the mobile phase through the chromatography column) – in the form of the number of perfectly mixed cells N_{ec}. Note that the recycling system contains only the mobile phase and no chromatography is occurring in it. The band spreading in this volume is caused by the axial mixing, which can be characterized by the number of perfectly (ideally) mixed cells. In the connecting tubing, the rate of axial mixing decreases with increasing length to diameter ratio. With a sufficient length of the connecting tubing, the band spreading in it may be less than in the column, that is $N_{ec} > N$.

2.1. Derivation of theoretical equations for eluting profiles

Consider recycling chromatograms recorded at two points of the closed-loop (Fig. 1): at the inlet of the column (point A) and at the outlet of the column (point B). The sample is repeatedly introduced at the inlet of the column (at point A). Counting of time and cycles is carried out from the moment of the first sample injection.

After the first injection, the position of a peak on the time axis $t_R(n)$ and its variance $\sigma^2(n)$ after n cycles are determined by the following equations [40,41]:

At point A:

$$t_R(n) = \left(\frac{1}{a} + b\right) n, \tag{1}$$

$$\sigma^2(n) = \frac{n}{N_{ef} a^2} \tag{2}$$

$$a = \frac{1}{1 - S + SK_D}, \tag{3}$$

$$N_{ef} = \frac{NN_{ec}}{N_{ec} + Na^2 b^2} \tag{4}$$

At point B:

$$t_R(n) = \frac{n}{a} + b(n-1) \tag{5}$$

$$\sigma^2(n) = \frac{1}{Na^2} + \frac{n-1}{N_{ef} a^2} \tag{6}$$

For the first injection, the peak equation at point B after n cycles is [41]:

$$X_0(n,t) = \frac{a}{\sqrt{2\pi}} \sqrt{\frac{NN_{ef}}{N_{ef} + N(n-1)}} \exp\left[-\frac{(n + ab(n-1) - at)^2}{2N_{ef} + 2N(n-1)} NN_{ef}\right] \tag{7}$$

Eq. (7) does not take into account the interaction of the peaks of previous cycles. This is taken into account in Eq. (8), which describes the transformation of the elution profile of the solute at the outlet of the column during the recycling process in the closed-loop from the first cycle to the current cycle n:

$$X_{n,0}(t) = \frac{a}{\sqrt{2\pi}} \sum_{i=1}^{n} \sqrt{\frac{NN_{ef}}{N_{ef} + N(i-1)}} \exp\left[-\frac{(i + ab(i-1) - at)^2}{2N_{ef} + 2N(i-1)} NN_{ef}\right] \tag{8}$$

In Eqs. (1)–(8), $b = V_{ec}/V_c$ is the ratio of the extra-column volume V_{ec} (connecting tubing, pump, valves) and the column volume V_c; a and N_{ef} are the parameters defined by Eqs. (3) and (4), respectively; N is the number of equilibrium stages in the column; N_{ec} is the number of perfectly mixed cells representing the extra-column dispersion; $S = V_s/V_c$ is the fractional volume of the stationary phase in the column; $K_D = y/x = constant$ is the equilibrium distribution ratio; $t = \tau(F/V_c)$ is the dimensionless time; F is the volumetric flow rate of the mobile phase; $X = x/\bar{x}$ is the dimensionless concentration of a solute in the mobile phase; $\bar{x} = Q/V_c$ is the average concentration of the solute in the column; Q is the amount of the solute in the sample injected; τ is the actual time.

From the physical nature of the process, it follows that the difference between a CLR CCC process with a single sample injection and a process with repeated sample injections will be expressed simply in the time shift. Thus, if, after the first injection at the time $\tau = 0$ ($t = 0$), the solute is reinjected at the time $\tau = \tau_r$ ($t = t_r$), the response to this injection (the circulating peak of the reinjected solute) at point B will be described by the following equations:

$t > t_r$

$$X_r(n,t) = \frac{a}{\sqrt{2\pi}} \sqrt{\frac{NN_{ef}}{N_{ef} + N(n-1)}} \exp\left[-\frac{(n + ab(n-1) + a(t_r - t))^2}{2N_{ef} + 2N(n-1)} NN_{ef}\right] \tag{9}$$

$$X_{n,r}(t) = \frac{a}{\sqrt{2\pi}} \sum_{i=1}^{n} \sqrt{\frac{NN_{ef}}{N_{ef} + N(i-1)}} \exp\left[-\frac{(i + ab(i-1) + a(t_r - t))^2}{2N_{ef} + 2N(i-1)} NN_{ef}\right] \tag{10}$$

To concentrate the target component K_{Dt} by repeated injections of the sample, the injection time of the second and subsequent injections should match the time when the circulating peak of this component will be at point A of the closed-loop (Fig. 1). This means that the injections should be performed at the time points determined by the equations:

$$t_{rt} = t_{Rt}(r) = \left(\frac{1}{a_t} + b\right) r \tag{11}$$

$$a_t = \frac{1}{1 - S + SK_{Dt}} \tag{12}$$

$$N_{eft} = \frac{N_t N_{ec}}{N_{ec} + N_t a_t^2 b^2} \tag{13}$$

where r is the number of the cycle, after which the sample is reinjected. The cycle numbers correspond to the numbers of passages of the target component through point A. Taking these conditions into account, Eqs. (9) and (10) can be transformed to:

$$X_r(n,t) = \frac{a_t}{\sqrt{2\pi}} \sqrt{\frac{N_t N_{eft}}{N_{eft} + N_t(n-1-r)}} \exp\left[-\frac{(n - r + a_t b(n-1-r) + a_t(t_{rt} - t))^2}{2N_{eft} + 2N_t(n-1-r)} N_t N_{eft}\right], \quad n \geq r+1 \tag{14}$$