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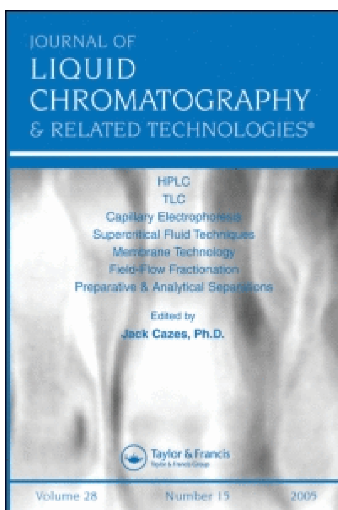

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Toroidal Coil Countercurrent Chromatography: A Fast Simple Alternative to Countercurrent Distribution Using Aqueous Two Phase Partition: Principles, Theory, and Apparatus

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To cite this Article Sutherland, Ian A. , Heywood-waddington, Deborah and Peters, Timothy J.(1984) 'Toroidal Coil Countercurrent Chromatography: A Fast Simple Alternative to Countercurrent Distribution Using Aqueous Two Phase Partition: Principles, Theory, and Apparatus', Journal of Liquid Chromatography & Related Technologies, 7: 2, 363 — 384
To link to this Article: DOI: 10.1080/01483918408073973
URL: http://dx.doi.org/10.1080/01483918408073973

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(2), 363-384 (1984)

TOROIDAL COIL COUNTERCURRENT CHROMATOGRAPHY: A FAST SIMPLE ALTERNATIVE TO COUNTERCURRENT DISTRIBUTION USING AQUEOUS TWO PHASE PARTITION

Principles, Theory and Apparatus

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ABSTRACT

The principles, theoretical basis and equipment for continuous two phase toroidal coil chromatography are described. Rat liver homogenates were subjected to analytical subcellular fractionation by toroidal coil chromatography in a phase mixture of 3.3% (w/w) dextran T500, 5.4% (w/w) poly(ethylene glycol) 6000, 10 mM sodium phosphate-phosphoric acid buffer, pH 7.4, in 0.26 M sucrose containing 0.05 mM Na_2EDTA and 1 mM ethanol. The distribution of organelles, as reflected by their marker enzymes, was compared to that obtained by discrete counter-current partition in a 17 transfer apparatus. Toroidal coil chromatography showed enhanced resolution of certain organelles. In particular, almost complete separation of plasma membrane from endoplasmic reticulum was achieved and some resolution of plasma membrane from lysosomes was obtained. It is concluded that toroidal coil chromatography offers a potentially useful alternative approach to organelle separation techniques.

INTRODUCTION

The toroidal coil centrifuge is conceptually a simple piece of apparatus, comprising a helically wound tube mounted

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circumferentially round a horizontal rotating disc. While it can be classified as a counter current chromatography process (1), its operation is similar to a continuous flow zonal rotor and its separation principles are those of a standard liquid-liquid chromatography column. Whereas centrifugation techniques separate on size, shape and density, the toroidal coil rotor offers the potential of separation based on partition between two immiscible liquids.

Separation of sample components with markedly different partition coefficients can be achieved in one or two test-tube partition steps involving mixing, settling and subsequent transfer of one of the phases to another test tube. However, samples with similar partition coefficients can only be resolved by using multiple discrete transfer or extraction techniques, such as counter-current distribution (2) or flow-through methods, such as liquid-liquid chromatography (3). The prime factor that makes toroidal coil chromatography different from the above techniques is that it uses centrifugation to hold one of the immiscible liquids stationary in the outer segments of the helical coil while the other phase is eluted through it.

Toroidal coil chromatography therefore can be considered as a continuous form of counter current distribution or a form of liquid-liquid chromatography without the solid support. It maintains the high retention volume of a phase partition process without an upper limit on the number of transfers. Adsorption problems and column contamination are minimised as the column requires no solid support to retain the stationary phase.

While applications using aqueous/organic phase systems have been described (4), the major application of the technique will be using polymer phase systems. The prime advantage of these polymer phases is that they are aqueous and the interfacial tension between the phases is extremely low - between 3 or 4 orders of magnitude less than aqueous/organic systems. However the very properties that make the phase systems an ideal

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partitioning medium for cells and macromolecules create problems when it comes to the implementation of the phase systems as a separation technique. For example a partition step in a test tube with a typical aqueous/organic phase system would take less than a minute, while with a polymer phase system could take anywhere from twenty minutes to an hour, because of the prolonged settling time of these phase systems.

The major advantage of the toroidal coil centrifuge over conventional phase partition techniques is its simplicity of setting up and operation, the reduced operating time and its potential for far greater resolution. Cell separations are possible with apparatus of this kind, provided the coils slowly rotate relative to the centrifugal acceleration field to avoid sample sedimentation. The principle of cell separation using such an apparatus has already been demonstrated (5) using a non-synchronous flow-through coil planet centrifuge. However, this apparatus is much more complicated to construct and the principle of phase mixing more complex.

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This publication describes the construction and operation of the toroidal coil centrifuge, examines the theory and demonstrates its reproducibility and resolving power by showing a clear fractionation of endoplasmic reticulum from plasma membrane in rat liver homogenate. The distribution of organelles is compared to a similar fractionation performed on a 17 transfer discrete counter current distribution apparatus, where the settling stage is enhanced using centrifugation (6). Detailed analysis of the phase system, operating and machine parameters will be described elsewhere (7).

