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Vortex counter-current chromatography

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ABSTRACT

A novel counter-current chromatographic system is developed by mounting a vortex column on a type-I coil planet centrifuge. The column is fabricated from a high-density polyethylene disk (16 cm diameter and 5 cm thick) by making multiple holes of various diameters (3–12.5 mm) each arranged in a circle and connected with narrow transfer ducts. The performance of this vortex column is tested with three different two-phase solvent systems with a broad range in hydrophobicity. The results indicated that the smallest diameter column (3 mm diameter, 120 units with 42.8 ml capacity) yielded the best separation with the height equivalent to a theoretical plate of 2 cm compared with 20 cm required by the conventional multilayer coil column of high-speed CCC. By avoiding the use of an Archimedean Screw Force, the system shows a low column pressure which would permit safe operation of a large preparative column without a risk of leakage of solvent and column damage. Published by Elsevier B.V.

1. Introduction

High-speed counter-current chromatography (HSCCC) has been widely used in the separation of natural and synthetic products [1–5]. It uses a type-J coil planet centrifuge which produces planetary motion of the multilayer coiled column in such a way that the column rotates about its own axis while revolving around the central axis of the centrifuge both in the same direction. This synchronous planetary motion generates an Archimedean Screw effect to create a favorable hydrodynamic effect on the two-phase solvents in a multilayer coil to produce efficient mixing and retention of the stationary phase. Type-I planetary motion, in which the coiled column synchronously counter-rotates about it own axis, can also perform efficient CCC separation in a small diameter coil similarly using an Archimedean Screw effect [6,7]. One disadvantage of utilizing the Archimedean Screw effect is that it produces high pressure in the separation column which would limit the application of a long column for preparative separations. Another disadvantage is its mixing pattern of the two phases along the column axis which tends to cause solute band broadening.

In the past it has been demonstrated that type-I planetary motion could be utilized for performing CCC without the Archimedean Screw Force by mounting a locular column on a gyrator [8]. However, the short revolution radius (2.5 cm) of the apparatus caused a problem of carryover of the stationary phase due to a lack of centrifugal force to stabilize hydrodynamic motion of the two phases in the column against high frequency vibration at 1500 rpm. In order to solve this problem, we have constructed a new compact type-I coil planet centrifuge with a 10 cm revolution radius to stabilize the vortex motion of the two phases in the column to improve the performance of this CCC system.

The present paper describes the results of our preliminary studies on this vortex CCC using the type-I coil planet centrifuge equipped with a novel column design. Performance of the vortex CCC system is examined by a set of two-phase solvent systems with a broad range of hydrophobicity each with suitable test samples, and evaluated in terms of theoretical plate number and peak resolution together with % retention of the stationary phase.

2. Mechanism of vortex CCC

The type-I planetary motion is identical to that of a vortex mixer which produces circular motion of the centrifugal force to vigorously mix a liquid in a test tube (Fig. 1). The only difference between these two systems is that the type-I planetary motion with a larger revolution radius can produce a greater centrifugal force to form a vortex between two immiscible liquid phases, whereas the vortex mixer can form a vortex between liquid and air, but fails to move two liquid phases enclosed in a test tube. This vortex motion of the two phases produced by the type-I planetary motion can be utilized

