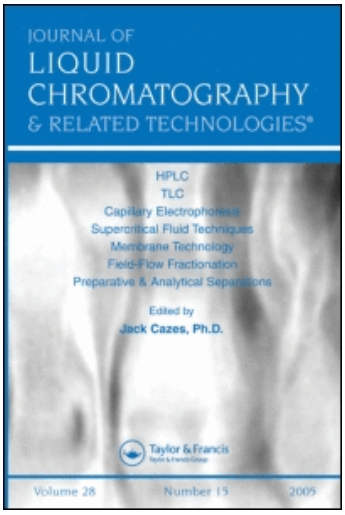

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Spiral Countercurrent Chromatography Studies Using the Spiral Disk Assembly

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Abstract: The first rotor proposed for high speed countercurrent chromatography (HSCCC), the spiral disk assembly, consisting of a stack of 8 high density polyethylene plates with spiral flow channels, has been studied with various solvents and peptide separations to understand its application to polar compounds and solvent systems. Mostly polar solvent systems comprised of the heavy alcohols, were studied because of their suitability for peptide and larger molecule separation. Peptide mixtures were separated to correlate partition coefficients ranging from 0.3 to 2.8 with elution in spiral CCC. With some peptides, scale-up purification experiments were performed with sample loadings up to 85 mg in the 153 mL volume rotor. These studies characterized polar solvent systems with high stationary phase retentions that can be used for separation and purification of compounds with suitable partition coefficients. The solvent system, sec-butanol-1% aq.trifluoroacetic acid with a 73.3% stationary phase retention proved to be useful for most separations. This is a very useful method to substitute for the use of acetonitrile in preparative chromatography.

Keywords: High speed countercurrent chromatography, Partition coefficient, Peptide purification, Polar solvent systems, Preparative chromatography, Single-spiral disk assembly rotor, Spiral countercurrent chromatography

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INTRODUCTION

In the last few years new CCC rotors have been designed based on a spiral flow pathway. We have now completed experiments with the first larger volume rotor assembly of 8 single-spiral high density polyethylene disks, described previously.[1–4] There are new spiral disk assembly rotors with more complex spiral channels, including 4 interweaved spirals per plate[1,3,5] and channels with mixing and settling compartments.[6,7] The capabilities of these are being studied. Systematic solvent system and resolution studies are important to accomplish for all the rotors proposed in spiral countercurrent chromatography.

The multi-layered coils used in HSCCC are able to chromatograph small molecules and natural products with organic-aqueous two-phase solvent systems. However, it was found that with the heavy alcohol-aqueous (polar) two-phase solvent systems, low stationary phase retention occurred that made it difficult to operate in HSCCC conditions. The polar solvent systems, such as n-butanol-acetic acid-water (4:1:5 v/v) or sec-butanol-water, useful for peptides, proteins, and other high molecular weight entities were not well retained in multi-layered tubing coils in the type-J planetary centrifuges. However, in spiral CCC these solvent systems have high stationary phase retention at the flow rates used in HSCCC.

The potential for this was identified previously in polar and non-polar solvent system retention measurements in model single spiral layer studies.[8–10] The stationary phase retention is dependent on the tubing screw direction and upon the planetary force induced by the centrifugal rate. Moderate stationary phase retentions were identified for some hydrophilic polar solvents in certain flow directions only, either H (head) to T (tail) or T to H. In the spiral separation rotor that is a stack of plates all in the same screw direction (as compared to alternating screw direction for wrapped coil tubing) the same effects are expected to be found. Therefore, the retention of many polar solvent systems are measured here to determine the actual values and if high enough for suitable function. Under similar running conditions, the elution of the upper phase and then the lower phase through the spiral rotor was done, with the stationary phase retention measured. The purpose of these studies was to determine the conditions of highest stationary phase retention of polar solvent systems, and to compare with a few other non-polar or less polar solvent systems commonly used in CCC.

Separations of peptides with various partition coefficients (K) were performed to observe the resolution of CCC in the spiral rotor. With different peptides available, the capability of a range of K values that could be separated with good efficiency was determined. Two groups of peptides were separated to see how the elution volumes correlate to the K.

Since the primary use of CCC is the preparative purification of compounds, this application was demonstrated here for two synthetic peptides with sample loadings ranging from 10 to 85 mg. In solvent systems developed for each peptide, the high purity level yields were determined from the chromatography in the 153 mL volume rotor.

EXPERIMENTAL

Instrument

The new spiral disk separation rotor was operated in a type-J planetary centrifuge with a 10 cm revolutionary radius, manufactured by Conway CentriChrom Inc. (Williamsville, NY, USA) Model DP-1000 (Ser. No. 107) (Figure 1). The tabletop instrument is approximately 47.5 cm high × 44 cm deep × 62 cm wide with a rotor chamber open to the front covered by a sliding Lexan (polycarbonate) window. A side panel controller has a digital readout to 1000 rpm and directional settings CW "Rev" and CCW "Fwd". A balancing ring surrounds the central shaft above the gears. This is filled with steel balls to provide compensation of up to 50 g imbalance. For the single rotor counterbalanced with brass weights (approximately 2.7 kg), the flow tubes pass through an opening and are clamped on the top roof.

