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Separation of a binary mixture by sequential centrifugal partition chromatography☆

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ABSTRACT

Sequential centrifugal partition chromatography (sCPC) is a novel continuous cyclic liquid–liquid chromatographic separation technology. Each cycle of the process comprises two steps, which differ by the liquid phase used as mobile phase (upper or lower phase) and its flow direction. The feed is introduced continuously in the unit and two product streams are collected alternately, in each step of the cycle. In this work, the sCPC was modeled using the stage (cell) model. The model was used to simulate a separation of a model binary mixture consisting of pyrocatechol and hydroquinone. The solutes distribution constants, system hydrodynamics and mass transfer parameters were determined experimentally and implemented in the model. Furthermore, a parameter study (variation of the feed concentration and step times) was performed by experiments and simulation. A recently developed method was used to select the operating parameters of the sCPC unit.

1. Introduction

In liquid–liquid chromatography, also called centrifugal partition chromatography or more common counter-current chromatography, the mobile and the stationary phase are liquid. The two phases of a biphasic liquid system, obtained by mixing two or more solvents, are used as mobile and stationary phase. One of the phases is kept stationary by means of centrifugal forces [1,2]. One advantage of this technology is the free choice of the stationary phase. Namely, the upper or the lower phase of the biphasic liquid system can be used as a stationary phase. Furthermore, the role of the stationary phase can be switched during the separation run [3]. This concept is utilized to perform continuous separation in a process called sequential centrifugal partition chromatography (sCPC) in this work. The idea of this process was patented by Couillard et al. [4]. The sCPC is a cyclic chromatographic process. Each cycle of the process comprises two steps, which differ by the liquid phase used as mobile phase (upper or lower phase) and its flow direction. The feed is introduced continuously in the unit and two product streams are collected alternately. For the past few years, a sCPC unit with hydrostatic columns has been commercially available at Armen Instrument (France).

Recently the sCPC concept was also applied in units with hydrodynamic columns. In these works the feed was introduced continuously [5] or as a bolus in the semi-continuous mode [6–8], and the same concept was called intermittent dual counter-current chromatography [8] or intermittent counter–current extraction [5–7].

In our previous works we have presented and validated a systematic procedure for the selection of the operating parameters of a sCPC unit [9,10]. The experimental results proved the concept of this novel separation technology [9]. This work focuses on the modeling and simulation of the sCPC operation. The stage model (equilibrium cell or plate model) is used to simulate a separation of a mixture of pyrocatechol and hydroquinone. In the first part of the paper the experiments used for the determination of the model parameters are presented. In the second part, the experimental validation of the model is presented, and the parameters affecting the unit performances are discussed.

2. Theory

2.1. Sequential centrifugal partition chromatography

The sCPC is a cyclic process. One cycle consists of two steps: descending and ascending step.

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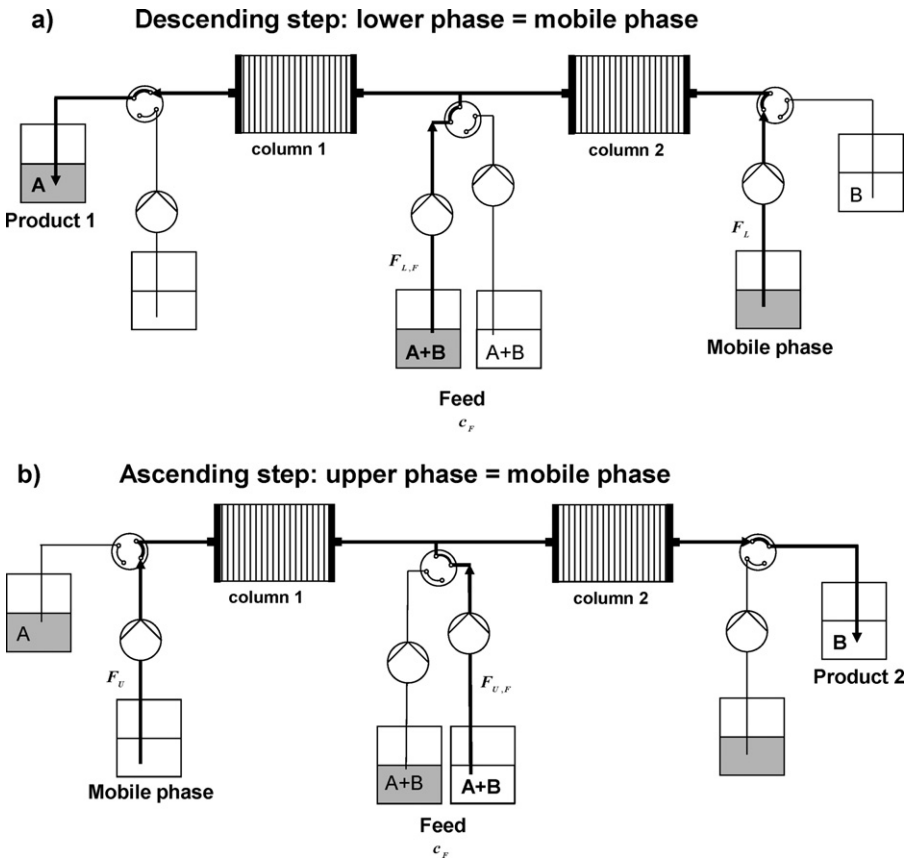

Fig. 1. Schematic presentation of the sCPC unit operating in (a) descending step and (b) ascending step.