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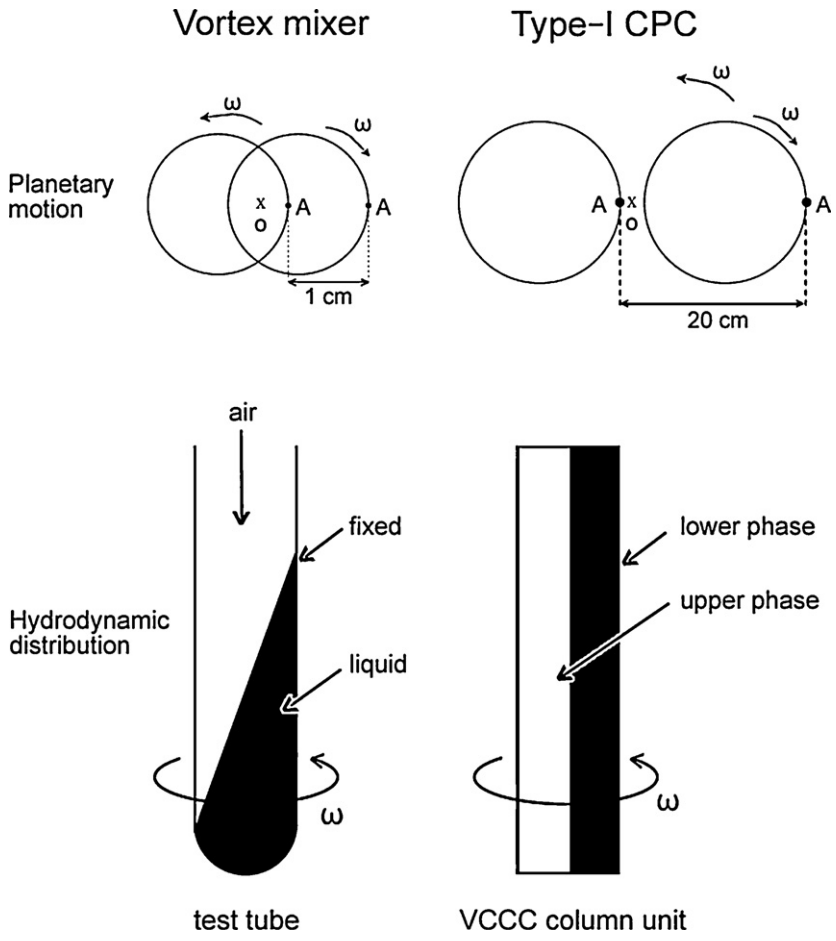

Fig. 1. Comparison in vortex motion between vortex mixer and type-I coil planet centrifuge.

for performing counter-current chromatography using a column design illustrated in Fig. 2 where a set of cylindrical column units is connected in series in such a way that a side terminal (inlet) of the unit joins the central terminal (outlet) of the next unit. In this column design, either lighter or heavier phase introduced from the side inlet (left) will retain near 50% of the other phase in each unit and exits through the center outlet (right).

The vortex CCC system has the following advantages over HSCCC which is based on the type-J planetary motion: under the uniformly circulating centrifugal force field, two phases in each unit undergo vortex motion where mixing takes place within a plane perpendicular to the column axis so as to prevent the longitudinal spreading of the sample bands. Efficient solute partitioning takes place in each unit not only at the interface between two phases, but also all over the internal wall surface since one of the phases (organic phase in the present case) having an affinity to the wall material will coat the wall surface. Also it is important to note that the vortex liquid motion does not produce any back pressure to the column so that a long column can be used without a risk of column damage due to elevated pressure. Since in the type-I planetary motion every point on the column is subjected to an identical centrifugal force field, the whole column space is efficiently used for separation, and the column capacity can be increased by eliminating the central shaft to accommodate a large column for preparative-scale separations.

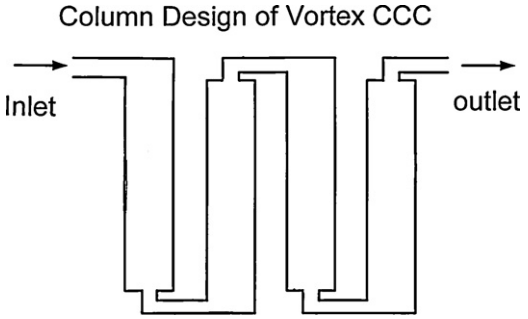

Fig. 2. Column design of vortex CCC.

3. Experimental

3.1. Apparatus

The type-I coil planet centrifuge used in the present studies was designed in our laboratory and fabricated in the machine shop in the National Institutes of Health, Bethesda, MD. USA. Fig. 3 schematically illustrates the cross-sectional view through the central axis of the apparatus. The apparatus holds a separation column and a counterweight symmetrically on the rotary frame at a distance of 10 cm from the central axis of the apparatus. The motor drives the rotary frame through a pair of pulleys coupled with a toothed belt. The stationary pulley mounted on the bottom plate around the central shaft is coupled to an identical planetary pulley fixed at the lower end of the column holder shaft. This pulley arrangement causes the column holder to synchronously counter-rotate on the revolving rotary frame. The flow tubes from the separation column are passed through the hole on the column holder shaft and

