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A multilayer coil in type-I counter-current chromatography

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Abstract

In the present study, a novel model of type-I counter-current chromatography was developed to simplify the separation column manufacture and extend its application ranges. In this system, a multilayer coil was introduced as the separation column of type-I counter-current chromatography for the first time. The chromatographic performance of this apparatus was evaluated in terms of retention of the stationary phase (*S_f*), theoretical plate (*N*) and peak resolution (*R_s*) using dipeptides and DNP-amino acids as test samples with two classic solvent systems composed of 1-butanol-acetic acid-water (4:1:5, v/v) (BAW) and hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v) (HEMW), respectively. The results indicated that the performance was optimal at the revolution speed of 200 rpm. The lower flow rate was more beneficial to retention of stationary phase and resolution. Over all results of our study revealed that the new type-I counter-current chromatography with a multilayer coil has a high application potential.

Keywords

multilayer coil; type-I counter-current chromatography; dipeptides; DNP-amino acids

1. Introduction

As a support-free liquid-liquid partition chromatography, counter-current chromatography (CCC) has been widely applied for the separation and purification of complex samples [1–4]. Type-I counter-current chromatography with the high efficiency, one of the important

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branch of CCC, was developed in 1971 [5]. However, its study was limited due to the original bulky centrifuge design [6].

In the past decade, a compact type-I coil planet centrifuge with a shorter revolution radius and a decreased column holder height was developed for counter-current chromatography to widen and extend its application ranges [7]. Subsequently, the column orientation, the mounted column model and the coiled column configuration were evaluated to better understanding of its performance [8–10]. Nevertheless, in the original design, the separation column was made by winding a single piece of tubing (0.85 mm I.D.) onto 47 pieces of nylon pipes (5 mm O.D. and 5 cm long) in such a way that every other coil unit has opposite handedness, which was mounted around the holder hub [7]. Because of complex procedure of column manufacture, it is still difficult to enormously widen the application ranges. In addition, larger O.D. tubing and special modified tubing were limited in the procedure of winding the tubing onto pipes. Larger O.D. tubing is more beneficial to increase the sample size [11, 12], the same as HPLC. The tubing modifications sometimes help to improve the column performance [13–15].

The multilayer coil separation column was widely used in the system of type-J CCC with high performance [16, 17]. In order to simplify the column design and increase the sample loading capacity the multilayer coil was applied to type-I counter-current chromatography in the present study. The performance was investigated at different experimental conditions using two typical solvent systems with suitable sets of dipeptide and DNP-amino acid samples.

2. Experimental

2.1. Apparatus

The type-I coil planet centrifuge used in the present study was fabricated at the NIH Machine Shop, Bethesda, MD, USA. It holds a separation column on one side and a counterweight on the other side of the rotor symmetrically at 10 cm from the central axis of the apparatus. The column holder undergoes a type-I synchronous planetary motion, i.e., the holder synchronously counter-rotates about its own axis once during one revolution around the central axis of the centrifuge. The multilayer column was made by winding a single piece of polytrifluoroethylene (PTFE) tubing with a diameter of 1.6 mm (Zeus Industrial Products, Orangeburg, SC, USA) onto the column holder (Fig. 1). The length and capacity of the tubing are approximately 30 m and 80 mL, respectively. The revolution speed of the apparatus is regulated from 0 to 1200 rpm with a speed controller (Model Leeson Speed Master, Washguard, USA).

A metering UFLC pump (Shimadzu LC-20ADVP, Columbia, MD, USA) was used for pumping the solvents, and the effluent was continuously monitored with a UV detector (LKB Instruments, Stockholm, Sweden). The elution curve was traced using a strip-chart recorder (Pharmacia, Stockholm, Sweden).

2.2. Reagents

1-Butanol, hexane, ethyl acetate and methanol were HPLC grade and purchased from Fisher Scientific, Fair Lawn, NJ, USA and other solvents such as acetic acid and hydrochloric acid of an analytical grade from Mallinckrodt Chemicals, Phillipsburg, NJ, USA. Test samples including tryptophyl-tyrosine (Trp-Tyr), valyl-tyrosine (Val-Tyr), N-2,4-dinitrophenyl-L-alanine (DNP-L-ala), N-2,4-dinitrophenyl- β -alanine (DNP- β -ala), N-2,4-dinitrophenyl-DL-glutamic acid (DNP-DL-glu) were all obtained from Sigma Chemicals, St. Louis, MO, USA.

2.3 Distribution Coefficient Measurement

The distribution coefficients (K_U) of each sample in the two-phase solvent system was determined using the conventional test tube method with a UV spectrophotometer (Genesis 10 UV, Thermo Spectronic, Rochester, NY, USA) at 280 nm [14]. The absorbance of the upper phase was recorded as A_U and that of the lower phase as A_L . Then, partition coefficient (K_U) was calculated according to the following equation: $K_U = A_U/A_L$.