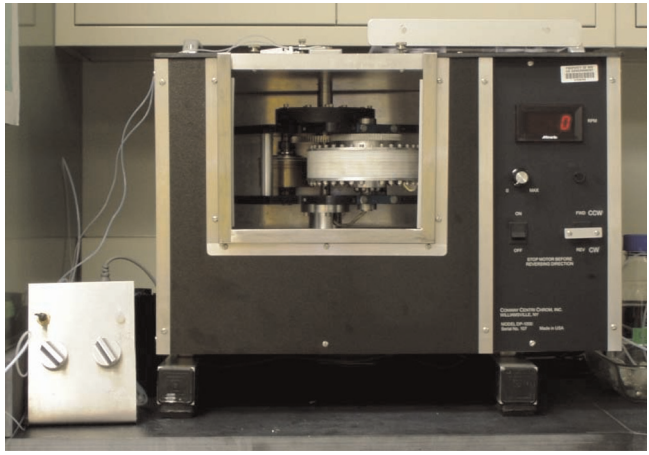

Figure 1. The single spiral 8-plate rotor mounted in the benchtop planetary centrifuge as described in the Experimental. Window cover is removed and lays on top right of machine. Flow tubing is clamped on top and enters the center shaft and exits below to the rotor shaft. Inside, the tubing enters the spiral disk assembly at its top and exits at lower end into the shaft and out to the central shaft (figure is provided in color online).

The spiral disk assembly rotor (Figure 1, National Institutes of Health, Machine Instrumentation, Design and Fabrication, Bethesda, MD, USA) is composed of eight 17.5 cm diameter high density polyethylene plates, each with a single spiral groove winding clockwise from 2.4 to 7.5 cm from the center. Each plate consists of 4 mm thick high density polyethylene with a single spiral channel 2.0 mm deep, 2.6 mm wide and with a 4 mm pitch throughout (Figure 2, photo of a similar polypropylene disk). The separation rotor and mode of use has been described in detail previously.[1,4] The planetary centrifuge is set for either CW or CCW revolution and the lower (heavy) mobile phase is pumped into the inner terminal of the rotor or the upper (light) phase is pumped through the outer end[4] during the elution. The revolution of the planet centrifuge was set at 800 rpm for these runs. The mobile phase delivery of 2 mL/min was provided by a D-1463 Knauer solvent pump (Berlin, Germany).

Materials

Solvents were HPLC grade from Mallinckrodt/Baker (Phillipsburg, NJ, USA) or Fisher Scientific (Fair Lawn, NJ, USA). Water was purified through a carbon and ion exchange filtration system with irradiation

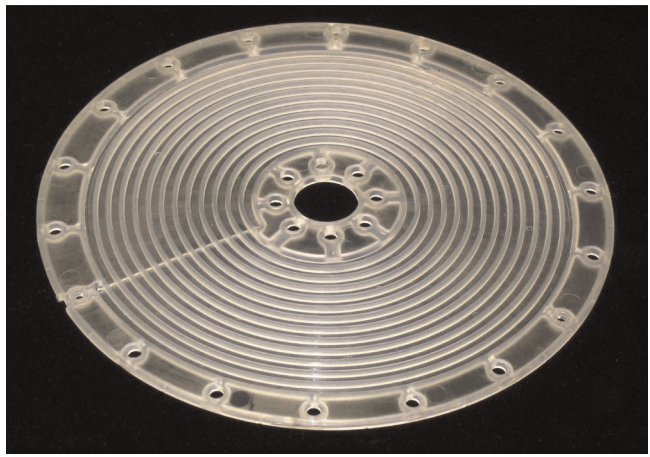

Figure 2. High density polypropylene single spiral disk fabricated by an injection molding process. The dimensions of the spiral channel and return channel on underside are described in the Experimental. The small cross section return channel carries the flow to the hole in the septum over the start of the spiral channel in the next disk. Eight disks sandwiched between Teflon-coated Viton sheets have been assembled into a new low priced separation rotor (figure is provided in color online).

(Neu-Ion, Baltimore, MD, USA). Peptides were previously synthesized at Peptide Technologies (Gaithersburg, MD, USA) using solid phase methods.[4,13] purified and stored for a few years. Some peptides were unpurified samples. For ease of detection, peptides with tyrosine were used. For these experiments, HPLC analysis was performed to assess the impurities present.