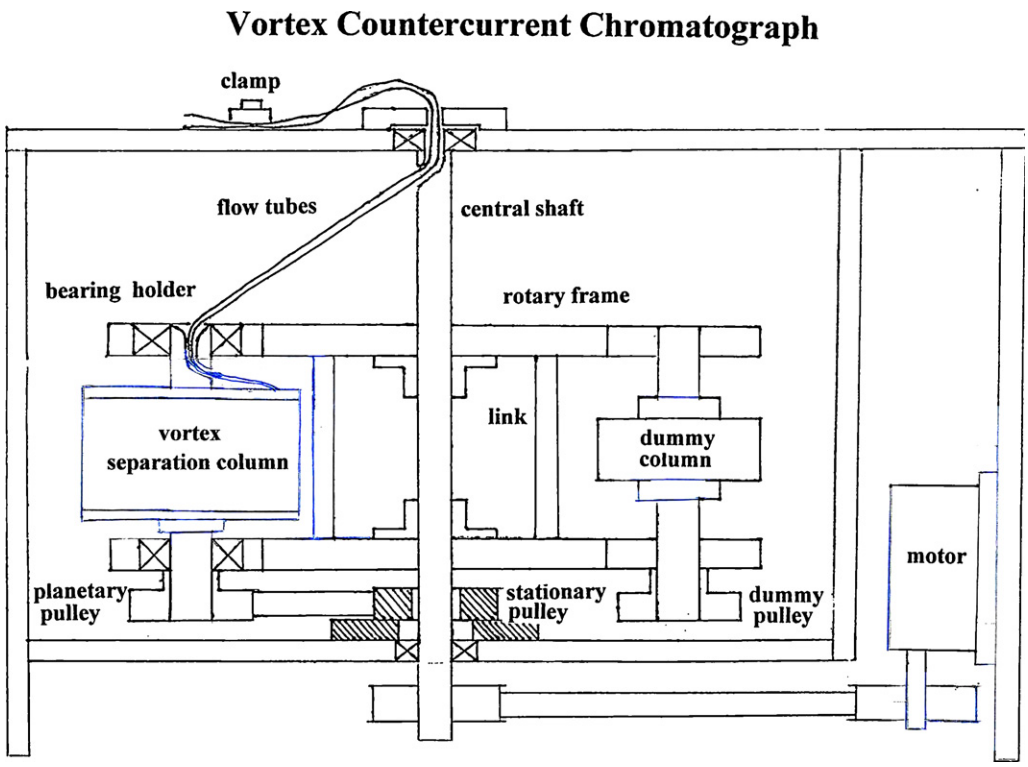

Fig. 3. Schematical drawing of vortex countercurrent chromatograph.

exit the centrifuge through the hole of the central shaft and then tightly supported on the upper plate of the centrifuge with a clamp as shown in the figure. These tubes keep their integrity for many runs if protected with a plastic sheath (Tygon tubing) at each hole and lubricated with grease.

The design of the separation column is shown in Fig. 4. The vortex column is made from a high-density polyethylene disk (17 cm in diameter and 5 cm in thickness) by making a set of holes in circle. Six columns are made each with different diameter (3 mm, 4 mm, 5 mm, 7.5 mm, 10 mm and 12.5 mm) which are arranged respectively from the outmost circle to the intermost circle on the disk as shown in the figure. In each column the cylindrical units are connected in side-center with a duct (1 mm wide groove). When this disk is sealed between a pair of PTFE sheets and metal flanges, it forms a series of cylindrical units as illustrated in Fig. 2. In the preliminary studies, a 3 mm diameter column located at the outermost circle was found to be most efficient. Therefore, the present experiments were performed exclusively with this smallest column. It contains 120 units and measures 600 cm in length with a total capacity of 42.8 ml excluding the dead space in the connection ducts.

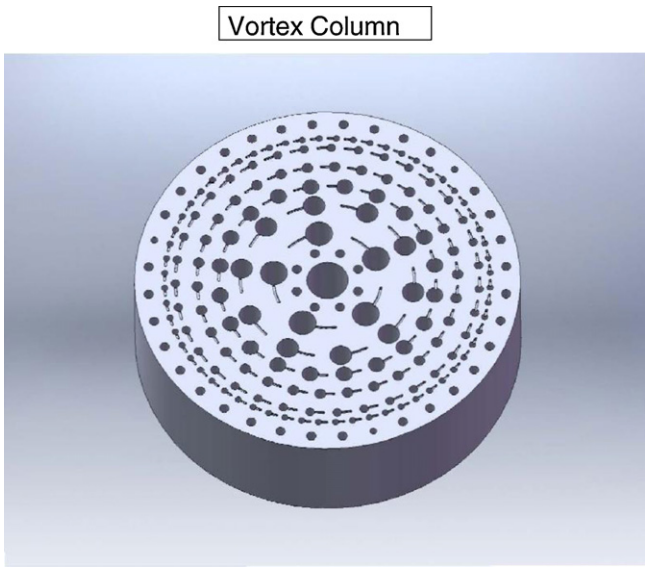

Fig. 4. Vortex column.