2.4 Two-phase Solvent Systems and Sample Solutions

Two typical two-phase solvent systems including hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v) (HEMW) and 1-butanol-acetic acid-water (4:1:5, v/v) (BAW) were used to separate test samples of DNP-amino acids (for HEMW) and dipeptides (for BAW), respectively. Each solvent mixture was thoroughly equilibrated in a separatory funnel by repeated vigorous shaking and degassing, and the phases separated shortly before use. Sample solution 1 was prepared by dissolving 5.3 mg of DNP-L-ala ($K=2.36$), 5.3 mg of DNP- β -ala ($K=1.18$) and 5.0 mg of DNP-DL-glu ($K=0.45$) in 10 ml of the upper phase of hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v) [18], and 0.4 ml was charged in each run. Sample solution 2 was prepared by dissolving 25 mg of Trp-Tyr ($K=1.69$) and 100 mg of Val-Tyr ($K=0.53$) in 20 ml of the upper phase of 1-butanol-acetic acid-water [18], and 0.6 ml was charged in each run.

2.5 Separation Procedure

The separation column was entirely filled with the upper stationary phase in each separation, followed by sample loading. Then, the column was rotated at a given revolution speed while the lower mobile phase was pumped into the separation column at a given flow rate. The effluent from the outlet of the coiled column was continuously detected at 280 nm. After the desired peaks were eluted, the separation system was shut down. Then, the column contents were collected into a graduated cylinder by pressured air to determine the volume of the stationary phase retained in the column. The retention of the stationary phase (S_f) was computed by dividing the volume of the retained stationary phase by the total column volume.

2.6 Evaluation of Partition Efficiency

The partition efficiency of separation column was evaluated by computing theoretical plate number (N) for each peak and the resolution (R_s) between the peaks using the following equations:

$$N=(4t_R/W)^2 \quad (1)$$

$$R_s=2(t_{R2}-t_{R1})/(W_1+W_2) \quad (2)$$

where t_R and W indicate the retention time and the baseline peak width in Eq 1 and Eq 2, respectively.

3. Results and discussion

As a mainstream separation column, the multilayer coil with the excellent performance was widely used in high-speed counter-current chromatography, especially for type-J CCC [16,19]. In the previous study, type-I CCC showed very good performance in the separation of dipeptide and DNP-amino acid samples each with the suitable two-phase solvent, although its manufacture of column is complex. In order to simplify the separation column design and extend its application ranges, the multilayer coil was introduced into the system of type-I CCC for the first time (Fig. 1). The performance of this novel design was evaluated in the separation of dipeptides and DNP-amino acids, which were classic systems for evaluating the performance of type-I CCC.

The performance on the separation of DNP-amino acids at the different revolution speeds with the different flow rates was investigated (Fig. 2). DNP-amino acid samples included DNP-ala, DNP-β-ala, and DNP-glu. Their K values reported in the previous study were 2.36, 1.18, and 0.44, respectively, in the two-phase solvent system composed of hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v) [20]. In general, S_f was decreased with the increased flow rates (0.25–1.50 mL/min) which was consistent with the literatures [21, 22]. However, to our surprise, S_f was increased with the decrease revolution speeds, except for revolution speed at 0 rpm. Fig. 2A shows that the S_f at 200 rpm was better than the others, although the highest value of S_f was lower than 20% (Table 1).

On the other hand, peak resolution at 200 rpm with the flow rates from 0.25–1.50 mL/min was better than that the other (Table 1). As we known, for the optimized and successful cases, the range of revolution speeds was 800–1000 rpm in the traditional type-J CCC experiments [23, 24]. However, it is strange that type-I CCC does not work well at high revolution speeds, which was very different from the traditional type-J CCC. The resolution of three compounds was very poor and lower than 0.5 at the revolution speed of 400 rpm with the flow rate of 0.25 mL/min. However, when the revolution speed decreased to 200 rpm, type-I CCC with the multilayer coil exhibited the very good performance (Fig. 3). When the flow rate was 1.50 mL/min, the three compounds were partially separated. One of R_s was 0.68 and another one was less than 0.50 (Fig. 3A and table 1). With the decreased flow rates, S_f , N , and R_s gradually improved (Fig. 3B and C). When the flow rate was decreased to 0.25 mL/min, three DNP-amino acids were separated completely in a couple of hours and R_s was 1.64 and 1.44, respectively (Fig. 3D and table 1).