The separation is carried out in a specially designed unit [4], which consists of two hydrostatic centrifugal partition columns and 4 pumps. The unit configuration during the descending and ascending step is presented in Fig. 1a and b, respectively.

The principle of the sCPC operation will be explained for a separation of a binary mixture of component A and B. Component A prefers the lower phase and component B prefers the upper phase. It is assumed that the separation is started in the descending step (Fig. 1a). The lower (mobile) phase – is introduced through column 2 and the feed components, A and B, dissolved in the lower phase are introduced between columns 1 and 2. Component A travels faster with the mobile phase and is collected in product 1. Before component B elutes from column 1, the unit is switched to the ascending step (Fig. 1b). The upper (mobile) phase is introduced now through column 1 and the feed components, A and B, dissolved in the upper phase are introduced between columns 1 and 2. The component B is eluted from column 1 and since B travels faster than component A, it is collected in product 2. Before A elutes out of column 2, the unit is switched back to the descending step.

2.2. Method for selection of sCPC unit operating parameters

The sCPC process includes six operating parameters: the feed flow rate during the descending ($F_{L,F}$) and ascending ($F_{U,F}$) step, the mobile phase flow rate in ascending (F_U) and descending step (F_L) as well as the duration of the ascending (t_{As}) and descending (t_{Des}) step. These parameters cannot be selected independently, what makes their selection not an easy and straightforward task. Recently, we developed a method for the selection of the operating parameters of a sCPC unit for a complete separation of a binary mixture [10]. Constraints on the sCPC operating parameters were derived using the assumption of instantaneous equilibrium between the two phases. The constraints are presented in Table 1, where V_U and V_L are the volume of the upper and lower phase, respectively, and K_k is the distribution constant of solute k.

The distribution constant is defined as ratio of the concentration of solute k in the stationary phase, c_k^S, and the concentration of solute k in the mobile phase, c_k^M:

$$K_k = \frac{c_k^S}{c_k^M} \tag{1}$$

One of the assumptions considered in the derivation of the above mentioned constrains is that K_k is constant, i.e. independent of the

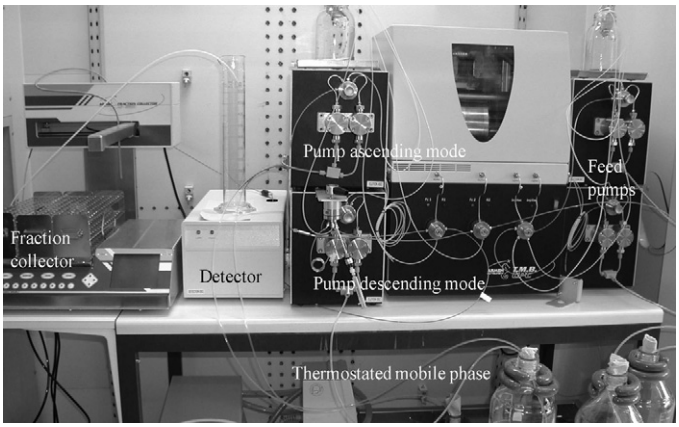

Fig. 2. Picture of the CPC set-up (model Armen TMB-250). Only the detector and fraction collector at the side of the product collection point in the ascending mode are shown.

Table 1
sCPC unit operating restrictions and operating parameters constraints for a complete separation of a binary feed mixture, with $K_A^{Des} < K_B^{Des}$ and $K_A^{As} > K_B^{As}$.

Restrictions	Operating parameters constraints
At the end of the descending step component B should stay in column 1	$t_{Des}^{max} < \frac{V_L + K_B^{Des} V_U}{F_L + F_{L,F}}$ (4)
At the end of the ascending step component B should be completely eluted from column 1	$\frac{t_{Des}}{t_{As}} < \frac{F_U}{F_L + F_{L,F}} K_B^{Des}$ (5)
At the end of the ascending step component A should stay in column 2	$t_{As}^{max} < \frac{V_U + (1/K_A^{Des}) V_L}{F_U + F_{U,F}}$ (6)
At the end of the descending step component A should be completely eluted from column 2	$\frac{t_{Des}}{t_{As}} > \frac{F_U + F_{U,F}}{F_L} K_A^{Des}$ (7)

solute concentration. So, in the descending step the distribution constant of solute k is:

$$K_k^{Des} = \frac{c_k^U}{c_k^L} \quad (2)$$

and in the ascending step is the reciprocal value of K_k^{Des}, namely:

$$K_k^{As} = \frac{1}{K_k^{Des}} = \frac{c_k^L}{c_k^U} \quad (3)$$

where c_k^U and c_k^L are the concentrations of component k in the upper and lower phase, respectively.

The method validation was presented in a previous paper [9].

2.3. Modeling

The most common approaches for the modeling of liquid–liquid chromatographic processes are: counter-current distribution model [11,12], plug flow model with axial dispersion [13,14], the equilibrium cell (stage) model [15,16], and non-equilibrium cell (stage) model [17].