Elution was performed using an HPLC pump (Shimadzu, Kyoto, Japan), the effluent was monitored with a UV detector (model Uvicord SII, LKB, Stockholm, Sweden), and the chromatogram was traced with a strip-chart recorder (Millipore model CR112, Billerica, MA, USA).

3.2. Reagents

Organic solvents including hexane, ethyl acetate, methanol, acetonitrile, 1-butanol are of chromatographic grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA) and acetic acid and hydrochloric acid from Marllinckrodt Baker (Paris, KY, USA). Test samples including Sudan I and II dyes, N-2,4-dinitrophe-L-alanine (DNP-ala), N-2,4-DNP-DL-glutamic acid (DNP-glu), L-valyl-L-tyrosine (Val-Tyr) were purchased from Sigma (St. Louis, MO, USA), and L-tryptophyl-L-tyrosine (Trp-Tyr) from Bachem (Peninsula Laboratories, San Carlos, CA, USA).

3.3. Preparation of two-phase solvent systems and sample solutions

The following three biphasic solvent systems were prepared: hexane–acetonitrile for separation of Sudan I and II dyes; hexane–ethyl acetate–methanol–0.1 M hydrochloric acid (1:1:1:1, v/v) for separation of DNP-glu and DNP-ala, and 1-butanol–acetic

acid–water (4:1:5, v/v) for separation of Val-Tyr and Trp-Tyr. Each solvent system was prepared in a separatory funnel by repeating vigorous shaking and degassing several times. The sample solutions were prepared by dissolving a given amount of sample mixture in the upper phase used for separation.

3.4. Experimental procedure

In each separation, the separation column was entirely filled with the stationary phase, either lighter or heavier phase, followed by sample injection with a syringe. Then the column was rotated at the indicated speed and eluted with the mobile phase at a desired rate. The effluent from the outlet of the column was monitored with a UV detector to trace the chromatogram and collected into a graduated cylinder to measure the volume of the stationary phase displaced with the mobile phase. In order to improve tracing of the chromatogram in some runs, ethanol was added to the effluent at the inlet of the monitor at a flow rate of 1/5 that of the mobile phase flow rate.

3.5. Partition efficiency evaluation

Using the following conventional equations, performance of the vortex CCC was evaluated in terms of theoretical plate number of each peak (TP or N), peak resolution (R_S) and the height equivalent to a theoretical plate (HETP) from the chromatogram:

$$N = \left(\frac{4V_R}{W}\right)^2 \quad (1)$$

$$R_S = 2\frac{V_{R2} - V_{R1}}{W_1 + W_2} \quad (2)$$

where V_R is the retention volume of each peak and W is the peak width. When the two peaks are only partially resolved, the front half width of the first peak (W_1') and the rear half width of the second peak (W_2') are used to approximate both N and R_S using the following modified formula:

$$N = \left[\frac{4V_R}{W_1' + W_2'}\right]^2 \quad (3)$$

$$R_S = \frac{V_{R2} - V_{R1}}{W_1' + W_2'} \quad (4)$$

HETP was computed by dividing 600 cm with the averaged N value of two peaks.

The % retention of the stationary phase (S_f) was calculated from the displaced stationary phase volume and the total column capacity. It is also computed from an equation:

$$S_f = 100\frac{V_{R2} - V_{R1}}{(K_2 - K_1)\,V_C} \quad (5)$$

where K is the partition coefficient of the specified peak ($K_2 > K_1$), and V_C is the total column capacity.

4. Results and discussion

4.1. Selection of column diameter

In the preliminary studies the peak resolution of test samples in each two-phase solvent system has been examined in cylindrical separation units with different diamers. The results clearly showed that the smallest diameter column with 3 mm I.D. produced the best peak resolution. This may be explained by the ratio between the column volume and the interface area between the two phases where mass transfer takes place. In the vortex CCC system, the solute partitioning takes place in two different interface areas between the two phases: one is a vertical rectangular interface formed between the rotating two phases and the other is the internal wall surface of the separation unit where one of the phases – organic phase or more hydrophobic phase – forms a thin layer against the rotating aqueous phase. Both of these interface areas available for mass transfer increases relative to the column volume as the internal diameter of the cylindrical column unit is decreased. Therefore following series of experiments were performed exclusively with the 3 mm diameter column units.

