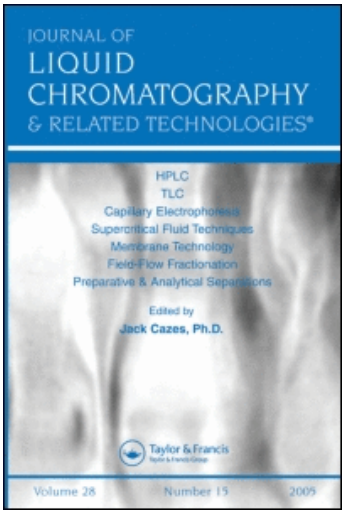

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Monolithic Integrated Flow Circuit (Mifc): A New Column Design for Countercurrent Chromatography

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MONOLITHIC INTEGRATED FLOW CIRCUIT (MIFC): A NEW COLUMN DESIGN FOR COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

A novel technique to produce a monolithic integrally formed system for countercurrent chromatography is introduced here. Through a molding technique, complex repetitive flow channels were formed into sheets of polypropylene. Two of these sheets were fused to form a closed separation chamber with a complete set of locules and integral transfer tubes. This system, called *monolithic integrated flow circuit* (MIFC), was produced in various configurations and tested while rotated, gyrated, or oscillated so as to promote mixing of the two solvent phases. Performance of each scheme was examined by separations of DNP-alanine and DNP-glutamic acid with a two-phase solvent system composed of chloroform: acetic acid: 0.1 N HCl (2:2:1). Under optimal conditions, the efficiencies of the schemes using gyration and oscillation were 94% and 74%, respectively. Simple rotational mixing was considerably less efficient.

This compact MIFC system was found to be a simple yet ideal way of producing flow channels of great complexity for conventional droplet CCC and locular CCC. The performance of the tested configurations was outstanding.

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INTRODUCTION

Countercurrent chromatography (CCC) (1-4) may be conveniently divided into helix CCC and nonhelix CCC. In helix CCC the separation column was prepared from a long piece of tubing wound helically onto a suitable core. In nonhelix CCC the column consisted of multiple tubular units connected with thin transfer tubes in series. Column preparation was difficult and required hundreds of junctions, each a potential source of leakage and constriction. Preparation of the locular column was particularly tedious and time consuming because each column unit was made of multiple centrally perforated discs inserted one by one into a straight tubular column.

The present paper introduces a novel method of producing complex repetitive flow channels formed of thin plastic sheets. This system, called monolithic integrated flow circuit (MIFC), was found to provide an ideal locular column for performing CCC.

PROCESS OF MIFC PRODUCTION

The forming was similar to vacuum forming but was specifically adapted to achieve high detail reproduction. Molds of one half of the flow channels, and mirror images of the same were machined into a ½" thick aluminum jig plate using a numerically controlled milling machine (Fig. 1A). The polypropylene sheets (0.015") were dried at 220°F for 2 to 6 hours to remove any moisture. Two such sheets were aligned in the mold. The purpose of the 1/8" thick silicone rubber pad shown in Fig. 1B was to force each polypropylene sheet in a hydraulic press into the machined cavity after a suitable temperature has been reached (280°F).

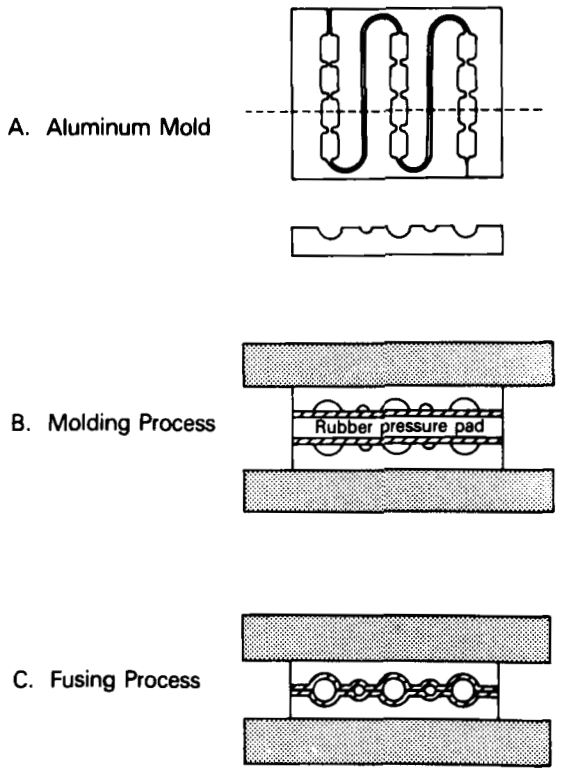

Figure 1. MIFC forming process. A: Aluminum mold for MIFC, B: Forming process; C: Fusing process.