DESCRIPTION OF APPARATUS

The Toroidal Coil (Figure 1)

The toroidal coil consists of 18 gauge PTFE *tubing* (i.d. 1.07 mm, o.d. 1.63 mm) wound onto a flexible 4.85 mm diameter

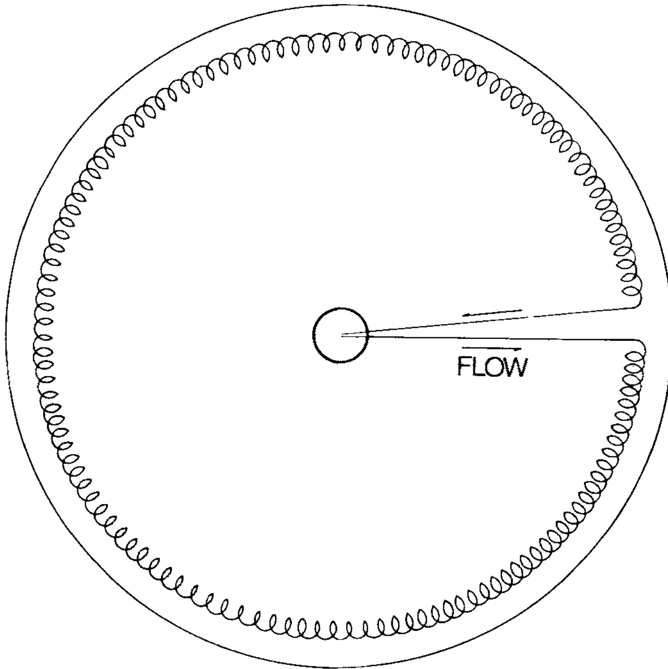

FIGURE 1

Plan view of toroidal coil, showing right handed helix with clockwise flow and rotation.

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former. The helical coil so formed is mounted circumferentially on a disc at a radius of 21 cm. The disc has a shoulder to support the toroidal coil radially. There are, in addition, six clamps locating it. The coil can be continuous with the inlet and outlet leads. Alternatively, 24 gauge tubing (i.d. 0.72 mm, o.d. 1.07 mm) can be used. This can be sleeve joined without connectors and considerably reduces the dead volume of the inlet/ outlet system.

The leads supplying liquid to and from the rotor have to be connected to the outside. Rotating seals could be used, but are prone to leakage. Methods eliminating the use of seals are simple to use and offer a number of advantages including reduced leakage and wear, and better sterility. One disadvantage is that

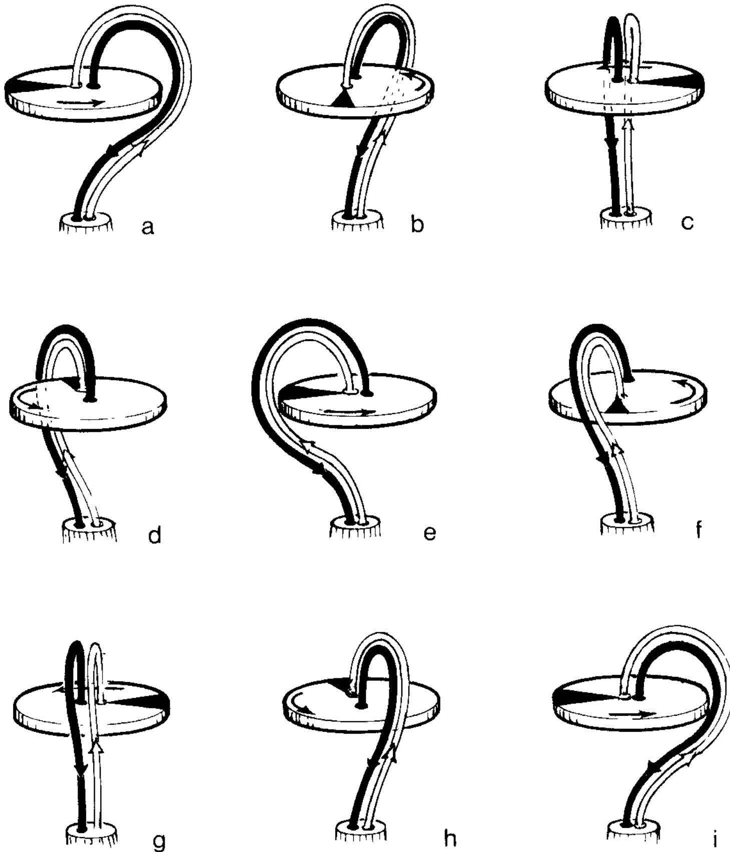

FIGURE 2

Sequencial diagrams showing the principle of seal-less connections to a rotating disc.

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the rotor is more complex. The principle of connecting tubes to and from a rotating disc (8) is a simple one (figure 2). The leads enter the centrifuge along the centre line of rotation above the disc, pass round the outside and enter it from underneath. If the disc is then rotated at, say, 1000 rev/min and the leads are constrained to rotate about the same axis at 500 rev/min around the outside of the disc, then the leads will not twist.