4.2. Separation of test samples with three different two-phase solvent systems

Performance of the vortex CCC system is examined by a set of two-phase solvent systems with a broad range of hydrophobicity each with suitable test samples, and evaluated in terms of theoretical plate number (TP or N), peak resolution (R_S) between two peaks and height equivalent to a theoretical plate (HETP) together with % retention of the stationary phase (S_f).

Fig. 5 shows the results of vortex CCC separation of Sudan I and II dyes with a hexane–acetonitrile binary two-phase solvent system in the 6 m long vortex column. In this nonaqueous binary system, both upper and lower phases have a low viscosity with a relatively small difference in density between the two phases (0.755–0.665 = 0.090 g/cm^3). The separation was performed under various flow rates (0.5, 1, 2 and 3 ml/min) and revolution speeds (600, 800, and 1000 rpm). The results show that the low flow rate at 0.5 ml/min produced the best separation in most cases. One remarkable finding is that the present system yields very small HETP values of less than 2 cm compared with 20 cm required by conventional HSCCC with the multilayer coiled column. This small HETP value of vortex CCC is due to its unique mixing pattern of the two phases which is limited in a plane perpendicular to the column axis to minimize the solute band spreading along the length of the column. On the other hand, the retention of the stationary phase is mostly less than 50% which decreases with the application of higher flow rates of the mobile phase. It is seen from these data that theoretical plate number (TP) is somewhat higher in the lower phase mobile whereas the higher peak resolution (R_S) is obtained by the upper phase mobile.

Separation of DNP-amino acids with hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v) solvent system is shown in Fig. 6. This moderately hydrophobic solvent system can be widely applied for separation of natural and synthetic products by changing the mutual solvent volume ratio. In the present study the two-phase solvent system was acidified by HCl since DNP-amino acids form skewed peaks in the neutral condition. As shown in Fig. 6, this solvent system is also effectively used in vortex CCC. Similar to the hexane–acetonitrile system, the separations with the lower mobile phase yield higher TP values but lower R_S than those with the upper mobile phase, while HETP values are substantially increased probably due to higher viscosity of the lower aqueous phase.

Fig. 7 similarly shows the separation of dipeptides with a polar two-phase solvent system composed of 1-butanol–acetic acid–water (4:1:5, v/v) which is most useful for separation of polar peptides. Because of its physical properties characterized by high viscosity of the organic phase, small difference in density between two phases (0.05 g/cm^3) and low interfacial tension, this system is not well retained in the conventional multilayer coil separation column. However, it was found that the vortex column can retain around 50% of the stationary phase with a lower mobile phase at a low flow rate. It is interesting to note that the peak resolution of dipeptides in 1-butanol–acetic acid–water (4:1:5, v/v) shows higher peak resolution in the upper phase mobile than that in the