Following this and while in the press the temperature was raised to 310°F to relieve stress in the formed sheet. After cooling, the rubber pad and teflon sheets were removed and the molds containing the polypropylene sheets realigned. The sheets were then fused under pressure at 330°F (Fig. 1C). When cooled, the completed plastic molding was removed and polypropylene tubing fused to the inlet and outlet ports. After testing for leaks, the unit was ready for use.

APPARATUS AND MECHANISM OF CCC

The present study utilized three mechanical devices, each performing a particular form of locular CCC, i.e., rotation locular CCC (RLCCC), gyration locular CCC (GLCCC), and oscillation locular CCC (OLCCC). The design of each instrument and the mechanism involved in each CCC scheme are described below.

RLCCC

The apparatus used for performing RLCCC had been reported earlier (5,6). It consisted of a cylindrical column holder, 5cm o.d. and 30cm in length, concentrically mounted around a rotary shaft which was equipped with a rotary-seal at each end to facilitate continuous elution. The rotary shaft was mounted with a pair of ball bearings on an aluminum frame which was positioned at a desirable angle from the horizontal plan. A motor (Electro-Craft Corporation, Model 0650) drove the rotary shaft through a pair of toothed pulleys coupled with a toothed belt, all mounted on the aluminum frame. The rotational speed of the column holder was regulated up to 500 rpm with a control unit (Electro-Craft Corporation, Model E-650M).

The mechanism of RLCCC is schematically illustrated in Fig. 2 which shows a cross-sectional view of the locular column. The column, inclined at angle α from the horizontal plane, is first filled with the stationary lower phase and the upper phase is introduced from the lower end of the locular column while the column rotated around its axis. Then, the upper phase displaced the lower phase to the level of the exit leading to the next locule, the process being continued throughout the column. Once the hydrostatic equilibrium is established, further elution of the mobile phase only displaces the upper phase in each locule leaving a large

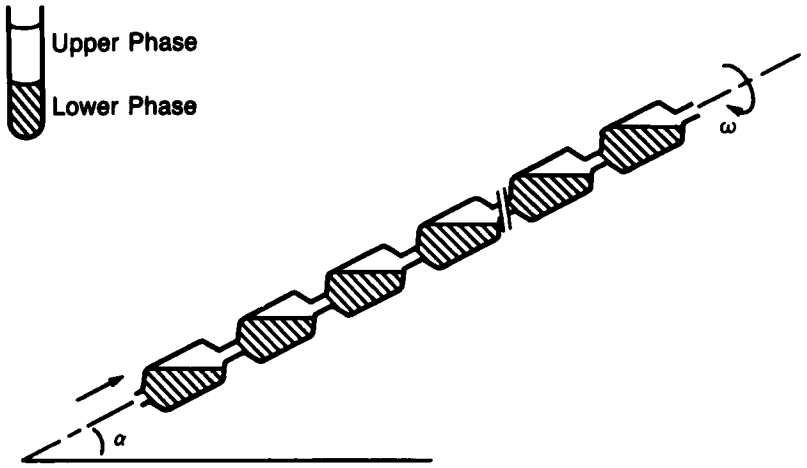

Figure 2. Mechanism of RLCCC.

volume of the lower phase stationary in the column. When the relationship between the upper and the lower phases is reversed at the beginning, the elution of the lower mobile phase from the upper end of the column produced a similar countercurrent process, leaving a large amount of the upper stationary phase in the column. Consequently, solutes introduced into the column are subjected to an efficient partition process in each locule and finally eluted out in the order of their partition coefficients.

GLCCC:

The original GLCCC apparatus (5) was modified and used for the present study. The motor synchronously drove a pair of vertical rotary shafts positioned 30 cm apart and bridged with a pair of links, one horizontally across the upper terminals and the other similarly across the lower terminals. The connection of the pins eccentrically mounted at each terminal of the rotary shafts to the hole of the ball bearings

embedded in each end of the links produced gyration or nonrotational circular motion of the links and the column holder, supported vertically between the links. The radius of gyration was determined by the distance between the pin and the axis of the rotary shaft at each terminal and can be adjusted at 1", ½" or 1/4" by mounting the pins into the respective holes. In order to balance the apparatus, the proper size of a counterweight was mounted at the middle portion of each rotary shaft on the side opposite to the location of the pin eccentrically mounted at each terminal. The gyrational speed of the apparatus is adjustable to 1000 rpm.