Methods

The stationary phase retention after elution of the mobile phase was measured for various two-phase solvent systems used in CCC. This was done by filling the rotor with the stationary phase, starting the centrifugation at 800 rpm and eluting the other, or mobile phase, at 2 mL/min. The emergence of the mobile phase or solvent front is noted and measured. After a few minutes, the centrifugation and flow is stopped and the contents are expelled with helium pressure into a graduate cylinder. The retained stationary phase volume as the per cent of the total volume is calculated. This is determined for the flow conditions as listed in Table 1: the lower mobile phase is eluted (through the inner entry) in the T to H direction (L-i-T, CCW rotation of instrument), then the rotor is filled again with the stationary phase and the lower phase is eluted in the H to T direction (L-i-H) by changing the direction of centrifugation. Next, the rotor is filled with the lower phase as the stationary phase and the upper phase is eluted in the outer entry in the H to T direction (U-o-H) and stationary phase determined, then the upper phase is eluted T to H (U-o-T) again by changing the centrifugation direction. In this fashion, the stationary phase per cent is measured for the solvent systems listed in Table 1. For the polar solvent systems, previous studies show there is low stationary phase retention with the other elution mode of upper phase into the inner terminal and lower phase into the outer terminal.[1,12]

In the development of methods for the separation of peptides, the K (concentration in upper phase/concentration in lower phase) was determined by HPLC as previously described.[4] The $K_{s/m}$ (stationary phase/mobile phase) calculated from the elution of a compound, is the ratio of the elution volume of the chromatographic peak minus the excluded volume of the coil to the total volume of the coil minus the excluded volume of the coil.[14]

The countercurrent purification procedures were as described previously:[4] briefly the peptide sample was dissolved in about 2 mL of each phase plus a 1 mL wash of each phase of the solvent system and loaded inline into the inner or outer terminal of the spiral coil, already filled with the stationary phase. The sample was loaded via an 8 mL loop

Table 1. Polar and non-polar solvent systems in spiral CCC

Solvent system composition (v/v)	Mobile phase flow direction (elution mode)	Rotation	Stationary phase retention (%)
n-Butanol/acetic acid/water (4:1:5)	L-i-T	CCW	78.90
	L-i-H	CW	39.90
	U-o-H	CCW	78.40
	U-o-T	CW	22.80
sec-Butanol/water (1:1)	L-i-T	CCW	76.60
	L-i-H	CW	46.50
	U-o-H	CCW	66.70
	U-o-T	CW	63.60
sec-Butanol/1% aq. trifluoroacetic acid (1:1)	L-i-T	CCW	70.30
	L-i-H	CW	54.10
	U-o-H	CCW	66.40
	U-o-T	CW	73.30
n-Butanol/*sec*-butanol/1% aq. trifluoroacetic acid (1:1:2)	L-i-T	CCW	58.40
	L-i-H	CW	53.20
	U-o-H	CCW	54.50
	U-o-T	CW	49.40
n-Butanol/1% aq. trifluoroacetic acid (1:1)	L-i-T	CCW	10.00
	L-i-H	CW	57.50
	U-o-H	CCW	42.30
	U-o-T	CW	51.60
n-Butanol/water (1:1)	L-i-T	CCW	1.60
	L-i-H	CW	67.60
	U-o-H	CCW	27.50
	U-o-T	CW	68.60
tert-Butyl methyl ether/ *n*- butanol/acetonitrile/0.1% aq. trifluoroacetic acid (2:2:1:5)	L-i-T	CCW	0
	L-i-H	CW	74.30
	U-o-H	CCW	20.30
	U-o-T	CW	53.70
tert-Butyl methyl ether/ acetonitrile/1% aq. trifluoroacetic acid (2:2:3)	L-i-T	CCW	0
	L-i-H	CW	75.20
	U-o-H	CCW	15.80
	U-o-T	CW	85.70
n-Hexane/ethyl acetate/methanol/water (3:5:3:5)	L-i-T	CCW	0
	L-i-H	CW	81.10
	U-o-H	CCW	11.30
	U-o-T	CW	91.30

in a manifold of an LC 2-way valve attached to a 2-way solvent/gas selector valve (Valco VICI Instruments, Houston, TX, USA) (Figure 1). The centrifugation was set at 800 rpm then the mobile phase was pumped at 2 mL/min. After the elution of up to 2 or 3 column volumes, the centrifugation was stopped and the contents were pushed out with helium gas. An Advantec SF-2120 fraction collector (Varex, Rockville, MD, USA) was used to collect 2 min fractions (4 mL per tube). Manual determinations of the absorbance of the fractions were made in a UV/Vis spectrophotometer (Ultrospec 2100 pro, GE Healthcare Bio-Sciences, Piscataway, NJ, USA). The absorbance measurements were plotted using Excel©. The peaks were identified by HPLC analysis in a D-Star binary gradient system (Manassas, VA, USA) with a variable UV detector and StarChrom data analysis software. The column, YMC AP-302 s-5, 5 μm, C18, 300 Å, 0.46 × 15 cm (Waters, Milford, MA, USA) was used with 0.1% aq.TFA (solvent A) and gradients of 0.1%TFA/acetonitrile (solvent B). The fractions containing pure peptide were dried in a Savant centrifugal evaporator connected to a Virtis lyophilizer and Heto −90°C condenser and vacuum provided by a Precision pump (ATR, Laurel, MD, USA).