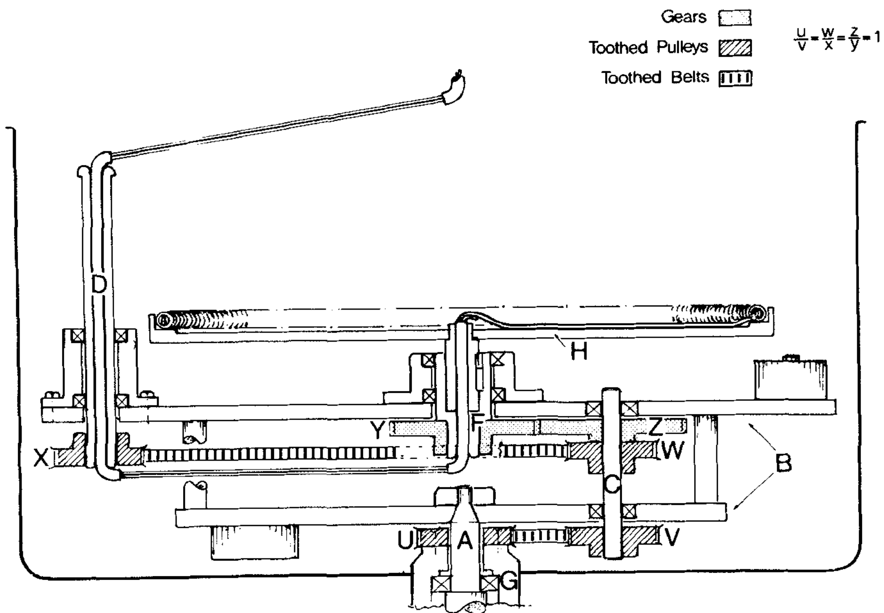

FIGURE 3

Diagram showing assembly of toroidal coil and seal-less connections. The drive shaft (A) rotates the carriage (B). The gear system (C) is constrained to rotate in planetary motion. Likewise the tube retaining shaft (D) is also constrained to rotate in planetary motion due to the toothed belt link between pulleys (X) and (W). The rotor shaft (F) will rotate in the opposite direction relative to shafts (C) and (D) due to the gear link between (Y) and (Z).

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The current rotor is shown in figure 3, mounted in the bowl of a Beckman J6 centrifuge. This centrifuge is particularly suited to the installation of the rotor as the drive shaft is not integral with the motor, and can easily be replaced by a modified drive shaft with a stationary tooth pulley for the toroidal coil rotor.

The points of maximum stress on the input and output leads occur at the bottom of the rotor shafts and at the inlet to the centrifuge bowl, due to their tumbling and rolling motion. Wear

is minimised by mounting the inlet and outlet leads in a Tygon tubing sheath, rounding the exit holes of the shafts and applying a light covering of silicon grease. Tube life in excess of 250 hours (15,000,000 cycles) is common. It is important to ensure that the tension in the leads is not excessive or wear rates will dramatically increase. The rotor is dynamically balanced in two planes by counterweights.

The Operating System

The operating system is shown schematically in figure 4. A roller peristaltic pump (Gilson minipulse 2) is used to pump the phases from the reservoirs into the toroidal coil. The proportion of lower phase flow (α) is regulated by the relative sizes of the peristaltic tubing. A counter-flow, fine bore mixing chamber ensures efficient mixing of the phases. A 4-way slider valve (Altex) is used to inject the sample (loop volume, 1 ml). A fraction collector (Gilson TDC 80), in drop count mode, collects the eluent in 1.5 ml plastic centrifuge tubes (Ependorf).

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THEORY

The fluid dynamics of two phase flow have been extensively studied for gas-liquid systems but for liquid/liquid systems still remain obscure, particularly when complicated by enhanced gravity and unconventional geometrical constraints.

A typical coil cross-section was constructed in Perspex, mounted on a specially adapted rotor and the behaviour of the phase system under the action of enhanced gravity observed with a stroboscope. The cross-section of the coil is shown in figure 5a-d. The coil is initially filled (a) with the lower phase followed (b) by the upper, lighter phase. The upper phase displaces the lower, heavier phase until it reaches point P1 when it cascades much like a waterfall to form (c) a new interface at Q1. Further flow of upper phase again displaces the lower phase in the next coil until new interfaces