The mechanism of GLCCC has been described earlier (5,6). Fig. 3 shows a schematic diagram of the apparatus (A) and the effects of the gyration on the two immiscible solvent phases and their interface in an individual locule. On cross-section (B) through a middle portion of a locule, successive positions of one locule are shown as it gyrates about the central point where the upper phase (clear) and the lower phase (shaded) are seen separated by centrifugal force forming an interface perpendicular to the force vector. The direction of the centrifugal force changes continuously, and both solvents and interface rotate with respect to the X marked on the column wall. Thus, the drag between the solvent and the internal surface of the locule induces stirring in each phase to accelerate the partition process. On the longitudinal section (C) through the center of the locules, the solvents form an interface perpendicular to the vector given by the sum of the centrifugal and gravitational accelerations as illustrated in the figure. The angle α determines both volume ratio and interfacial area between the two phases in the locule. Since the mobile phase usually covers the entire area of the exit hole in the locule, the maximum interface area is achieved only

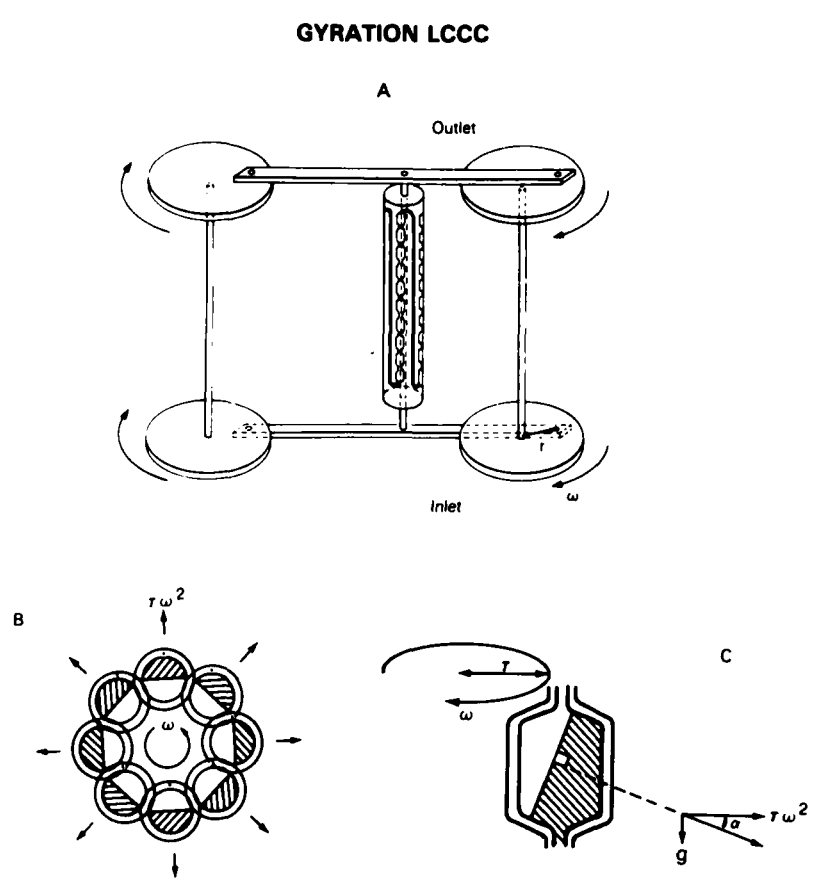

Figure 3. Mechanism of GLCCC. A: Schematical diagram of the apparatus. B: Cross-sectional view through the middle portion of a locule. C: Longitudinal section through the axis of the locule.

by eluting with either the upper phase upwards or the lower phase downwards through the column.

OLCCC:

The formed MIFC was firmly taped onto a suitable holding frame such as light polystyrene foam, and mounted to a vertically positioned reciprocating frame for enhanced mixing and shaking (Fig. 4A). This

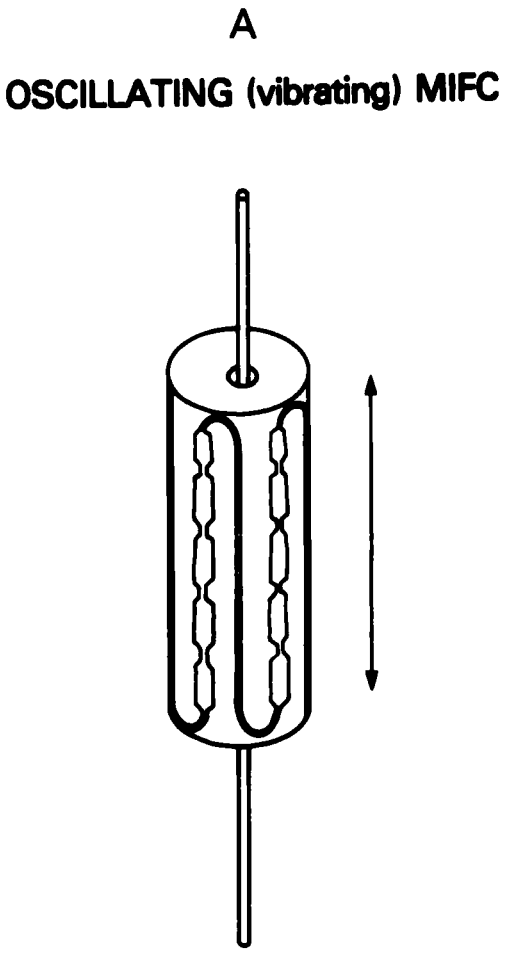

Figure 4. Mechanism of OLCCC. A: Overall view of the oscillating locular column. B: Effects of oscillation on the interface of the two solvent phases.