RESULTS AND DISCUSSION

Solvent System Studies

The stationary phase retention values expressed as the percent of the total volume capacity are listed in Table 1 in the decreasing order of the upper stationary phase retention in the condition of the lower phase pumped into the inner terminal in the tail to head direction set by CCW rotation (L-i-T, CCW). Organized in this way, the solvent systems seem to follow in the order of decreasing polarity or increasing hydrophobicity. Significantly, for all the solvent systems there is over 50% stationary phase retention for all the mobile phases at 2 mL/min in either H to T or a T to H flow direction. Thus, larger molecules that have useful partition coefficients in these solvent systems can be separated with either phase mobile. The first 4 solvent systems have high stationary phase retentions ranging from 78.9% to 58.4% for the upper phase in the L-i-T elution mode; whereas in this condition for the last 4 solvent systems in the table, this is the lowest retained condition from 15.8% to 0%. In the U-o-T elution mode, the highest stationary phase per cent was attained in the non-polar or hydrophobic solvent systems at the bottom of the list. The retentions ranged from 68.6% to 91.3%. As the solvent system becomes more polar or hydrophilic, the higher stationary phase retention for the upper phase mobile shifts to the U-o-H elution mode (54.5% to 78.4%).

In the multi-layer coil, the organic non-polar solvent systems display a unilateral hydrodynamic distribution of the upper phase to the head end and lower phase to the tail end, at a critical high flow and centrifugal rate.[8,9,11] Thus, for a stationary phase retention, the mobile upper phase has to be pumped from the tail to the head for the lower phase to be retained. Conversely, the lower phase has to be pumped from the head to the tail for the upper phase to be retained. This is the case for the spiral coil, as well, as seen in the solvent systems in the bottom of the list. The intermediate and polar solvent systems have a more complex distribution in the multi-layer coil, the phases going in the opposite direction with less stationary phase retention, generally. In this spiral coil, these solvent systems have high retentions in both directions as seen in Table 1 for the solvent systems in the upper half of the list.

The characteristics of viscosity and interfacial tension have to do with the different hydrodynamic tendencies of the solvent systems. Generally, the polar solvent systems have higher viscosity and lower interfacial tension indicated by long settling times after mixing.[10] Solvent systems with settling times from 34 to 60 sec are at the top of the list and those with settling times of 24 to 12 sec are below. These results are in conformance with the previous model studies of single layer spirals that differentiated the solvent system behaviors.[8–10] This rotor represents a unidirectional spiral flow path in a manifold to provide a larger volume for chromatographic use. Importantly, the advantage of the spiral rotor is that there is high stationary phase retention for all the solvent systems in a particular elution direction such that any mobile phase can be used. Table 1 is a useful guide that gives the operation conditions for high stationary phase retention for the polar solvent systems in spiral CCC.

Peptide Separation Studies

In order to correlate the elution volume of compounds with their partition coefficient or K, peptides with a range of K values were separated. Peptides 6877, 7020, and 507 with different charges had different K values (Table 2) and were separated in a HPLC gradient useful for identifying the separation results (sample standards in Figure 4). The

Table 2. Partition coefficients of peptides in 1%TFA/sec-butanol

ID No. – sequence	$K_{U/L}$	$K_{S/M}$
6877 SAGSADQYLAVPQHPYQA	0.29	0.61
7020 LYKYKVVRIEPLGVA	1.64	1.96
507 TAENPEYLGLDVPV	2.42	2.8

order of elution of the peptides in HPLC was in the same order as their K values (Table 2). In the CCC separation, a mixture of 10 mg of each peptide was loaded in 3 mL of each phase of the solvent system (sec-butanol/1% aq.Tfa, 1:1 by vol) onto the spiral disk rotor filled with upper phase, and the lower phase was eluted as the mobile phase as described in the Methods. The absorbance readings of the fractions are plotted in Figure 3. The solvent front eluted at 43 mL (fraction 11); thus the stationary phase retention was 72%. In Figure 4, HPLC analysis of peak fractions showed a complete separation of peptide 6877 in the first CCC peak (fractions 20–30), the next major peak starting at fraction 58 contained mostly 7020 and the last major peak contained mostly 507 with the last 2 peptides not completely separated, but eluting in the expected order according to their K. The $K_{s/m}$ values calculated from the run are close to the $K_{u/l}$ values (Table 2). The first peak was pooled and dried down. The recovery of this peptide, 6877, was 8.8 mg from 9.8 mg loaded.

Another set of peptides was chromatographed on the spiral CCC in the same elution conditions to observe the separation with this combination of K values (Table 3). Small amounts of the peptides were combined and loaded in 3 mL of each phase. The absorbance of every 5th fraction was read manually as shown in Figure 5. By HPLC analysis shown in Figure 6, the content of the first peak was 6877 and 7131 was identified between fractions 45–65, followed by 5595 in the last peak starting at fraction 92. All three peptides of this group were completely separated