In this work the stage model (also called equilibrium cell or plate model) of Martin and Synge [15] is used to describe the continuous operation of the sCPC unit and to model the elution profile of pulse injection experiments performed in a batch mode. In the stage model the column j (j = 1,2) is modeled as a sequence of N ideally mixed equilibrated cells, i.e. the solute partitioning between the mobile and the stationary phase occurs instantaneously. The band broadening effects such as axial dispersion and mass transfer resistance are represented by the number of stages (plates), N. The – sCPC – stage model is presented in detail in [10].

The main – assumptions of the model are: K_k is constant (independent of the concentration of the solute), V_U and V_L are constant and equal, and N is equal in the ascending and descending step. The sCPC model equations are summarized in Table 2, where $c_{k,in}^U$ is the concentration of component k in the upper phase at the inlet of the column (during the ascending step) and $c_{k,in}^L$ is the concentration of component k in the lower phase at the inlet of the column (during the descending step). The definition of the remaining model parameters and variables in Eqs. (8)–(16) was introduced at the beginning of this section.

The generic mass balance equation (Eq. (8) in Table 2) is solved using the specific conditions for each step, which are defined by Eqs. (9)–(12) for descending step and Eqs. (13)–(16) for ascending step. The distribution constant in the descending and ascending step is defined by Eqs. (2) and (3), respectively. Additionally, it is assumed that at time zero there are no solutes inside the sCPC columns.

The model can be easily adapted and used to simulate a batch elution separation (pulse injection elution profiles) in two columns connected in series, in ascending or descending mode.

For example to simulate a batch elution in ascending mode, the mass balance (Eq. (8)) should be solved using the following boundary and initial conditions:

$$c_{k,in}^U = 0 \quad (17)$$

$$F_L = 0 \quad (18)$$

$$t = 0: \quad c_{k,1}^U = \frac{NQ}{\left(V_U + V_L K_k^{As}\right)}; c_{k,2}^U = c_{k,3}^U = \ldots = c_{k,N}^U = 0 \quad (19)$$

where Q is the mass of the solute, which is introduced with a sample pulse injection into the first cell (stage) of the column [16].

The commercial software gPROMS V3.3.1 [18] was used to solve the model equations.

3. Material and methods

3.1. Chemicals

Heptane, methanol and ethyl acetate (analytical grade) used in the sCPC experiments were supplied by Merck, Germany. Hydroquinone and pyrocatechol with a purity ≥99% were purchased from Sigma–Aldrich, USA. Water was deionized.

Methanol used in the high-performance liquid chromatography (HPLC) analysis (gradient grade for liquid chromatography, ≥99.9%) was purchased from Merck, Germany. Millipore 18 MOhm water was used in the HPLC analysis.

3.1.1. Solvent system and feed solution

A biphasic solvent system heptane/ethyl acetate/methanol/water with the global composition 1/2/1/2 (v/v/v/v) was used. The selection of the solvent system was discussed in our previous work [9]. A model mixture of pyrocatechol and hydroquinone was used as sample solution.

The biphasic liquid system was prepared by mixing certain volume portions of heptane, ethyl acetate, methanol and water and shaking vigorously. The system was equilibrated at room temperature (23 ± 2 °C) for 2–3 h. In the following, the two liquid phases were separated and placed in two separated reservoirs. For the preparation of the feed mixture, a specific amount of hydroquinone

Table 2
sCPC model equations.

Generic mass balance of component k in column j

$$\frac{V_U}{n}\frac{dc_{k,i,j}^U}{dt} + \frac{V_L}{n}\frac{dc_{k,i,j}^L}{dt} = F_{U,j}\left(c_{k,i-1,j}^U - c_{k,i,j}^U\right) + F_{L,j}\left(c_{k,i+1,j}^L - c_{k,i,j}^L\right) \quad (8)$$

Descending step			Ascending step		
Stationary phase = upper phase	$F_{U,j} = 0 \quad j = 1, 2$	(9)	Stationary phase = lower phase	$F_{L,j} = 0 \quad j = 1, 2$	(13)
Mobile phase (=lower phase) introduced in column 2	$c_{k,in,2}^L = 0$	(10)	Mobile phase (=upper phase) introduced in column 1	$c_{k,in,1}^U = 0$	(14)
Flow rate and mass balance at the feed node	$F_{L,1} = F_{L,2} + F_{L,F}$	(11)	Flow rate and mass balance at the feed node	$F_{U,2} = F_{U,1} + F_{U,F}$	(15)
	$F_{L,1}c_{k,in,1}^L = F_{L,2}c_{k,1,2}^L + F_{L,F}c_{k,F}^L$	(12)		$F_{U,2}c_{k,in,2}^U = F_{U,1}c_{k,n,1}^U + F_{U,F}c_{k,F}^U$	(16)

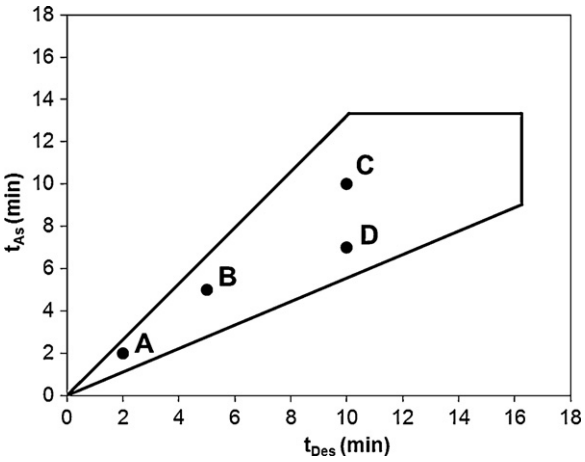

Fig. 3. Region of complete separation of pyrocatechol and hydroquinone. Point A: $t_{As} = t_{Des} = 2$ min; Point B: $t_{As} = t_{Des} = 5$ min; Point C: $t_{As} = t_{Des} = 10$ min and Point D: $t_{As} = 7$ min, $t_{Des} = 10$ min. Feed flow rates: 2 ml/min $F_{L,F} = F_{U,F}$, mobile phase flow rates: 10 ml/min $F_L = F_U$.

and pyrocatechol was added in each phase (upper and lower phase) and mixed vigorously.