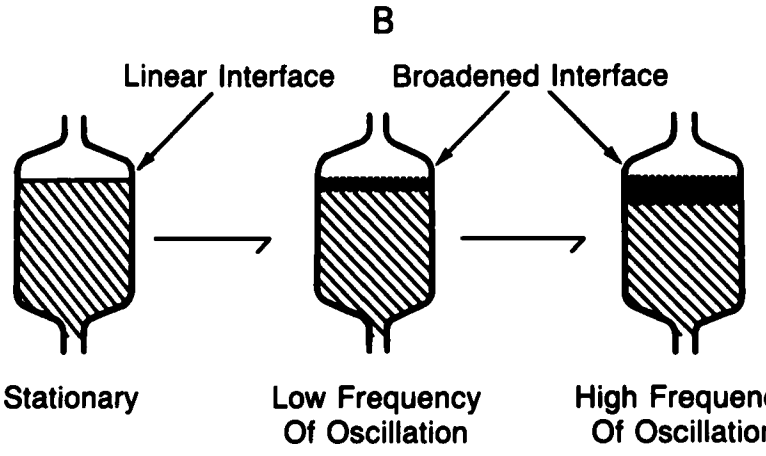

Figure 4B.

apparatus consists of a platform connected through linear bearings to a motor-driven rotating flywheel. The resulting linear motion displayed a sinusoidal undulation while both the amplitude and frequency of oscillation could be adjusted over a wide range. With a speed control the frequency of oscillation could be varied from zero to 900 cycles per min.

In operation, the system was primed as described under RLCCC. On starting to shake, there was active mixing of the two phases in every locule. The most active mixing was at the interface which appeared as a broad band rather than a discrete line. Under stroboscopic observation the interface became broader as the frequency and/or the amplitude of oscillation increased (Fig. 4B).

EXPERIMENTAL

Column

Two types of MIFC plate were used for performing LCCC. Each plate was made of two 0.015" thick polypropylene sheets measuring 26cm x 40 cm.

One plate (spherical locular column) consisted of 1174 spherical locules, each 6 mm in diameter, with a total capacity of 134 ml including the volume of the transfer channels. The second type (cylindrical locular column) consisted of 642, cylindrical locules, each measuring 6 mm in diameter and 10 mm long, with a total capacity of 175 ml. Each column was wrapped with aluminum foil to minimize permeation loss of the organic solvents through polypropylene.

Preparation of Two-Phase Solvent System and Sample Solution

A two-phase solvent system composed of chloroform, acetic acid, and 0.1N hydrochloric acid (2:2:1 by volume) was used in these studies. Chloroform was of glass-distilled chromatographic grade (Burdick and Jackson Laboratories, Inc., Muskegon, MI) while both glacial acetic acid (J. T. Baker Chemical Co., Phillipsburg, NJ) and hydrochloric acid (Fisher Scientific Company, Fair Lawn, NJ) were of reagent grade. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature before use.

Two dinitrophenyl (DNP) amino acids (Sigma Chemical Co., St. Louis, MO) were selected as test samples for the above solvent system. They were N-2,4-DNP-DL-glutamic acid (DNP-glu) (1.9) and N-2,4-DNP-L-alanine (DNP-ala) (0.56); the partition coefficient of each sample in the above solvent system expressed as the ratio of solute concentration in the upper phase to that in the lower phase is in parentheses. The sample solution was prepared by dissolving the above samples in the upper phase to obtain a concentration of each component at 0.5g%.

Test Procedures

In each separation the column was first filled with the stationary phase. This was followed by injecting 2 ml of sample solution at the sample port located at the inlet of the column. Then the mobile phase was pumped into the column at a selected flow rate while the apparatus

was operated at a selected rate. The eluate from the outlet of the column was continuously monitored through an LKB Uvicord S at 275 nm and then collected into a graduated cylinder to measure stationary phase retention. Both upper aqueous and lower nonaqueous phases were used as the mobile phase. In each LCCC scheme, the experiments were repeated for each column by changing operational conditions such as the mobile phase and its flow rate, speed of the apparatus, orientation of the column, etc.