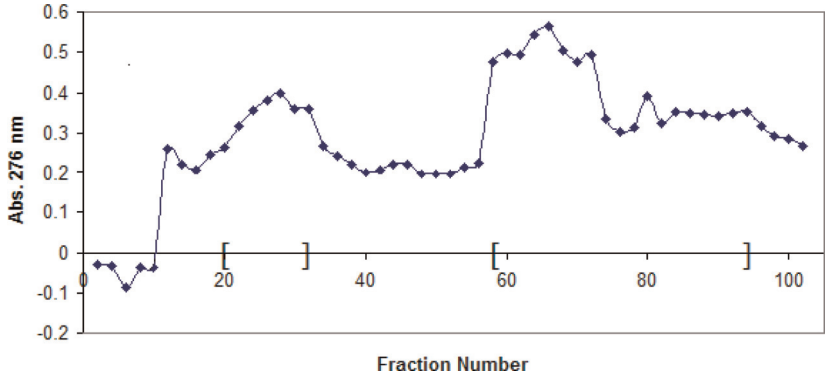

Figure 3. Separation of approximately 10 mg each of the peptides in Table 2 in the solvent system sec-butanol/1% aq.Tfa with the lower phase mobile run in the conditions L-i-T (800 rpm, CCW). Fractions of 2 min with flow of 2 mL/min total to 4 mL/fraction. Solvent front came out at fraction 11. The brackets indicate the fractions containing peptide. In fractions 20–30, the peptide 6877 was located. Peptides 7020 and 507 were incompletely separated starting at fraction 58. Further details are described in the Results and Discussion (figure is provided in color online).

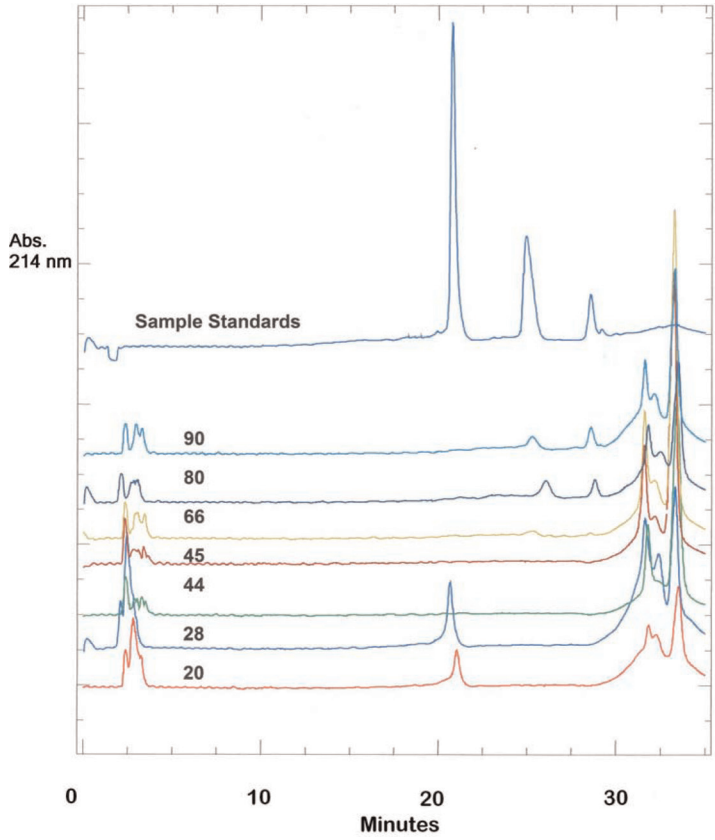

Figure 4. Overlay of HPLC analyses of CCC fractions of Figure 3. Absorbance at 214 nm plotted against min. Retention times of 6877, 7020, and 507 were 20.7, 25.3, and 28.9 min, respectively (Sample Standards). Analyses were approx. 50 µL of CCC fractions prior to drying and removal of solvent, which show absorbing peaks at void volume and end of gradient wash at 30 min. HPLC conditions are A = 0.1% aq.TFA, B = 0.1% TFA/acetonitrile with a gradient of 5% to 20% B in 20 min and wash from 20% to 50% B at 30–35 min at a flow of 1 mL/min. Further details of HPLC are in the Experimental. Fractions 20 and 28 contain peptide 6877; 66 shows mostly 7020 and fraction 90 has both peptides, but more of 507 (figure is provided in color online).

Table 3. Partition coefficients of peptides in 1%TFA/sec-butanol

ID No. – sequence	$K_{U/L}$	$K_{S/M}$
6877 SAGSADQYLAVPQHPYQA	0.29	0.44
7131 SAGSADQYLAVPQAPYQWA	0.91	1.45
5595 YVDKFAEF	2.8	3.09

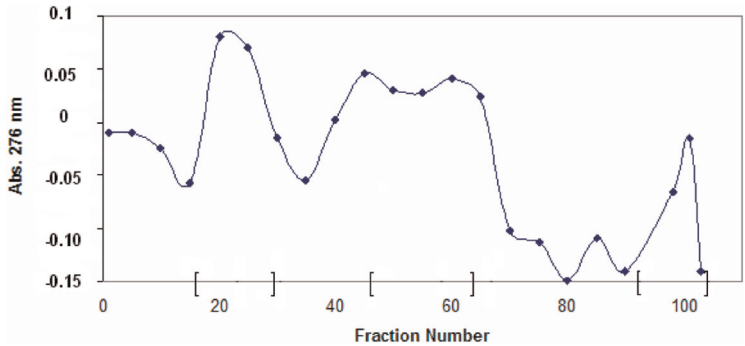

Figure 5. Spiral CCC of the peptides of Table 3 using the same conditions as in Figure 3. Amounts of 14 mg 6877, 5 mg 7131, and 8 mg of 5595 were dissolved in 3 mL of each phase. Solvent front occurred in fraction 5 for an excluded volume of 19.5 mL. The localization of each purified peptide identified by HPLC is shown. Fractions 25–30 contained 6877, fractions 60–67 had 7131, and finally 5595 was included in 92–109 (figure is provided in color online).