A another series of experiments was investigated to evaluate effects of dipeptide separation with the polar BAW solvent system on Rs, N and Sf. Trp-Tyr and Val-Tyr were used as the test samples. Their K values were 1.69 and 0.53 in BAW system, respectively [20]. Higher revolution speeds were bad for Rs and Sf, which was similar with HEMW system. With the decreased revolution speed, the performance of multilayer coil was improved. The revolution speed at 200 rpm brought the best Sf and Rs (Fig. 4). However, when the revolution speed decreased to 0 rpm, the worst Sf and Rs were obtained. When the flow rate was 1.50 mL/min, the two dipeptides were partially separated. One of Rs was 1.00 (Fig. 5A and table 1). With the decreased flow rates, Sf, N, Rs improved gradually (Fig. 5). When the flow rate was decreased to 0.25 mL/min, Trp-Tyr and Val-Tyr were separated completely and Rs was 1.92 (Fig. 5D and table 1).

As described above, the present experiment shows that the type-I planetary motion can yield the best separation at a low revolution speed of 200 rpm compared with the conventional type-J high-speed CCC in which best separation is obtained at a high revolution speed of over 800 rpm. This can be explained on the basis of difference in the centrifugal force distribution between these two planetary motions. The type-J planetary motion produces a complex force distribution which changes the amplitude and the direction of force vector according the distance of tube location on the column holder. In this system the best separation is obtained at the coil location at the β value between 0.5-0.8 which distributes the lighter phase toward the head and the heavier phase toward the tail. ($\beta = r/R$ where r is the distance between coil and the holder shaft and R is the distance between the column holder shaft to the central axis of the centrifuge.) Consequently, higher revolution speed accelerates the motion of the mobile heavier phase toward the tail to yield a high retention of the stationary phase and good peak resolution. In contrast, in the type-I planetary motion, all location of the coil is subjected to the equal centrifugal force so that the two phases competitively move toward the head of the column. Therefore, application of high revolution speed inhibits the motion of the mobile phase toward the tail resulting in a low retention of the stationary phase and reduced peak resolution.

4. Conclusions

Multilayer coil can be successfully applied to the separation of DNP-amino acids, and dipeptides with excellent peak resolution to replace the traditional coiled unit column in type-I counter-current chromatographic system. The overall experimental results indicated that the multilayer coil was very good performance in type-I counter-current chromatography. Although the retention of stationary phase was not much higher than before, Rs was improved because of enhanced interaction between solvent system and samples. In addition, the simple manufacture model for column makes it possible to use bigger diameter or modified tubing in type-I CCC.

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Figure 1.
The holder of a multilayer coil separation column for type-I counter-current chromatography.

Figure 2.

Comparison of retention of stationary phase of various flow rates in DNP-amino acid separation using the multilayer coil in type-I counter-current chromatography. Solvent system: n-hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v); stationary phase: upper phase; mobile phase: lower phase; test sample: DNP-DL-glu, DNP- β -ala and DNP-L-ala; revolution speed: 200 rpm.

Figure 3. DNP-amino acid separation by multilayer coil in type-I counter-current chromatography. Solvent system: n-hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v); sample: DNP-DL-glu, DNP-β-ala and DNP-L-ala; revolution speed: 200 rpm; detection: 280 nm; flow rate: (A) 1.50 ml/min; (B) 1.00 ml/min; (C) 0.50 ml/min; (D) 0.25 ml/min.

Figure 4.

Comparison of retention of stationary phase of various flow rates in dipeptide separation using the multilayer coil in type-I counter-current chromatography. Solvent system: 1-butanol-acetic acid-water (4:1:5, v/v); stationary phase: upper phase; mobile phase: lower phase; test sample: trp-tyr, val-tyr; revolution speed: 200 rpm.

Figure 5. Dipeptide separation by multilayer coil in type-I counter-current chromatography. Solvent system: 1-butanol-acetic acid-water (4:1:5, v/v); sample: trp-tyr, val-tyr; revolution speed: 200 rpm; detection: 280 nm; flow rate: (A) 1.50 ml/min; (B) 1.00 ml/min; (C) 0.50 ml/min; (D) 0.25 ml/min.

Table 1

The performance of multilayer coil in type-I counter-current chromatography for DNP-amino acid and dipeptide separation.

Sample	Solvent system	Flow rate (ml min ⁻¹)	Sf (%) ^b	N ^c	Rs ^d
DNP-DL-glu NP-β-ala DNP-L-ala	HEMW ^a	0.25	17.33	4096/2069/1219	1.64/1.44
		0.50	11.31	4011/1873/1422	0.78/0.85
		1.00	8.04	5041/1250/—	0.76/<0.50
		1.50	6.94	5228/1041/—	0.68/<0.50
Val-Tyr Trp-Tyr	BAW ^a	0.25	11.14	4937/1579	1.92
		0.50	9.10	3328/605	1.69
		1.00	6.94	887/275	1.22
		1.50	5.82	341/250	1.00

Note:

^aHEMW: hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v);

^bBAW: 1-butanol-acetic acid-water (4:1:5, v/v);

^cSf: the retention of stationary phase;

^dN: theoretical plate number;

^eRs: resolution. Revolution speed: 200 rpm.