Analyses of Partition Efficiency

Performance of each column was evaluated from the elution profile of the two peaks in the chromatogram in several different ways by calculating theoretical plate number n, partition efficiency per locule, time required for yielding one theoretical plate, and peak resolution $R\sigma$. The theoretical plate number was obtained from the retention time of the peak maximum (R) and width (W) for each peak according to the conventional equation

$$n = (4R/W)^2 \quad (1).$$

The first and second peaks often showed substantially different n values which were then averaged. Partition efficiency per locule was then given by n over the number of locules. Time required for yielding one theoretical plate was given by retention time of the solvent front divided by the average n value. Peak resolution was obtained from the equation

$$R\sigma = 8(R_2 - R_1)/(W_2 + W_1) \quad (2)$$

where R_1 and R_2 denote the retention times and W_1 and W_2 denote the width of the first and second peaks provided that all values are expressed in the same units.

RESULTS AND DISCUSSION

Chromatograms illustrated in Fig. 5 were obtained from three different locular CCC schemes under the optimized operational conditions. All separations were performed with a standard MIFC column consisting of 642 cylindrical locules with a total capacity of 175ml at a flow of 120 ml/h with each of the phases as the mobile phase. Sample volume was 2 ml in each separation.

In all chromatograms two DNP amino acid components were resolved as symmetrical peaks and eluted in two to three hours. As shown in Fig. 5, the use of the upper phase as the mobile phase (rather than the lower phases) yielded substantially higher partition efficiency and peak resolution. This can be explained on the basis of solvent-wall interaction in the locule. In all LCCC schemes the two solvent phases are distributed by gravity while the flowing mobile phase steadily forms droplets in the stationary phase at the inlet of the locule. These droplets are quickly dispersed into the stationary phase by the column motion to provide both broad interface area and efficient mixing of the two phases. However, droplet formation may be reduced or prevented altogether by wall-affinity of the mobile phase which only tends to hug the internal wall surface of the locule, without forming droplets. Since the lower chloroform-rich nonaqueous phase has a strong affinity to the column wall made of polypropylene, the use of such a lower phase as the mobile phase may result in the reduced efficiency.

Selected experimental data obtained from each scheme are summarized in Table 1. In RLCCC, the inclination of the column axis and rotational speed were found to be the major variables. The optimal inclination of

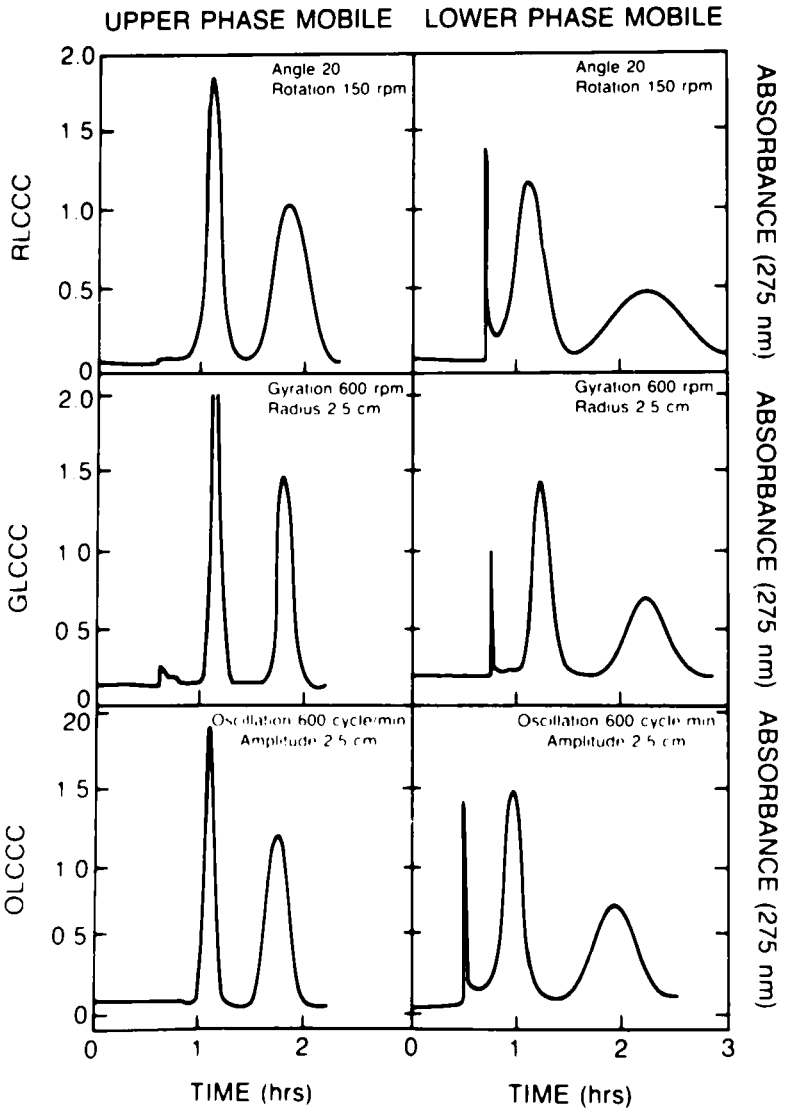

Figure 5. Typical chromatograms obtained from three different LCCC schemes. A: RLCCC; B: GLCCC; and C: OLCCC.
In all separations the experimental conditions are as follows:
Column: MIFC with 642 cylindrical locules and a total capacity of 175 ml.
Sample: DNP-glu and DNP-ala mixture 2 ml each component at 0.5 g% in the upper aqueous phase.
Flow Rate: 120 ml/h.