and in the expected elution order (Table 3). Except for one peptide, 7131, the values are close to the theoretical values. Interestingly, the peptide 5595 elutes before peptide 7131 in reverse phase HPLC (Figure 6), whereas it elutes after 7131 in CCC (Figure 5). The selectivity of HPLC and CCC is different for some molecules.

Preparative Purification of Peptides

After studying the resolution of the spiral CCC for separation of 5 to 10 mg of peptides, it was next important to assess the method as a preparative tool for higher mass loads of synthetic peptides. An amount of 30 mg of peptide 507 was chromatographed in 1% aq.TFA-sec-butanol in the L-i-T mode and the absorbance of the fractions plotted in Figure 7. Peptide was eluted in fractions 70–97 with 80–87 being the most highly pure fraction (Figure 8). The fractions 72–79 seem to have a concentrated hydrophilic impurity eluted by the aqueous phase. The total mass recovery was about 50%. For a sample load of 85 mg, run at the same flow rate, peptide was eluted at the same elution volume from fractions 70 to 110. Most of pure peptide was recovered from pooled fractions 83–94, 23 mg. From the run, a total of 57 mg was recovered or 67%. The analysis is shown in Figure 8 as well. The 85 mg sample represents a mass load of 57.5 mg/100 mL of rotor volume. Another peptide, 7131, was purified by spiral CCC in 30 to 62 mg loads to obtain pure peptide for studies. The values of K for this peptide measured in various solvent systems are given in Table 4. The crude peptide had a dark pink

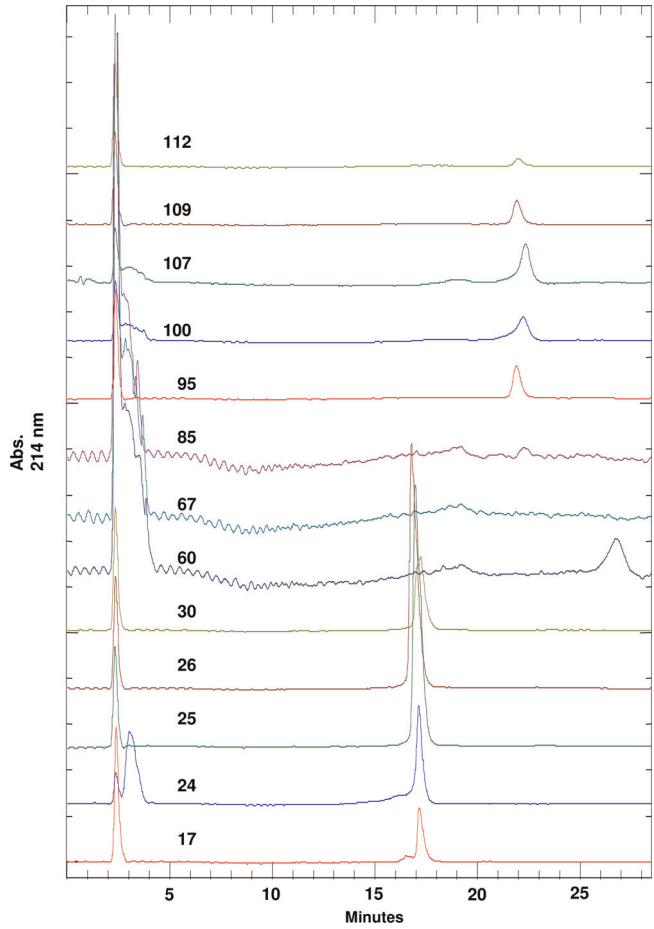

Figure 6. HPLC analyses of fractions in the CCC separation in Figure 5. HPLC method similar to Figure 4, except that the gradient conditions are 5% to 20%B in 15 min followed by a wash from 20% to 50% at 25–30 min. Retention times of peptides, 6877, 7131, and 5595 are 17, 27, and 21.5 min, respectively. The time of analyses are shown up to 25 min (figure is provided in color online).

color due to tryptophan side products, which polymerize in drying from trifluoroacetic acid and other reagents used in the last synthetic step. Although a basic solvent system would be more optimal for its purification, the K in basic solvent systems was too low. Therefore, we modified the aq.TFA-sec-butanol solvent system to 0.1% aq.TFA-sec-butanol and chromatographed to obtain pure peptide for further experiments. In all the runs, homogeneously pure peptide (white powder) was obtained (Figure 9).