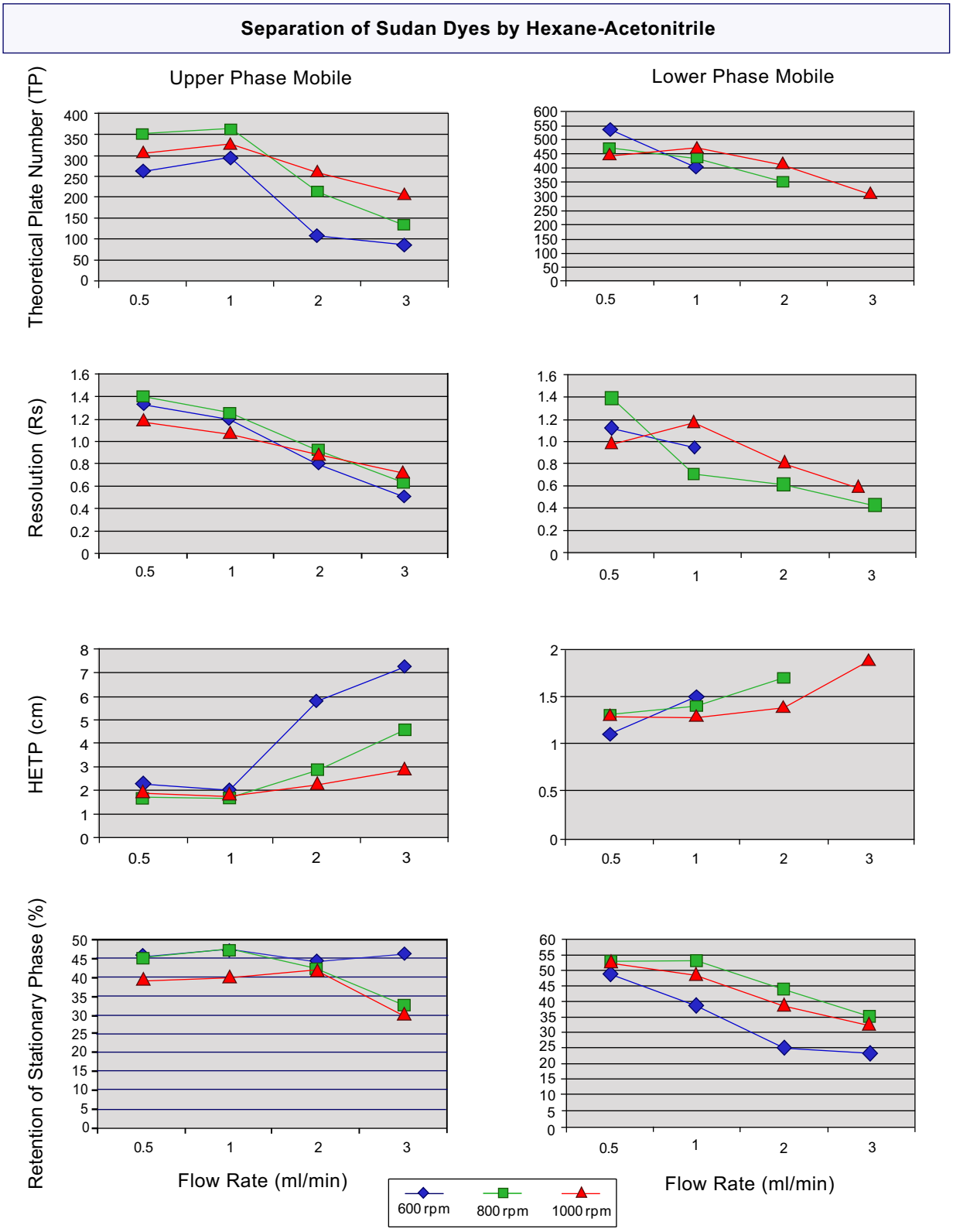

Fig. 5. Performance of 3 mm vortex column on separation of Sudan I and II dyes in hexane–acetonitrile solvent system. Experimental conditions: apparatus: type-I coil planet centrifuge with 10 cm revolution radius; column: 3 mm diameter, 5 cm deep, 120 cylindrical units with 42.8 ml capacity; solvent system: hexane–acetonitrile; sample: Sudan I and II each 0.5 mg in 0.5 ml upper phase; flow rate: 0.5–3 ml/min as indicated in abscissa; revolution 600, 800 and 1000 rpm; detection: 280 nm.

lower phase mobile. This may be explained on the basis of difference in viscosity between the two phases. In the butanol solvent system, the upper organic phase has higher viscosity than the lower aqueous phase. This causes lower retention (30%) of the aqueous stationary phase against viscous organic mobile phase which has higher affinity to the internal wall surface of the separation column. Consequently, the lower stationary phase retention results in lower peak resolution with a higher theoretical plate number.