TABLE 1

Optimum Experimental Conditions and Partition Data for Three LCCC Schemes

LCCC SCHEME	Column (No. of Locules)	Column* Inclination	Column Motion (radius)	Mobile Phase U or L**	Retention of S.P.*** (%)	Partition Efficiency (T.P.)+	T.P./ Locule (%)	Time/T.P. (sec)	Peak Resolution (σ)
RLCCC	Spherical (1174)	35°	100 rpm (2.5 cm)	U	54	108	9	15.0	6.0
				L	56	51	4	33.9	4.7
	Cylindrical (642)	20°	150 rpm (2.5 cm)	U	56	200	31	10.8	7.0
				L	48	80	12	31.5	5.5
GLCCC	Spherical (1174)	90° (vertical)	600 rpm (2.5 cm)	U	20	1,126	96	1.4	7.6
				L	15	386	33	10	5.9
	Cylindrical (642)	90° (vertical)	600 rpm (2.5 cm)	U	40	602	94	3.6	11.4
				L	37	182	28	14.9	6.5
OLCCC	Spherical (1174)	90° (vertical)	600 rpm (1.25 cm)	U	26	423	36	5.4	9.9
				L	37	380	32	6.7	8.2
	Cylindrical (642)	90° (vertical)	600 rpm (1.25 cm)	U	43	474	74	2.7	9.6
				L	57	142	22	12.7	7.9

* Inclination from the horizontal plane
** U: Upper Phase, L: Lower Phase
*** S.P.: Stationary Phase
+ T.P.: Theoretical Plate Number

the column axis against the horizontal plane, for providing the greatest interfacial area of the two phases, varied with the shape of the locule. As indicated in Table 1, the optimum inclination for the cylindrical locule was 20° and that for the spherical locule was 35°. Smaller inclination decreased the stationary phase retention while greater inclination resulted in lower partition efficiency. In both types of the locular column the maximum column efficiency was observed at the rotational speed of 150 rpm.

In order to eliminate the need for the rotary seals, experiments were performed by manually reversing the rotation every 5 seconds or about 12 rotations at 150 rpm, which produced essentially the same results as obtained with the regular run at the same rpm. However, reversing the rotation every 110° (using the device for an automobile windshield wiper) resulted in much lower peak resolution.

In GLCCC best results were obtained at 450 rpm for the spherical locular column and at 600 rpm for the cylindrical locularcolumn, both with a gyrational radius of 2.5 cm. This gives angle α (see Fig. 3B) for the spherical locule at 10° and that for the cylindrical locule at 5°. Under these operational conditions flow rates of 120 ml/hr and 60 ml/hr yielded satisfactory results for both columns. Lower speed of gyration gave increased retention of the stationary phase but with broader peaks, whereas higher gyrational speeds produced excessive carryover of the stationary phase from the column resulting in lower peak resolution.

OLCCC was performed in a vertical column position at a 2.5 cm amplitude of oscillation. The results obtained under these conditions were found to be quite similar to those obtained with GLCCC. Compared with GLCCC, OLCCC gave substantially shorter retention time of the solvent front while it tended to produce more persistent carryover of the stationary phase in the later stage of elution.

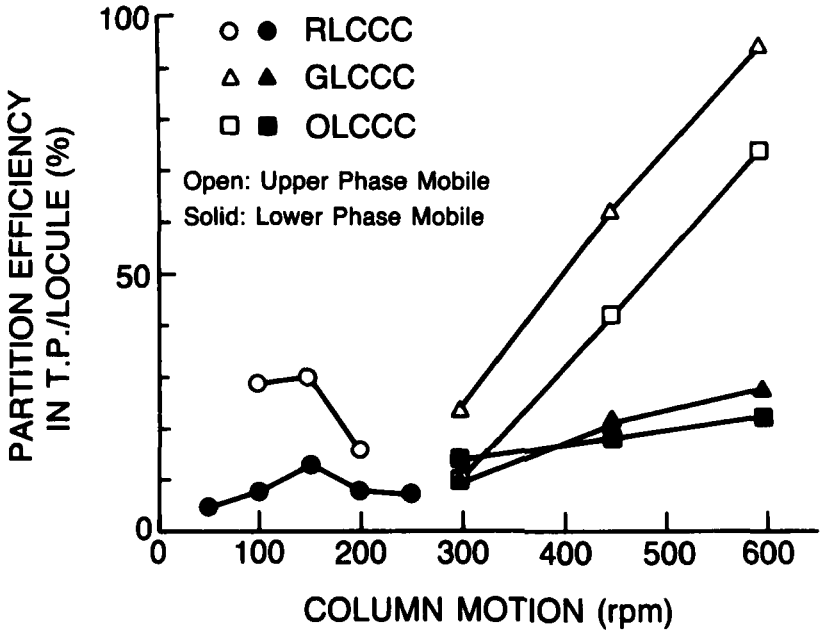

Figure 6. Effects of column motion on partition efficiency of each LCCC scheme.