3.2. Equipment

The sCPC experiments were performed in a Centrifugal Partition Chromatograph, model Armen TMB-250, produced by Armen Instrument (France) (Fig. 2). The unit includes 2 hydrostatic centrifugal partition columns (volume of 125 ml each; 930 cells each) placed on 2 separated rotors and 4 HPLC gradient pumps (maximum flow rate of 50 ml/min). The maximum rotational speed is 3000 rpm and the maximum pressure 80 bar. A UV detector ECOM DAD600 2 WL 200–600 nm (ECOM) and a fraction collector (model LS 5600, Armen Instrument, France) are connected in series at each of the two product outlet sites. The sCPC unit operation is controlled via software provided by the producer. The reservoirs with the mobile phase and feed are placed in a water bath Ecoline Staredition E 140 (Lauda, Germany) and equilibrated at 25 °C during the experiment.

A manual injection valve (Rheodyne, Model 7010) was installed after each of the two pumps used to deliver the mobile phase, in ascending and descending step, respectively. This injection valve was used in the pulse injection experiments, which were performed in batch mode as described in Section 3.3.2.

3.3. Methods

3.3.1. Determination of distribution constants by shake flask experiments

The distribution constant (partition coefficient) of each solute, hydroquinone and pyrocatechol, in the biphasic system heptane/ethyl acetate/methanol/water 1/2/1/2 was determined with the shake flask method at room temperature (23 ± 2 °C). For each component the shake flask experiment was repeated four times. The concentration of the upper and lower phase was analyzed by HPLC as described in Section 3.4. The distribution constant of each component was calculated as the ratio of the concentration (i.e. peak area) of the solute in the upper and lower phase. More details can be found in [9].

3.3.2. Pulse injection experiments

Pulse injection experiments were carried out, both in ascending and descending mode. To perform this experiment, the unit was run in batch mode. The columns were connected in series and the feed flow rate was set to 0. An injection valve (Rheodyne, Model 7010) was installed at the inlet of the column series, respectively to ascending and descending mode. In each mode, the columns were entirely filled with 50/50 (v/v) upper and lower phase first, using one of the mobile phase pumps at a flow rate of 50 ml/min. After that the rotors were started. When the set rotation speed was reached, the mobile phase was pumped into the columns at a pre-set flow rate for a certain time to ensure that there is no loss of the respective stationary phase. A pulse of a mixture of hydroquinone and pyrocatechol, prepared in the corresponding mobile phase (upper phase in ascending mode and lower phase in descending mode), was injected and the elution profile was recorded with the UV detector placed at the column series outlet, respectively to ascending and descending mode.

3.3.3. sCPC experiments

In the sCPC experiments, the columns of the sCPC unit were filled with 50 vol% of each phase using one of the two mobile phase pumps at a flow rate of 50 ml/min and 0 rpm. After that, the rotors were started out and when the pre-set rotation speed was reached the unit was started up, in ascending or descending step. The step, at which the unit was started in each experiment, is given in the Figure captions. The elution profile of the product stream in each step was monitored on-line with the UV detectors and, additionally, fractions were collected for HPLC analysis (see Section 3.4).

The separation run was stopped as soon as the cyclic steady state (CSS) was achieved.

3.4. Analytics

HPLC analysis was used to determine the amount of hydroquinone and pyrocatechol, respectively in: (i) the equilibrated upper and lower phases from the shake flask experiments and (ii) the fractions collected during the sCPC experiment. The samples were diluted with methanol prior to the HPLC measurement.

HPLC analyses were carried out on a Gilson system (Middleton, WI, USA) consisting of 322H2 binary gradient pump, GX Direct injection module, 151 UV/VIS Detector, and Trilution LC control software. The analysis was performed isocratically using a Nucleosil 100-5 C18 column (125 mm × 3 mm i.d.) at 30 °C and 280 nm. A methanol/water (15:85, v/v) mixture was used as mobile phase, at a flow rate of 0.6 ml/min. The injected volume of the sample was 10 μl.

4. Results and discussion

The sCPC model (Eqs. (2), (3) and (9)–(16)) was used to simulate the separation of a binary mixture of pyrocatechol (P) and hydroquinone (H), which was performed in the sCPC unit described in Section 3.2. The mathematical model includes two parameters, the distribution constants of the components (K_k) and the number of stages (N), which must be determined experimentally.

This section is organized as follows. First, the results of the experimental determination of the model parameters are presented. After that, the validation of the model predictions with experimental sCPC data is shown. At the end, the results of the study on the influence of some of the operating parameters on the unit separation performances are presented and discussed.