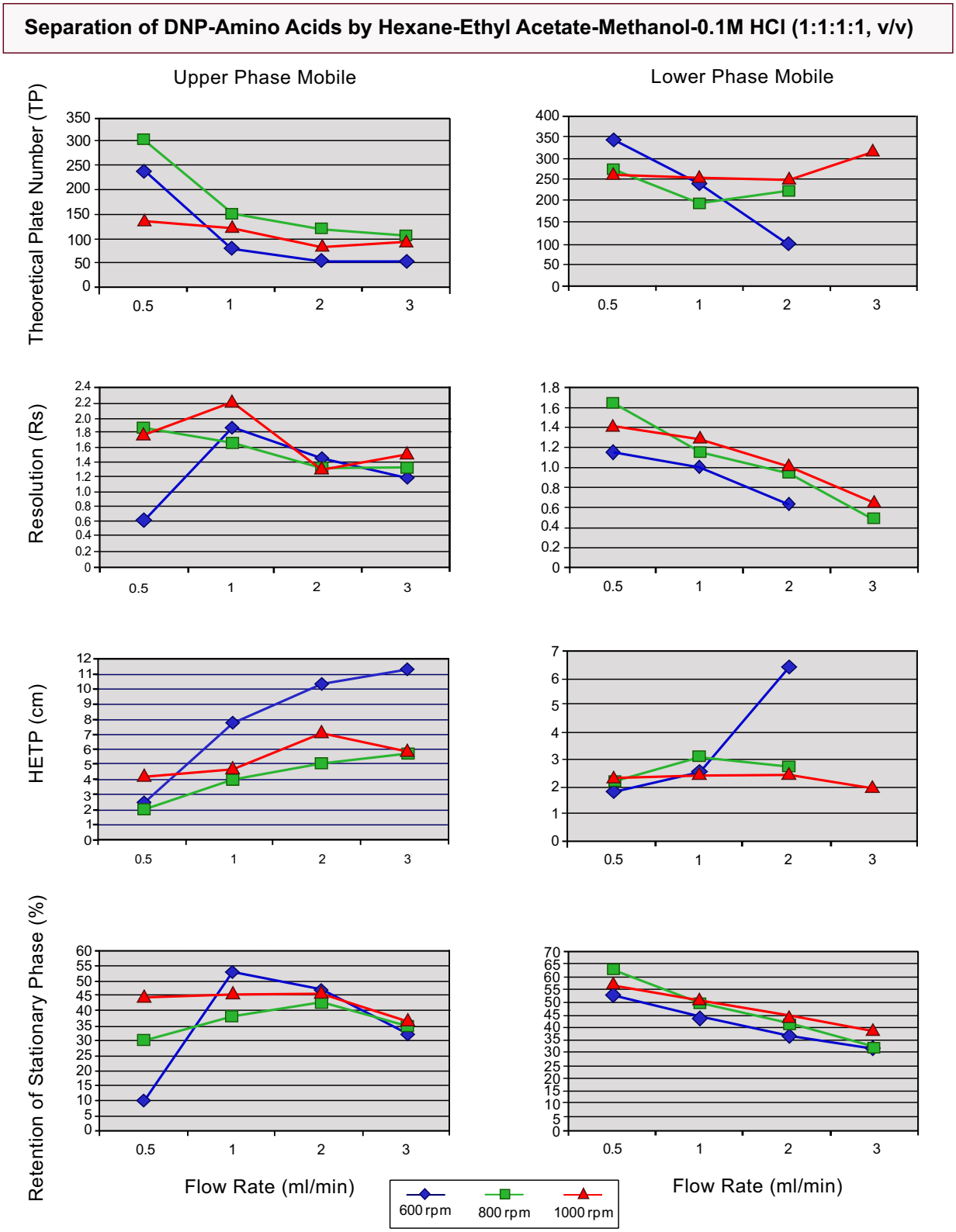

Fig. 6. Performance of 3 mm vortex column on separation of DNP-amino acids in hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v) solvent system. Experimental conditions: solvent system: hexane–ethyl aceteate–methanol–0.1 M HCl (1:1:1:1, v/v); sample: DNP-DL-glutamic acid and DNP-N-alanine each 1 mg in 0.1 ml upper phase. Other conditions are described in Fig. 5 caption.

The overall results of the above experiments indicate that the vortex CCC can be universally used for the organic-aqueous two-phase solvent systems with a broad range in hydrophobicity.

4.3. Column pressure in vortex CCC separation

One great advantage of vortex CCC over the conventional HSCCC is that the column pressure in these separations is only few psi