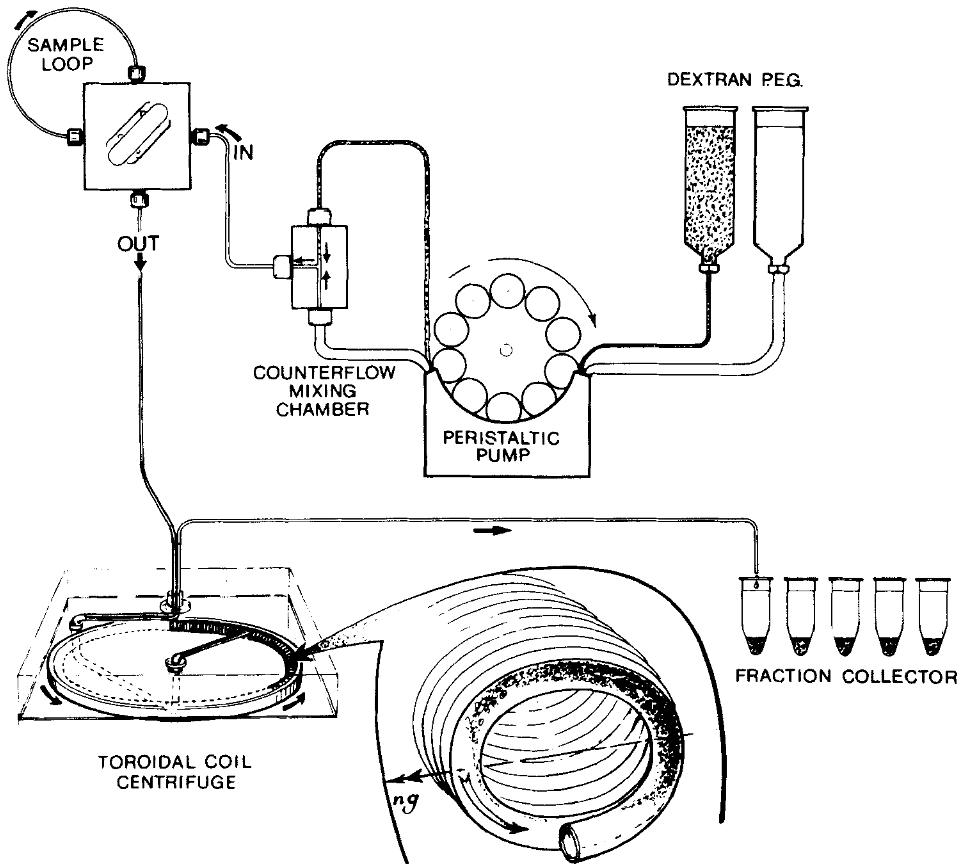

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FIGURE 4

Schematic layout of the toroidal coil centrifuge operating system.

are formed (d) at Q2 and P2. The segment of lower phase between P1 and Q2 is left as the retained phase while the upper phase continues to stream through it. Mixing takes place at and below the interface Q2 relative to the acceleration vector (shown arrowed in 5a).

In principle either phase can be the mobile phase. If the heavy phase is mobile the cascade would occur on the other side

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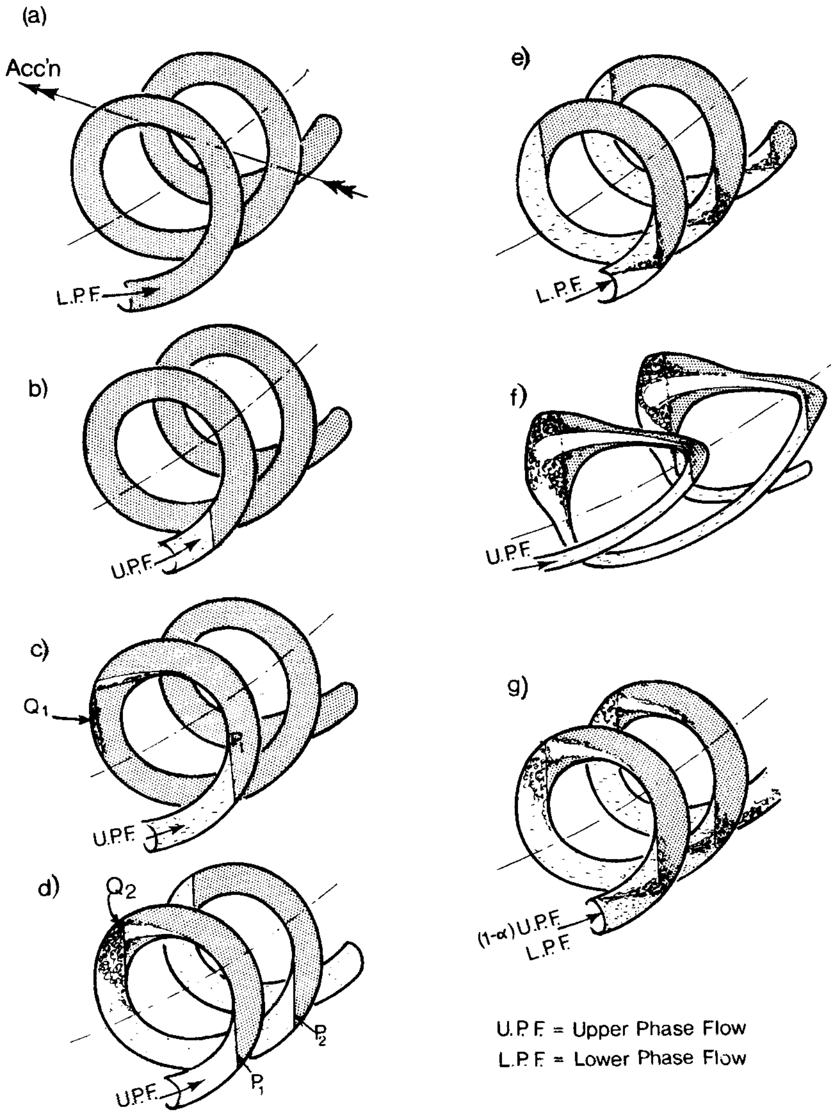

FIGURE 5

Diagram illustrating flow of 2 phase solutions through the helical coil. For detailed explanation see text. The shaded area represents the heavier dextran phase.

of the coil with mixing at and above the P interfaces (5e). It can be seen that mixing for light phase flow is limited to the Q interface areas and consequently is non-optimal. This can be improved by modifying the geometry (5f) or by flowing both phases at differing flow rates (5g). This has the advantage of avoiding "dead" zones in the retained phases giving mixing at P and Q interfaces and avoiding long retention times for low partition material.

If a number of linked coils are initially filled with the lower phase and the upper phase then pumped in, each coil will in turn approximately half fill with the upper phase displacing the excess lower phase through the coil system. The final result will be a retained phase, a mobile phase and mixing in each coil determined by the flow rate and the acceleration field.

The elution times of samples with varying partition coefficient will now be examined. The nomenclature is given in Appendix I.