4.1. Distribution constants

The distribution constants of pyrocatechol (K_P^{Des}) and hydroquinone (K_H^{Des}) in the heptane/ethyl acetate/methanol/water (1/2/1/2, v/v/v/v) system were measured by shake flask experiments as described in Section 3.3.1. The following values of the distribution constants were obtained: $K_P^{Des} = 2.2 \pm 0.2$ and $K_H^{Des} =$

Table 3
Column efficiency (i.e. number of stages, N), number of perfectly mixed cells (n) and number of mass transfer units (T) at different mobile phase flow rates, in descending and ascending mode; 1700 rpm.

Flow rate (ml/min)	Descending mode				Ascending mode			
	N_H (–)	N_P (–)	n (–)	T (–)	N_H (–)	N_P (–)	n (–)	T (–)
8	460	299	596	568	261	363	417	542
12	540[a]	358[a]	689	703	411[a]	568[a]	652	870
14	613[b]	419[b]	763	879	465[b]	615[b]	689	1114

[a] Mean values obtained from 3 pulse injection experiments.
[b] Mean values obtained from 2 pulse injection experiments.

0.6 ± 0.1. The standard deviation of the distribution constant is in the expected range according to the literature [19].

The maximum concentration of the solute in either system did not exceed 0.01 mol/l so that the Nernst partition law can be applied. In the low concentration range, it can be assumed that the distribution constant of solute k in the ascending mode, K_k^{As}, is the reciprocal value of the distribution constant of solute k in the descending mode, K_k^{Des}. At higher solute concentrations the value of the distribution constant could increase or decrease, depending on the solute solubility in each of the two liquid phases.

4.2. Column efficiency in batch mode

In order to evaluate the CPC columns efficiency in ascending and descending mode, pulse injection experiments of a pyrocatechol and hydroquinone mixture (5 mg/ml each) were performed at 1700 rpm using different mobile phase flow rates (8, 12, 14 ml/min) and an injection volume of 1 ml. The columns were filled with 50/50 (v/v) of upper and lower phase. This volume ratio was chosen because for the same mobile phase flow rate the retention times of the early and late eluting component in both modes would be similar, since $K_P^{Des} = 2.2$ and $K_H^{Des} = 0.6$ and $K_P^{As} = 0.5$ and $K_H^{As} = 1.7$. This implies a similar duration of the two steps of the sCPC cycle.

To access the column efficiency at different flow rates, the number of stages for pyrocatechol and hydroquinone were calculated from the UV detector signal using the momentum analysis:

$$N = \left(\frac{\mu_t}{\sigma_t}\right)^2 = \left(\frac{t_R}{\sigma_t}\right)^2 \tag{20}$$

where μ_t is the first absolute moment (i.e. mean retention time) and σ_t^2 is the variance determined by the second central moment of the elution peak. The number of stages at different mobile flow rates in ascending and descending mode, at 1700 rpm, is presented in Table 3.

The mass transfer in centrifugal partition chromatography occurs between two liquid phases in a centrifugal force field. The contact area between the two phases, i.e. mass transfer area, is a function of several parameters. The visualization of the flow regime in the cells of a CPC column have helped to better understand the influence of the physical properties of the biphasic liquid system, mobile phase flow rate and rotational speed (centrifugal force) on the stationary phase retention and the area of phase contact [14,20–23]. In general, for fixed mobile and stationary phase volumes (i.e. stationary phase retention) the mobile phase flow rate and the rotational speed increase the phase contact area and consequently increase the mass transfer rate and improve the column efficiency.

As expected, the column efficiency increases with the increase of the mobile phase flow rate (see Table 3). In general, for a component with distribution constant equal to 1 ($K_k^{Des} = K_k^{As} = 1$) similar column efficiency, i.e. number of stages would be expected in ascending and descending mode. Here, the column efficiency is different in each mode due to the different distribution constants of hydroquinone and pyrocatechol in each mode ($K_P^{Des} > K_P^{As}$ and $K_H^{Des} < K_H^{As}$).

The non-equilibrium stage model proposed by Villermaux [24] can be used to evaluate the contribution of the axial dispersion and the mass transfer resistance on the column efficiency. In this model the column is modeled as series of perfectly mixed cells in which due to the presence of mass transfer resistance the mass transfer between the mobile and stationary phase does not occur instantaneously (finite mass transfer rate). Kostanyan [17] adapted the model for liquid–liquid chromatography. In the case of equal volumes of the mobile and stationary phase (each 50% of the column volume), the number of stages for solute k, N_k, can be presented as follows [17]:

$$\frac{1}{N_k} = \frac{1}{n} + \frac{2}{T_k} \cdot \left(\frac{K_k}{1+K_k}\right)^2 \tag{21}$$

where n is the number of perfectly mixed cells and T_k is the number of mass transfer units for solute k. The n is related to the axial dispersion ($n \approx (Pe/2) = (uL/2D)$) and T_k is related to the mass transfer ($T_k = (k_{MT_K} a V_c)/F$) [17]. Pe is the Peclet number, u is the mobile phase velocity, D is the axial dispersion coefficient, k_{MT_K} the volumetric mass transfer coefficient, a is the specific surface of the phase contact and V_c is the column volume. Since hydroquinone and pyrocatechol have the same molecular weight, we assume that T_k is equal for both components ($T_k \approx T$). The calculated values of N_k, n and T are given in Table 3. Both, n and T increase with the increase in the mobile phase flow rate, demonstrating decrease of the influence of the axial dispersion and mass transfer effects on the band broadening.