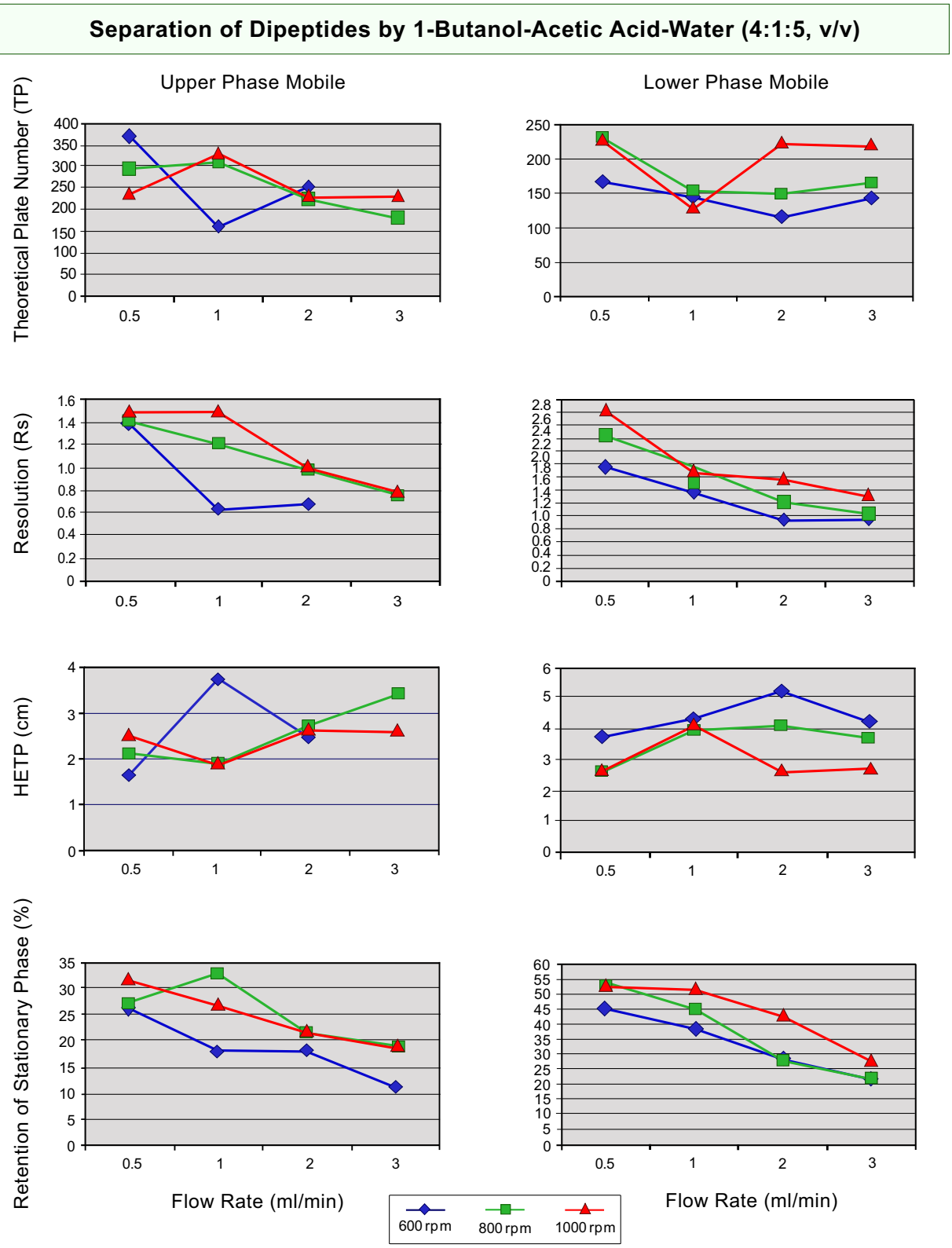

Fig. 7. Performance of 3 mm vortex column on separation of dipeptide in 1-butanol–acetic acid–water (4:1:5, v/v) solvent system. Experimental conditions: solvent system: 1-butanol–acetic acid–water (4:1:5, v/v); sample: Trp-Tyr (0.25 mg) and Val-Tyr (1 mg) in 0.2 ml upper phase. Other conditions are described in Fig. 5 caption.

where stopping the centrifugation run does not change the pressure at the outlet of the pump. This is due to the fact that the present system does not use the Archimedean Screw Force which imposes hydrodynamic column pressure in the conventional HSCCC with a multilayer coil separation column, and it also avoids the effect of high hydrostatic pressure generated by the density different between the two phases in the centrifugal partition chromatography [9]. Since this operating pressure of several psi in the present system is mostly caused by the resistance through the narrow flow tube, it is assumed that a large preparative column of 1 L capacity

(25 times of the present column capacity) can be operated with a low pressure below 50 psi at a high revolution speed hence the risk of leakage of solvent from the column is minimum.

5. Conclusions

The vortex CCC method has the following advantages over the conventional CCC techniques: (1) since the two phases are mixed in the plane perpendicular to the column axis, solute band spreading along the length of the column is minimized. (2) By eliminating the hydrodynamic Archimedean Screw effects in HSCCC and the hydrostatic force generated by the density difference between the two-phases in centrifugal partition chromatography, the system produces extremely low column pressure which permits the use of a long separation column without a risk of leakage of solvents. (3) Since the system produces a uniformly circulating centrifugal force field at every point on the holder, all space on the holder is efficient column space. Thus, the column capacity can be increased simply by eliminating the central shaft to accommodate a large column holder.

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