For upper phase flow continuity (figure 6)

$$(1-\alpha)V_f = u_u A(1-\beta)$$

$$\therefore\ u_u = \frac{V_f}{A}\frac{(1-\alpha)}{(1-\beta)} \qquad \text{---------} \ (1)$$

For lower phase flow continuity :-

$$\alpha V_f = u_1 A\beta$$

$$\therefore\ u_1 = \frac{V_f \alpha}{A\ \beta} \qquad \text{---------} \ (2)$$

Solute Partitioning

From partition theory, assuming adequate mass transfer between the phases; the mean linear velocity for a solute of partition coefficient (k) will be u_k where :-

$$u_k = u_u\left(\frac{k'}{k'+1}\right) + u_1\left(\frac{1}{k'+1}\right)$$

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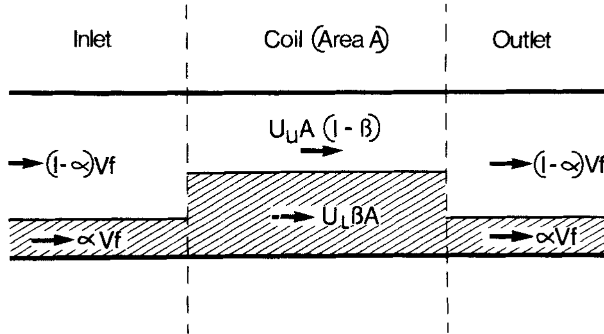

FIGURE 6

Representation of two-phase flow characteristics through the toroidal coil centrifuge. The inlet flow (V_f) is a mixture of lower phase flow (αV_f) and upper phase $(1-\alpha)V_f$ by arrangement. The volume flow through the coil will depend on the respective linear flows (u_u) and (u_1) and the relative proportion of the phases in the coils which can be considered constant in the steady state.

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and $k = k'\beta/(1-\beta)$

$$\therefore u_k = \left[\frac{u_u k(1-\beta) + u_1\beta}{k(1-\beta) + \beta}\right] \quad \text{---------} \ (3)$$

The time taken for solute of partition coefficient (k) to pass through the coil of length V^*/A will be V^*/Au_k and the volume eluted (V_k), taking into account the inlet and outlet tube volumes, will be :-

$$V_k = \frac{V_f V^*}{Au_k} + V_{in} + V_{out} \quad \text{---------} \ (4)$$

Substitution of u_k from (3) and u_u and u_1 from (1) and (2) gives :-

$$V_k = \left[\frac{k(1-\beta)+\beta}{k(1-\alpha)+\alpha}\right] V^* + V_{in} + V_{out} \quad \text{---------} \ (5)$$

As a check on equation (5), a solute with a partition coefficient of unity should be treated by the system as though it was filled with a single solution, and hence the elution volume should equal the system volume. Substitution of k = 1 into equation (5) verifies this, giving :-

$$V_{k=1} = V^* + V_{in} + V_{out} \quad \text{---------} \quad (6)$$

Other useful markers are :-

$$V_{k=\infty} = \left[\frac{(1-\beta)}{(1-\alpha)}\right] V^* + V_{in} + V_{out} \quad \text{---------} \quad (7)$$

and $$V_{k=0} = \frac{\beta}{\alpha} V^* + V_{in} + V_{out} \quad \text{---------} \quad (8)$$

as they mark the beginning and end of sample elution from the point of injection.

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Particle Partitioning

The partition ratio (G) is defined as the ratio of particles in the upper phase to those at the interface between the phases. Assuming that the interface is retained with the lower phase, then the linear velocity of a particle with partition ratio (G) is given by :-

$$u_G = u_u\left(\frac{G}{G+1}\right) + u_l\left(\frac{1}{G+1}\right) \quad \text{---------} \quad (9)$$

The volume eluted can be calculated by substituting for u_u and u_l in (9) and proceeding as for solutes to give

$$V_G = \left[\frac{\beta(1-\beta)(G+1)}{\beta(1-\alpha)G+\alpha(1-\beta)}\right] V^* + V_{in}+V_{out} \quad \text{-----}(10)$$

Note the elution volumes for G = ∞ and G = 0 are the same as for solutes in equations (7) and (8) while $V_{G=1}$ is given by :-

$$V_{G=1} = \left[\frac{2\beta(1-\beta)}{\beta(1-\alpha)+\alpha(1-\beta)}\right] V^* + V_{in}+V_{out} \quad \text{-----}(11)$$

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Calculation of Retention Volume (βV^*)

While all the above formulae are useful in determining the elution volumes if partition coefficients or ratios are known or alternatively to determine these partition values if they are not, the proportion of lower phase retained (β) still has to be determined in some way. This can be done in one of two ways. The first is at the beginning of a run: if the coil is initially filled with lower phase and a measuring cylinder is used to collect the displaced lower phase (V_E) then this value can be used to calculate the amount of lower phase left in the coil. Elution of the first amount of upper phase is equivalent to considering the elution volume of the $K = \infty$ peak in equation (7) so that $V_E = \left[\frac{(1-\beta)}{(1-\alpha)}\right] V^* + V_{in} + V_{out}$ rearranging

$$\beta = 1 - \left[\frac{(1-\alpha)\ \{V_E - (V_{in} + V_{out})\}}{V^*}\right] \text{----- (12)}$$

Alternatively at the end of run, the retained dextran phase can be pumped out giving :-

$$V_p = \alpha(V_{in} + V_{out}) + \beta V^*$$

Rearranging :-

$$\beta = \frac{\left[V_p - \alpha(V_{in} + V_{out})\right]}{V^*} \text{--------- (13)}$$

In practice the average value of β from the two equations gives the most reliable values.