The elution profiles of the pulse injection experiments performed at different mobile phase flow rate in ascending and descending mode (presented in Table 3) were also used for the calculation of the distribution constant of pyrocatechol and hydroquinone. The following average values were obtained: $K_P^{Des} = 1.9 \pm 0.2$ and $K_H^{Des} = 0.6 \pm 0.1$. These values are in a good agreement with the values obtained from the shake flask experiments (see Section 4.1).

4.3. Modeling of batch elution

One of the assumptions of the sCPC model is that the number of stages is equal in the ascending and descending step. Thus, it was investigated, if the use of a mean number of stages (obtained from ascending and descending experiments) for a simulation of the sCPC operation is acceptable. The batch elution experiments, performed in descending and ascending mode using different flow rates (Table 3), were simulated using the batch stage model described in Section 2.3. The number of stages used in the simulation was the average value of the number of stages in ascending and descending mode at a given mobile phase flow rate. A good agreement between the experimental and simulated elution profiles was achieved.

Table 4
sCPC unit operating parameters and system parameter.

Parameter	Value
Feed flow rate in descending step, $F_{L,F}$ (ml/min)	2
Feed flow rate in ascending step, $F_{U,F}$ (ml/min)	2
Mobile phase flow rate in descending step, F_L (ml/min)	10
Mobile phase flow rate in ascending step, F_U (ml/min)	10
Total column volume, V_C (ml)	247
Feed concentration in ascending and descending step, c_F (mg/ml)	5 mg/ml (each, P and H)
Distribution constant of pyrocatechol in descending step (P), K_P^{Des}(–)	2.2
Distribution constant of hydroquinone in descending step (H), K_H^{Des} (–)	0.6
Mean number of stages of pyrocatechol, N_P (–)	463
Mean number of stages of hydroquinone, N_H (–)	476

The comparison of the simulated and the experimental elution profiles at a mobile phase flow rate of 12 ml/min is available in Supplementary data.

Next, the influence of the number of stages on the shape of the elution profiles of pyrocatechol and hydroquinone was investigated by simulation (results presented in Supplementary data).

The influence of the number of stages on the elution profile is insignificant for values above 400. The elution profile with 1000 stages is identical to the one with 700 stages.

4.4. Modeling of the sCPC separation

4.4.1. Selection of the sCPC unit operating parameters

The operating parameters constraints presented in Table 1 were used to select the operating parameters of the sCPC unit. As it can be seen from Eqs. (4)–(7) in Table 1, the flow rates and step times cannot be selected independently. The sCPC unit flow rates were selected first. For the used biphasic liquid system the maximum mobile phase flow rate at which the phase ratio of 50/50 (v/v) can be guaranteed in both steps is 16 ml/min. A mobile phase flow rate of 10 ml/min (descending and ascending step) and a feed flow rate of 2 ml/min (descending and ascending step) were selected. The unit operating parameters are summed up in Table 4. The mean distribution constants of the shake flask experiments were used.

It should be mentioned, that a rotational speed of 1700 rpm was selected in all experiments, because it was observed in previous experiments [9], that an increase of the rotational speed to 2000 rpm did not result in an improvement of the separation performances. Due to the pressure drop limitation of the unit (80 bar), it was not possible to increase the rotational speed more than 2000 rpm.

After the selection of the mobile phase and feed flow rates, the region of complete separation was calculated using the Eqs. (4)–(7) from Table 1. The region of the complete separation is presented in a working diagram $t_{As} = f(t_{Des})$ in Fig. 3. Pure products can be collected for all (t_{As}, t_{Des}) pairs located inside this region, if the solutes distribution equilibrium is reached instantaneously. Four sCPC experiments were performed with (t_{As}, t_{Des}) pairs corresponding to the points A, B, C and D in Fig. 3.

Detailed information about the selection of the operating parameters and the derivation of the operating parameters constraints can be found in [9,10].

4.4.2. sCPC model validation

The sCPC operation was simulated using the stage model (Eqs. (8)–(16) in Table 2). As input parameter, the mean number of stages, N_k, determined by pulse injections at a flow rate of 12 ml/min – and the distribution constant of the solute k, K_k^{Des}, measured by shake flask experiments were used in the simulation (Table 4). The distribution constant of the solute k in ascending mode, K_k^{As}, is the inverse value of K_k^{Des}.