As shown from Fig. 5 and Table 1, both GLCCC and OLCCC yielded much higher peak resolution than RLCCC. In Fig. 6 partition efficiency of the cylindrical locular column expressed in terms of percent theoretical plate per locule for three LCCC schemes are plotted against the rate of column motion in either rotation or oscillation per minute. Two curves were drawn for each scheme, one for upper phase mobile (open symbol) and the other for lower phase mobile (solid symbol). The column efficiency of RLCCC (circle) started to rise earlier and reached the maximum value of 12% to 15% at 150 rpm, followed by sharp decline with further increase of the rotational rate. In both GLCCC (triangle) and OLCCC (square) the efficiency started to rise later at between 200 and 300 rpm, but

continued to rise with increased column motion up to 600 rpm or oscillation/min and reached the maximum efficiency of near 100% (upper phase mobile).

The inferior performance of RLCCC is likely to be caused by the centrifugal force on the two solvent phases in the rotating column. Fig. 7 shows the cross-sectional view through the central axis of the cylindrical locular column for RLCCC (A and B) and GLCCC (C).

In Fig. 7A, the locular column wrapped around the column holder rotates at angular velocity ω with the holder inclination α from the horizontal plane. The two solvent phases in each locule are thus subjected to the combined force field, i.e., gravity g and relative centrifugal force vector $r\omega^2$ acting in an asymmetric manner around the column holder. In the lower position of the locule the centrifugal force is added to the gravity resulting in enhancement of the force field while in the upper position of the locule the centrifugal force opposes gravity causing reduction of the magnitude and deviation of the total force field. This reduces interface area and degree of circular mixing of the two solvent phases in the locule. With further increase of the rotational speed, the relative centrifugal force vector would eventually exceed the gravity resulting in loss of circular motion of the solvent phases with respect to the wall of the locule. Under these circumstances the two solvent phases are distributed in such a way that the lower phase always stays at the peripheral portion and the upper phase in the proximal portion of the locule as shown in Fig. 7B. Consequently, a high speed rotation interferes with solute partitioning process by limiting mixing of the two solvent phases in the locule. Consequently, the partition efficiency in RLCCC can still be improved by mounting the locular column closer to the center of rotation, thereby minimizing the undesirable effects of the centrifugal force.