MATERIALS AND METHODS

Phase System Preparation

Dextran T500 (batch FD16027) was obtained from Pharmacia (Uppsala, Sweden) and Breox poly(ethylene-glycol) 6000 from Hythe Chemicals (Southampton, England). Stock solutions of the polymers were used to prepare phase mixtures (150 g) containing 3.3% (w/w) dextran T500, 5.4% (w/w) poly(ethylene glycol) 6000, 10 mM sodium phosphate-phosphoric acid, pH 7.4, 0.26 M sucrose,

0.05 mM Na_2EDTA, ph 7.4 and 1 mM ethanol. When the phase system had cooled to 4^oC, it was shaken and allowed to settle overnight before separating the two phases. This phase system is an adaptation of that used by Morris & Peters (6) for fractionation of rat-liver homogenate. The purpose of the ethanol was to minimise inactivation of the catalase (9).

Sample Preparation

Non-fasted male Sprague-Dawley rats (150-200 g) were stunned and killed by cervical dislocation. The liver was immediately removed and 0.5 g perilobular tissue was minced with a razor blade. The minced tissue was then disrupted in a Dounce homogeniser (Kontes Glass Co., Vineland, NJ, USA) in 10 ml of ice-cold poly(ethylene-glycol)-rich upper phase, from which 0.4 g of water had previously been evaporated, with nitrogen, to allow for the water content of the liver tissue. Homogenisation was standardised with 10 strokes of a loose-fitting (type A) pestle, followed by 10 strokes of a tight-fitting (type B) pestle. Fibrous material was removed by passing the homogenate through a 50 micron nylon mesh. Upper phase, 0.9 ml, containing homogenate, together with 0.1 ml of lower phase, was used as the sample for partition experiments.

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Running Procedure

The coil was initially filled with dextran-rich lower phase. The centrifuge was then set to rotate the toroidal coil at 1000 rev/min. The mobile phase, comprising 94% poly(ethylen-glycol)-rich upper phase well mixed with 6% dextran-rich lower phase, was pumped into the coil at 14 ml/hr and the eluent collected in 1 ml fractions. When the system reached equilibrium, i.e. when the eluent also contained 6% dextran-rich lower phase, the sample was injected by means of a 4-way slider valve (Altex). After collection of about 40 x 1 ml fractions, the coil was brought to rest and the contents of the coil pumped out with

water. The volume ratio was analysed by measuring the height of the total phase and interface in each of the fractions. The fractions were stored at -20°C for subsequent analysis. All equipment was in the 4°C cold room.

Analytical Methods

Sucrose (0.25 M, 0.15 ml) was added to each fraction before analysis to give a single phase. The distribution of organelles was determined by assaying (10) the following marker enzymes in alternate fractions: N-acetyl-β-glucosaminidase (lysosomes); neutral-α-glucosidase (endoplasmic-reticulum); lactate dehydrogenase (cytosol); γ-glutamyl-transferase (plasma membrane).

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RESULTS

Figure 7 compares the fractionation of rat-liver homogenate by both a 17 transfer counter current partition apparatus (6) and a toroidal coil centrifuge. Comparison of the two techniques is complicated by the fact that CCD is a discrete process conventionally transferring upper-phase to the right, whereas TCC is an elution process where the upper-phase elutes first, hence inverting the partition spectrum. For the purposes of comparison, the counter-current distribution has been reversed to give upper-phase transfer from right to left. The fractions are compared on an equal volume basis giving a counter current distribution of 18 fractions (23.4 ml) and a toroidal coil distribution of 36 fractions (36 ml). Note that theoretical markers have been placed on both graphs. The counter-current experiment markers are based on a theory outlined by Albertsson (11), while those for the toroidal coil experiment are derived from equations (6), (7), (8) and (11).

Both results show that the organelle distribution curves are highly reproducible and qualitatively similar. The toroidal coil results show a significant improvement in