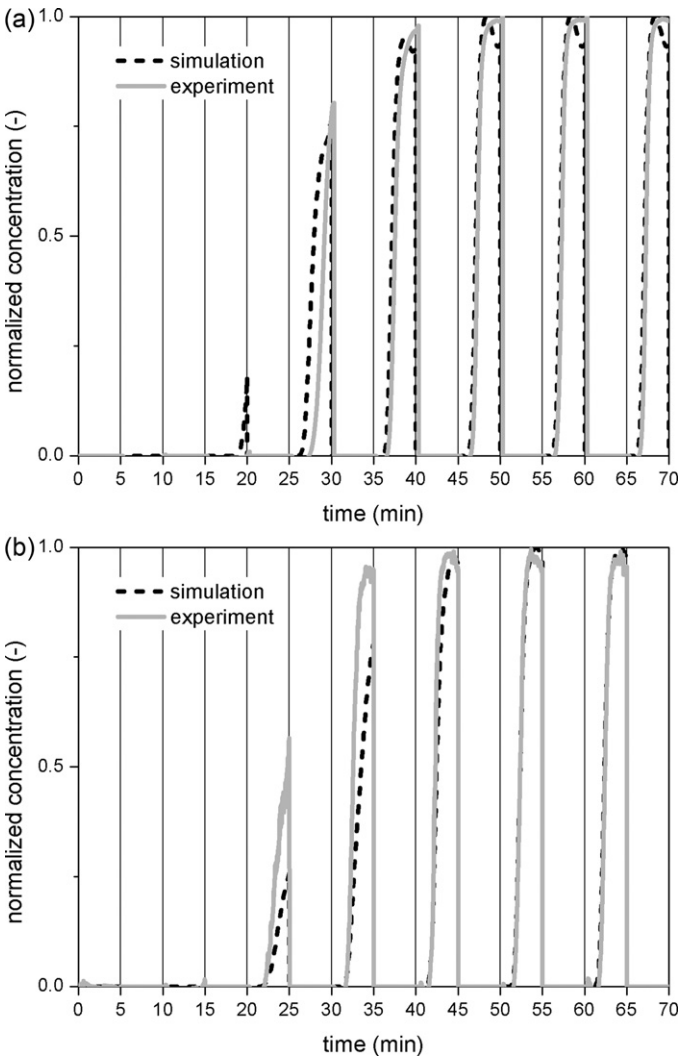

Fig. 4. Comparison of the normalized concentration history and normalized UV signal at the (a) product collection point in descending mode (b) product collection point in ascending mode. $t_{As} = t_{Des} = 5$ min (Point B in Fig. 3), feed flow rates: 2 ml/min ($F_{L,F} = F_{U,F}$), mobile phase flow rates: 10 ml/min ($F_L = F_U$), rotation speed: 1700 rpm. sCPC operation started in the ascending step.

The simulated and experimental data obtained for point B from Fig. 3 (i.e. $t_{As} = t_{Des} = 5$ min) using the operating parameters given in Table 4, are presented in Fig. 4. The simulated normalized concentration is compared with the normalized UV signal at the product collection point in descending mode (Fig. 4a) and at the product collection point in ascending mode (Fig. 4b). According to the simulation, the concentration of pyrocatechol at the product collection point in descending mode and of hydroquinone at the product collection point in ascending mode are equal to zero, what means that both products are pure.

The cyclic steady state in the simulation and the experiment was achieved approximately after six cycles. The model predicts very well the concentration elution profile in the cyclic steady state. The small discrepancies between the experiment and the simulation are in the range of the experimental deviation obtained in the sCPC unit separation reproducibility test in [9].

At cyclic steady state, the products were collected during the entire cycle and analyzed by HPLC. In agreement with the

Table 5
Influence of the cycle time duration ($t_{As} + t_{Des}$) on the product purity and number of cycles needed to reach cyclic steady state (CSS). Feed flow rates: 2 ml/min ($F_{L,F} = F_{U,F}$), mobile phase flow rates: 10 ml/min ($F_L = F_U$), rotational speed: 1700 rpm, sample concentration 5 mg/ml.

Point in Fig. 3	t_{Des} (min)	t_{As} (min)	CSS: time (min)	CSS: cycle number	Purity (ppm)
A	2	2	60	15	<1
B	5	5	60	6	<1
C	10	10	60	3	<1
D	10	7	60 (Des) 80 (As)	4 (Des) 5 (As)	<1

simulation, in which a purity of 100% was achieved, the collected products were pure (impurity <1 ppm).

4.4.3. Variation of the step times (cycle time)

For any point inside the separation region plotted in Fig. 3, pure products are expected. The constraints on the operating conditions are given as t_{Des}/t_{As} ratio, which can be fulfilled by different absolute values of t_{As} and t_{Des}. For the operating parameters presented in Table 4, the influence of the step times on the unit separation performances was investigated: (i) by changing the duration of the two steps in a way that their ratio stays constant, $t_{Des}/t_{As} = 1$ and (ii) by changing the t_{Des}/t_{As} ratio. The time and the cycle number needed to achieve the cyclic steady state and the purity of the products are summed up in Table 5.

Simulations were performed and compared with experimental results presented in Table 5. The same results as in the experiments were achieved.

Pure products were collected for all cases studied, including the point A which is very near to the border of the region of complete separation.

The time needed to reach the cyclic steady state is similar (approximately 60 min) for case A, B, C and the descending mode in case D. Approximately 80 min are needed in the ascending mode in case D until the unit reaches the cyclic steady state. The time needed to achieve a cyclic steady state is related to the effective velocity of the components in each mode and the duration of the steps [10].

The cycle number until the cyclic steady state is reached is the highest (15 cycles) for case A. As already discussed in the previous paper [9], from the operating point of view, the use of longer operation times is advisable to extend the valves lifetime and to have enough time for re-establishing the flow pattern inside the columns after the switch of the mobile phase type (upper or lower) and its flow direction.