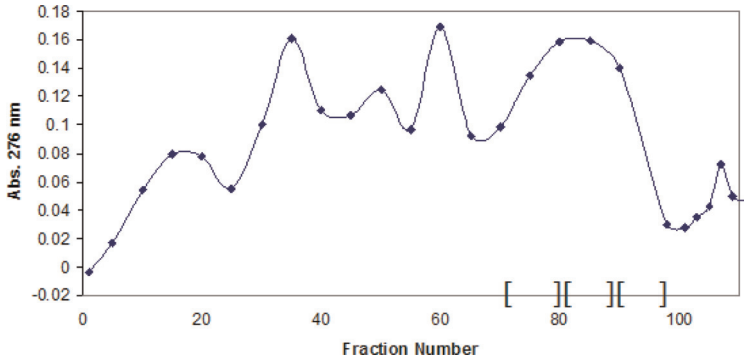

Figure 7. Preparative chromatography of 30 mg peptide 507 in the spiral CCC in the same conditions as Figure 3. Solvent front where mobile phase comes out is at fraction 10 or 39 mL. Stationary phase retention in this run was 74%. Contents pushed out at fraction 100. The combined fractions, dried down as indicated in the brackets were analyzed by HPLC (results in Figure 8 left). The pooled fractions 80–87 were the highest purity level recovered (figure is provided in color online).

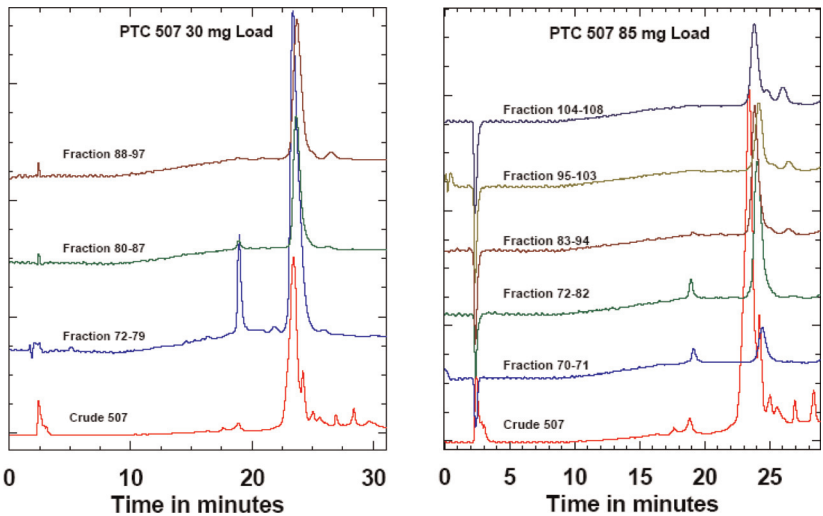

Figure 8. (Left) HPLC analysis of the recovered fractions of the 30 mg run of peptide 507. Overlay of HPLC chromatograms of approximately 50 μg with detection at 215 nm run in a gradient of 5% to 20% B in 20 min, other conditions as described in Figure 4 and the Experimental. (Right) HPLC analysis of fractions recovered from 85 mg run (CCC chromatogram not shown) (figure is provided in color online).

Table 4. Partition coefficient of peptide 7131 in CCC solvent systems

Solvent system (by vol.)	$K_{U/L}$
sec-Butanol/water (1:1)	0.47
sec-Butanol/1% aq.TFA (1:1)	0.91
sec-Butanol/0.1% aq.TFA (1:1)	0.88
sec-Butanol/*n*-butanol/pyridine/glacial acetic acid/water (2:6:2:1:9)	0.18
sec-Butanol/*n*-butanol/pyridine/0.1% aq.acetic acid (3:4:3:11)	0.18

Certain other solvent systems and elution modes were tried. The upper phase of 1% aq.TFA-sec-butanol was utilized as the mobile phase for a 13 mer peptide shown in Figure 10. The peptide with a $K_{u/l} = 0.9$ or $1/K = 1.1$, was eluted in the fractions 61–76 as indicated in the figure. This elution volume peak calculated to $K_{s/m} = 1.1$. The elution mode was U-o-H with a high stationary phase retention. For this solvent system as shown in Table 1, all elution modes have useful stationary phase retention. A more hydrophobic peptide was separated in one of the least polar solvent systems in Table 1. In Figure 11 the elution mode used was L-i-H, which is the only one that can be used for the lower phase mobile. The stationary phase retention in the run was 69%. The peptide was also