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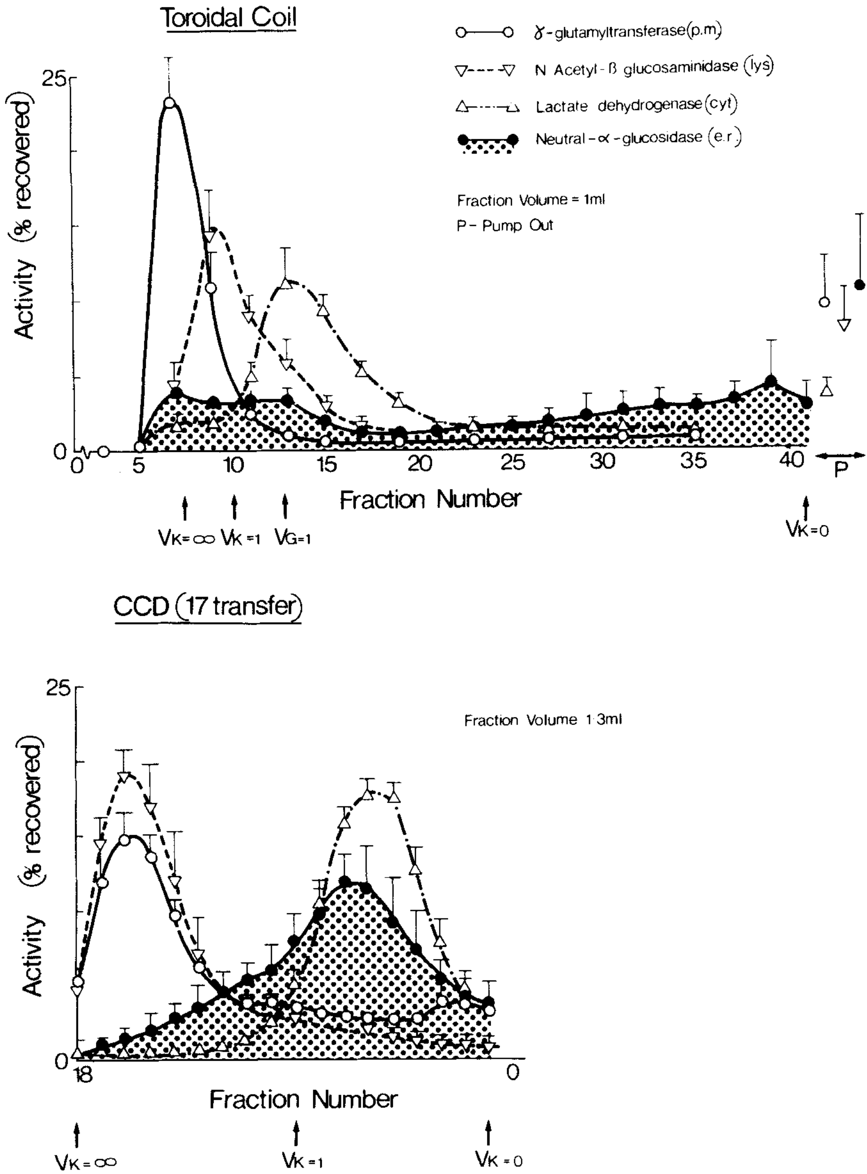

FIGURE 7

Analytical subcellular fraction of rat-liver homogenate by (a) 17 transfer discrete counter current apparatus (6) and (b) continuous toroidal coil centrifuge. Results show mean ± SD for (n) experiments with discrete CCD (n=3) and continuous TCC (n=7). The enzyme activities are given with the organelles in parentheses. Recovered enzyme activities range from 70-90%.

resolution, with a partial separation of lysosomes from plasma membrane and a clear separation of the cytosol peak from endoplasmic reticulum. Separation of plasma membrane from endoplasmic reticulum is achieved by both techniques although this is more complete for the toroidal coil experiment. The fact that the k = 1 elution point is biased towards the point of sample elution in the toroidal coil fractionation largely contributes to the shift of the soluble cytosol peak to the left.

When the CCD experimental results are compared with 17 transfer theoretical binominal distributions, the plasma membrane, lysosome and cytosol peaks are essentially single components, apart from small secondary peaks for plasma membrane and lysosomes at low k, and for cytosol at high k. The endoplasmic reticulum distribution shows signs of heterogeneity, with a major component at low k and one or possibly two components at high k. These are resolved in the toroidal coil distributions whereas the subsidiary lysosome components only appear as a shoulder and the plasma membrane component is not found.

The subsidiary peaks of the lysosomes and cytosol distribution are shifted to the left due to the asymmetrical partition distribution. This has the effect of concentrating the subsidiary cytosol peak and merging the lysosome one with the major peak. The absence of the plasma membrane subsidiary peak is anomalous, but could be explained by the small quantity of plasma membrane eluted at pump out. Any components affected by sedimentation in this way would necessarily be large and possibly anomalies from the homogenisation process.

Note that lysosomes and plasma membrane components are resolved by the toroidal coil experiment in contrast to the counter-current distribution experiment where they appear to have identical modal partition coefficients.

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DISCUSSION

The data in this paper indicates that resolution of the principle subcellular organelles of rat-liver homogenates can be achieved by toroidal coil chromatography. In particular, marked heterogeneity of endoplasmic reticulum and its resolution from plasma membrane is demonstrated. These organelles, having very similar size and density characteristics, are not readily separated by density-gradient centrifugation techniques (12). Resolution of the various organelles is enhanced, compared with discrete counter-current partition experiments reported previously (6). The fact that the enzyme distributions are qualitatively similar for both processes and that the elution profiles are within the bounds of the theoretical markers, confirms a TCC separation based on partition. The assumption that the interface is retained with the lower phase is shown to be valid by the retention of the endoplasmic reticulum in the coil. The early elution of the plasma membrane peak compared to the $V_{K=\infty}(V_{G=\infty})$ marker may be due to locally high β values. The theoretical predictions assume a constant value of β, and analysis of the phase proportions in each fraction shows evidence of lower phase carry-over between fractions 5-8 which could produce early elution of the high partition peaks.

The shift of the soluble cytosol peak to the left in the TCC distribution would be expected from the marked shift to the left of the theoretical k=1 marker. Likewise, the shift of the G=1 marker to the left in the TCC distribution emphasises the fractionation between the low partition and high partition components of the endoplasmic reticulum peak, which is only just noticeable in the CCD distribution. Low partition components are therefore fractionated more efficiently than high partition ones. The reverse would be true if the dextran-phase was used as the mobile phase. Such a procedure could form the basis for subfractionating the plasma membrane and lysosome peaks.