4.4.4. Variation of the feed concentration

The feed concentration should be selected such that the feed solutes do not affect the liquid–liquid equilibrium of the biphasic liquid system, since a change of the distribution constants (partition coefficients), the physicochemical properties of the biphasic liquid system and the ratio of the two phases in the sCPC columns may occur [25]. Furthermore, one assumption of the method used to select the sCPC unit operating parameters and of the sCPC mathematical model is that the distribution constant and the volumes of the mobile and stationary phase are constant (i.e. independent of the concentration of the solute).

The partitioning of hydroquinone and pyrocatechol in the biphasic system heptane/ethyl acetate/methanol/water (1/2/1/2, v/v/v/v) was measured with the shake flask method in a concentration range of the solute in the system between 0.1 and 150 mg/ml. The distribution equilibrium of hydroquinone is linear up to approximately 20 mg/ml and of pyrocatechol up to approximately 80 mg/ml.

According to these results, a sCPC separation with feed concentration of 15 mg/ml of each solute was performed. In Fig. 5 the measured normalized UV signal and simulated normalized concentration history at the product collection point in descending (Fig. 5a) and ascending mode (Fig. 5b) over the time is presented.

The products collected in cyclic steady state were pure (impurity <1 ppm) and the cyclic steady state was achieved in approximately six cycles. These results are in agreement with the simulation. In the first cycles there are discrepancies in the experimental and simulated values. The product elutes earlier in the simulation than in the experiment in descending mode, and in the ascending mode an opposite behavior is observed. One of the possible reasons for the observed discrepancy in the elution profiles could be a slight change of the distribution constant and a change of the

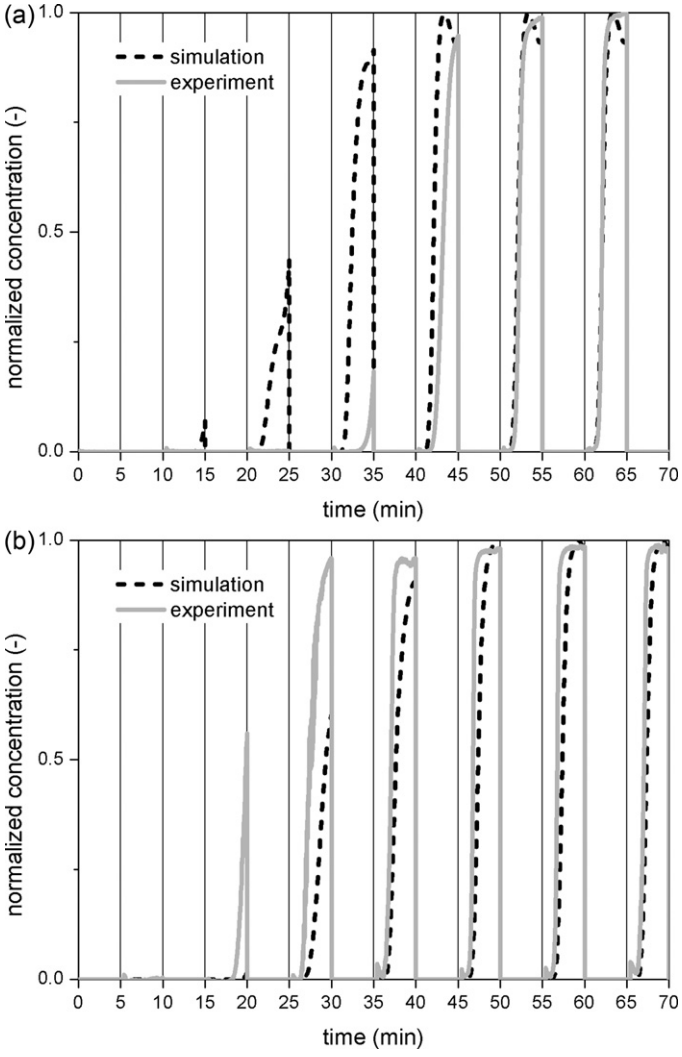

Fig. 5. Comparison of the normalized concentration history and normalized UV signal at the (a) product collection point in descending mode (b) product collection point in ascending mode. $c_F = 15$ mg/ml, $t_{As} = t_{Des} = 5$ min (Point B in Fig. 3), feed flow rates: 2 ml/min ($F_{L,F} = F_{U,F}$), mobile phase flow rates: 10 ml/min ($F_L = F_U$), rotation speed: 1700 rpm. sCPC operation started in the descending step.

column efficiency. This hypothesis will be systematically studied in future.

Considering that the feed is partially diluted in the sCPC unit, the use of a slightly higher feed concentration could also be possible. Anyhow, this should be proven experimentally.

5. Conclusion

A separation of a binary feed mixture of pyrocatechol and hydroquinone in a sequential centrifugal partition chromatographic (sCPC) unit was simulated using the stage model. The sCPC model parameters were determined experimentally. The distribution constants of the feed components were measured by shake flask method. The number of the stages, which gives information about the dispersion and mass transfer effects in the two sCPC columns, was determined from batch elution experiments performed in ascending and descending mode. The sCPC model was validated by experimental separation runs performed in a sCPC unit.

It was demonstrated that the stage model is suitable for simulation of sCPC separation as long as the concentration of the feed stream does not affect the distribution equilibrium of the feed components and the volume of the two phases in the columns. Under these restrictions, the stage model is predictable and can be used to perform parametric studies, select unit operating parameter and optimize the process performances.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.102.

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