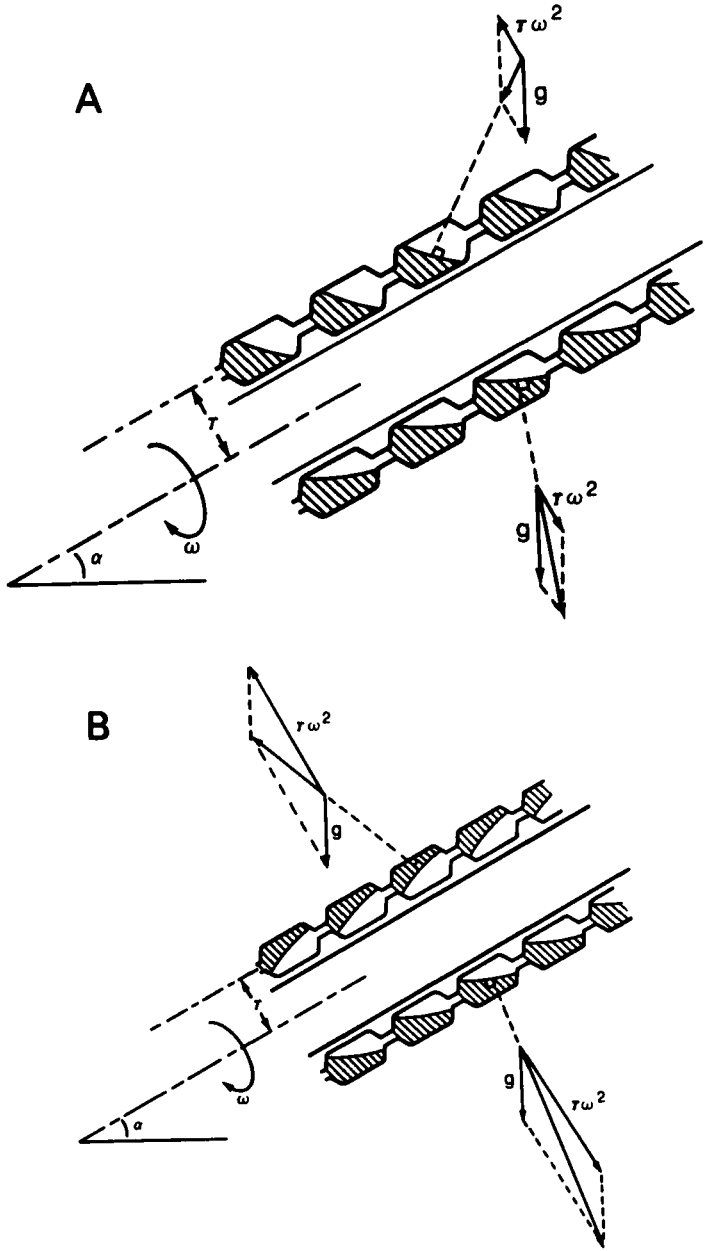

Figure 7. Effects of the rotational speed on the distribution of the two solvent phases in RLCCC (A and B) and GLCCC (C).

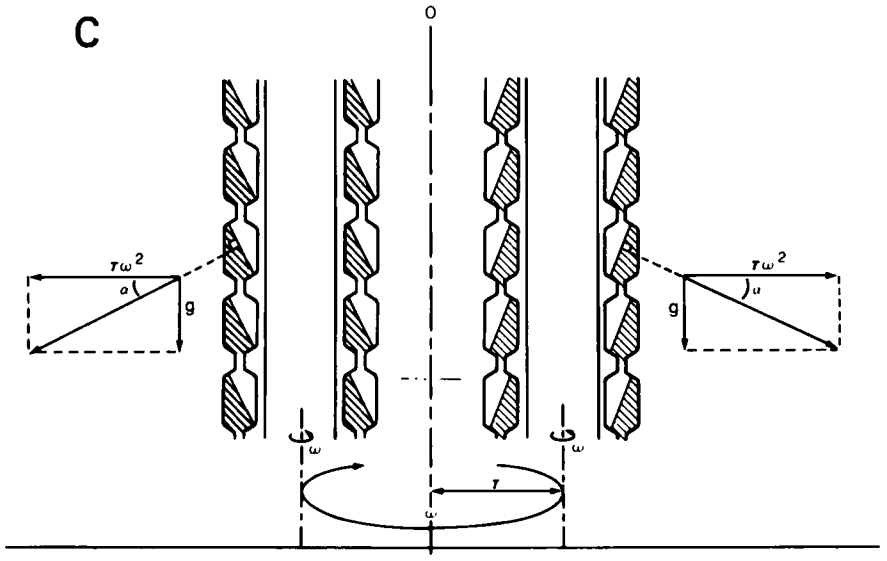

Figure 7C.

In Fig. 7C a similar cylindrical locular column wrapped on a vertical column holder is undergoing gyrational motion or nonrotational circular motion around central axis 0. In order to facilitate comparison with RLCCC in Fig. 7B, the gyrational radius and the angular velocity are similarly set at r and ω, respectively. Then gyration produces relative centrifugal force $r\omega^2$ which rotates around the vertical axis at a uniform angular velocity, ω. The total force acting on the holder is given by the sum of $r\omega^2$ and gravity g to determine the inclination of the interface of the two solvent phases in each locule. Consequently, gyration produces a symmetrical orientation of the interface around the central axis as illustrated in Fig. 5C. Since the column wall does not rotate with respect to the outside observer, the two solvent phases and their interface rotate against the wall to promote the partition

process. This permits application of a much higher gyrational speed to the column compared with the RLCCC described earlier.

In OLCCC, oscillatory motion produces a fluctuating force field acting to and fro along the axis of the locule. This motion agitates the two solvent phases at their interface to form multiple small droplets of one phase in the other to promote solute partition process along a broad interface. Substantial broadening of the interface with increased oscillatory frequency has been observed with a dyed mobile phase under stroboscopic illumination. In addition, oscillation and flow rate of the mobile phase may determine the size of the mobile phase droplets formed at the entrance of each locule to affect the partition efficiency. The higher the frequency of oscillation and the slower the flow rate, the smaller the droplets and the higher the partition efficiency. It is interesting to note that GLCCC and OLCCC yielded similar column efficiency at the same frequency, even though the force fields in these two schemes are quite different.

Overall results of our preliminary studies indicate great usefulness of MIFC as locular columns for CCC. The MIFC column provides the following advantages over the conventional locular columns.

1. The column is easily fabricated to a desired conformation.
2. The column has no internal connectors except for the inlet and outlet lines.
3. The column is light, compact, nonfragile and relatively inexpensive, and is suitable for mass production.

It is of great interest that columns can be made into a doughnut-shaped rotor for use in a centrifuge for elutriation of particles and for CCC with polymer phase systems.

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