The equipment described is moderately simple to construct and the toroidal coil rotor can be adapted for use in several commercially available centrifuges. The equipment has been routinely used for 3 years and for in excess of one hundred experiments. The seal-less method of connections between rotor and fraction collector/pump has proved particularly useful. It avoids heating problems, organelle/cell damage and technical failures, characteristic of many centrifuge seals. In addition, the same rotor can be used for particle separation, working on the same principle as a low speed zonal rotor. The toroidal coil will permit a large number of partitions between the two phases. The present rotor has approximately 550 turns and this represents a considerable improvement on existing two-phase partition machines (11), which are labour intensive and involve lengthy procedures. It would be relatively simple to mount two or more concentric toroidal coils on the rotor, in order to compare two separate samples under otherwise identical conditions. Direct comparison of the organelle separations achieved by the 17 transfer discrete partition apparatus with the same tissue source, enzyme analytic methods and essentially the same two-phase polymer systems indicates that, although the latter technique yields enhanced resolution, less than optimal separation was achieved for the greatly increased number of transfers expected within the toroidal coil. In other words the number of theoretical plates was less than expected. The reason for this is not clear but it is likely that complete equilibration between the two phases is not reached for each loop of the coil. Analysis of the peak shape suggests that mixing in the toroidal coil centrifuge is only about 5-10% efficient. There is thus clearly significant scope for improvement. Current experiments are aimed at enhancing organelle separation by varying such parameters as rotor speed, flow rate, phase mixture composition and relative proportions of the two phases and coil geometry. Similarly, a more detailed study of theoretical models for phase partition in the toroidal

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coil centrifuge should help in the design of more effective separation procedures and these are currently in progress.

The effectiveness of the toroidal coil centrifuge has been demonstrated in comparison with an equivalent enhanced gravity CCD technique. If coil efficiency can be improved, then the process could become a powerful organelle separation technique which is particularly suited to automation.

ACKNOWLEDGEMENTS

We would like to thank Dr Yoichiro Ito of the Laboratory of Technical Development, NIH; Dr Derek Fisher of the Biochemistry Department, RFPMS, London, and Denis Rothwell, Head of Engineering, NIMR, London, for their critical evaluation of the manuscript.

We would also like to thank Frank New and Kate Bunker for their respective contributions to the artwork and typing.

Finally, the staff of the Engineering Workshop at NIMR are gratefully acknowledged for their skill and craftsmanship in producing the toroidal coil rotor, and Beckman (UK) for supplying the J6 centrifuge.

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REFERENCES

1 Ito Y., Counter Current Chromatography, Jl. Biochem. Biophys. Methods, 5, 105-129 (1981)

2 Craig L.C., Comprehensive Biochemistry, 4, Elsevier, Amsterdam/London/New York (1962)

3 Snyder L.R. & Kirkland J.J., Introduction to Modern Liquid Chromatography, 2nd Ed, Wiley Interscience, New York, 323-348 (1979)

4 Ito Y. and Bowman R.L., Countercurrent Chromatography with the Flow-through Centrifuge without Rotating Seals, Anal. Biochem., 85, 614-617 (1978)

5 Sutherland I.A. and Ito Y., Cell Separation Using Two-Phase Polymer Systems in Nonsynchronous Flow-through Coil Planet Centrifuge, Anal.Biochem., 108, 367-373 (1980)

6 Morris W.B. and Peters T.J., Microanalytical Partition of Rat-Liver Homogenates by Poly(ethylene glycol)-Dextran Counter-current Distribution, Eur. J. Biochem., 121, 421-426 (1982)

7 Heywood-Waddington D., Sutherland I.A., Morris W.B. and Peters, T.J., Subcellular Fractionation of Rat-Liver Homogenates using Two-Polymer Phase Systems in a Toroidal Coil Centrifuge, submitted for publication (1983)

8 Ito Y., Suaudeau J. and Bowman R.L., New Flow-Through Centrifuge Without Rotating Seals Applied to Plasmaphoresis, Science, 189, 999 (1975)

9 Chance B., The Reactions of Catalase in the presence of the Notatin System, Biochem. J., 46, 387-402 (1950)

10 Smith G.D. and Peters T.J., Analytical Subcellular Fractionation of Rat-Liver with Special Reference to the Localisation of Putative Plasma Membrane Marker Enzymes, Europ. J. Biochem., 104, 305-311 (1980)

11 Albertsson P.A., Partition of Cell Particles and Macromolecules, Wiley Interscience, New York (1971)

12 Beaufay H., Amar-Costesec A., Thines-Sempoux D., Wibo M., Robbi M. and Berthet J., Analytical Study of Microsomes and Isolated Subcellular Membranes from Rat-Liver, J. Cell. Biol., 61, 213-231 (1974)

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APPENDIX I

Nomenclature

A = Coil cross-sectional area

G = partition ratio for particles

k = partition coefficient for solutes

k' = apparent partition coefficient (corrected for volume ratio)

u_l = mean linear velocity of lower phase

u_G = mean linear velocity of particle with partition ratio G

u_k = mean linear velocity of solute with partition coefficient k

u_u = mean linear velocity of upper phase

V^* = Total volume of toroidal coil

V_E = Elution volume

V_f = Volume flow into coil

V_p = Volume lower phase pump out

V_k	=	Elution volume of compact with partition coefficient k
V_{IN}	=	Volume of inlet tube
V_{OUT}	=	Volume of outlet tube
α	=	proportion of lower phase in total flow
β	=	mean proportion of lower phase retained in coil

Subscripts

G	=	particle partition ratio G
k	=	solute partition coefficient k
l	=	lower
u	=	upper

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