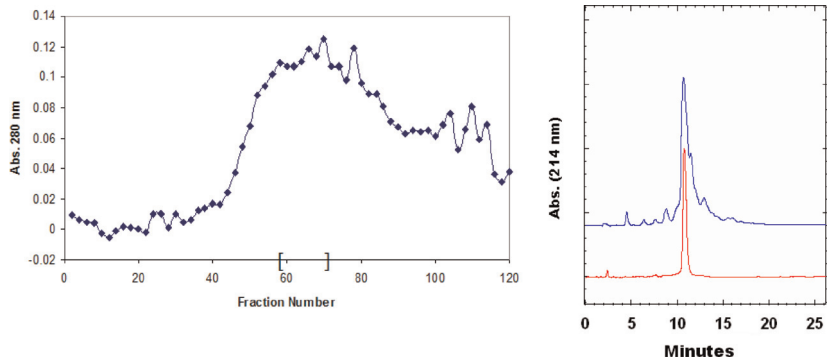

Figure 9. (Left) Chromatography of 62 mg peptide 7131C in 0.1%TFA/sec-butanol with lower phase mobile, L-i-T, 1 mL/min at 800 rpm and 4 mL fractions collected. Solvent front at 34 mL. Fractions from 58 to 100 were analyzed. Very pure peptide was recovered from fractions 58–70. (Right) HPLC analysis of a pure fraction 63–66 of the 7131C separation (lower trace) compared to crude (upper trace) in a gradient of 5%B at 0 min and 20% to 60%B from 5 min to 25 min (figure is provided in color online).

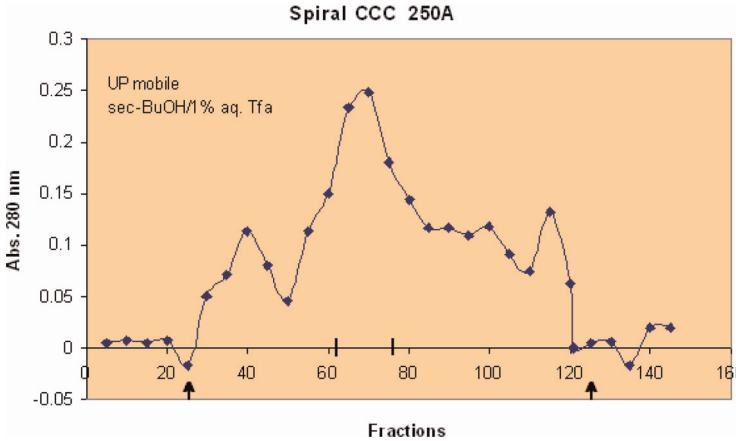

Figure 10. Preparative chromatography of 32 mg of 13-mer (GGEMRDNWR-SELY, 250A) in the U-o-H elution mode in the sec-butanol/1% aq.TFA solvent system with a flow of 1 mL/min, 2 min fractions. This was done by changing the entry of the mobile phase (upper) to the outer terminal (other end) and CCW rotation. Solvent front emerged at fraction 24 or 47 mL and the contents pumped out at 121. Purified peptide identified by HPLC analysis eluted between fractions 61–76 (figure is provided in color online).

chromatographed with the upper phase mobile in the U-o-T condition and the stationary phase retention was very high at 84% (not shown). The peptide came out after a long elution volume.

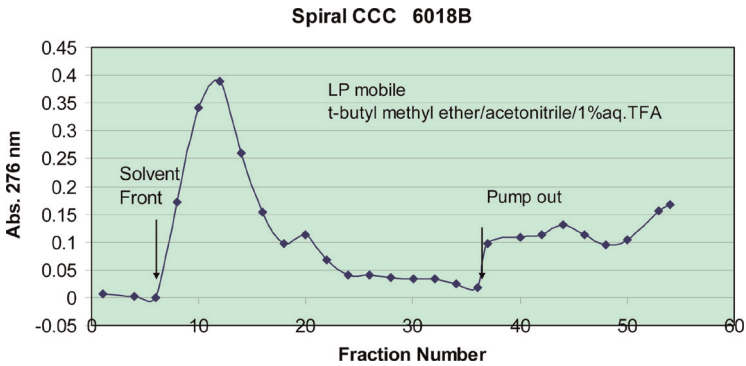

Figure 11. Separation of crude CQNIVPYTIIKDIHELF, 47 mg, in the solvent system, *tert*-butyl methyl ether-acetonitrile-1% aq. trifluoroacetic acid (2:2:3) in the L-i-H elution mode at 2 mL/min. In this experiment the fractions were 3 min. The stationary phase retention was 69%. The most purified fractions 12–16 were located in the second half of the first peak, and amounted to 10 mg (figure is provided in color online).

CONCLUSIONS

A spiral CCC rotor with 8 plates of a single spiral channel/plate having a total volume of 153 mL has been found to be versatile in holding a high stationary phase for many polar solvent systems in high speed conditions. Importantly, the operating conditions of this method are defined for the polar solvent systems, thus extending the application range of HSCCC to more polar molecules.

With a sec-butanol solvent system, peptide samples with partition coefficients ranging between 0.3 and 2.8 were mostly well separated. Synthetic peptides in sample loads up to 85 mg were purified to high purity levels with good recovery, further demonstrating the utility of this method for preparative purification. These results indicate that the spiral CCC can serve as a useful laboratory scale purification method that can use other organic solvents than acetonitrile, which is presently scarce and expensive. The use of the aqueous phase as the mobile phase simplifies the recovery by only requiring one lyophilization step of a smaller volume per mass of purified peptide compared to preparative HPLC.

The spiral disk assembly has just been manufactured with polypropylene disks, fabricated using an injection molding process and with sheeting of Teflon-coated Viton. A photo of one of these disks is shown in Figure 2. This lower cost rotor is being evaluated for the same capability and will be made available commercially.

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