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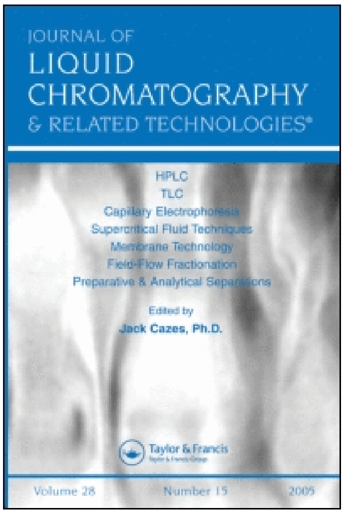

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Review of Progress Toward the Industrial Scale-Up of CCC

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Abstract: Considerable advances have been made in the last two years on the industrial scale-up of countercurrent chromatography. This paper briefly reviews the scale-up progress being made by three groups, two in France and one in the UK before giving details of advances being made at Brunel Institute for Bioengineering, Brunel University in the UK on the scale-up of their J-type centrifuges.

Keywords: Industrial scale-up, Countercurrent chromatography, Dynamic extraction, Centrifugal partition chromatography

INTRODUCTION

Brunel Institute for Bioengineering (BIB) received funding from the EPSRC's Innovative Manufacturing Initiative (IMI) research program for "Realizing Process Scale CCC". The EPSRC-IMI program encourages universities to work closely with industry to develop technology that fulfills an urgent industrial need. The university performs the research while the industry supplies the project management. As a result of this, an industrial hydrodynamic pilot scale coil planet centrifuge has been built by the pharmaceutical industry's supply chain companies. The hardware produced is therefore robust, reliable and readily serviceable. The pilot centrifuge has a rotor radius of 300 mm, a speed range of 100–850 rpm, a capacity of 4.6 liters, with tubing of 10 mm bore and 60 m length on two bobbins. The flow rate range varies from 0.1 to 1.6 liters/min leading to batch separation times of between 10–20 minutes per injection. Sample loading studies have shown that

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10% column volume loading capacities are feasible with up to 50% w/w sample concentrations of crude extracts. Processing rates of up to 3 kg/hour are feasible.

In a parallel study, the University of Nantes, with support from Institut Français du Pétrole (IFP) and Armen Instrument, have proved that the performance of hydrostatic centrifugal partition chromatography (CPC) instruments can be greatly improved by an appropriated design of the "column" (duct and channel shapes). Such an optimization leads to a more productive device with better stationary phase retention, a more efficient column and faster flow-rates leading to greater throughput. Further thought and experiments gave the French team the key to increase efficiency and achieve further scale-up of CPC hydrostatic instruments for industrial CCC applications. Furthermore, instruments can be used for classical elution, displacement and dual mode applications. In addition, the French team has developed a new method of operating the system, in such a way that sequential elution (alternative ascending and descending modes) with continuous injection of the sample in the middle of the 'CPC column' is possible. Components are separated into two fractions which are collected on each side of the CPC instrument (Patent pending). The first instrument will be offered as an explosion proof version and fulfills all the standards and regulations for industrial applications. Its total volume is 25 liters (12.5 liters for sequential applications) and operational flow-rate is around 1 liters/min.

In another independent French development by PARTUS Technologies, in collaboration with Couturier SAS and the University of Reims, an alternative design 25 liter hydrostatic CPC centrifuge is being developed with extremely rigorous industrial criteria being met. Margraff et al., in their paper in this issue,[1] describe their rotor and its performance. All wetted parts are made of titanium and the single component rotor can be operated up to 150 bar (2,000 psi).

Preliminary results on retention of the liquid stationary phases from non-aqueous and aqueous-aqueous biphasic solvent systems are given and discussed. They introduce a novel feature somewhat like wire wool into their cells to break up the Coriolis flow profile and drastically enhance mixing. They had two major aims: 1) to develop a machine that would reliably operate in semi-continuous mode for a succession of identical purification cycles over a period of at least 100 hours (one week); 2) to develop a high productivity and high flow through process at an industrial scale. They present chromatographic performances using a non-aqueous biphasic solvent system and an aqueous two phase system (ATPS) to validate their process.

Benefits of Manufacturing Scale CCC

Solid phase chromatography is not the method of choice for a process engineer. This is because it is expensive and, from the regulatory point of view, the process can change with time due to non-specific adsorption by the solid support. Liquid-liquid chromatography is much more analogous to liquid-liquid extraction and a methodology which is well understood and frequently used by process engineers in manufacturing processes. Countercurrent Chromatography (CCC) is either a liquid-liquid chromatography or extraction process depending on how it is used. It will therefore offer the process engineer an alternative to crystallization and liquid-liquid extraction and could in the future become the method of choice for a wide range of high resolution separation and extraction processes. Some unique advantages are listed below:

- *No sample loss.* As both phases are liquid, the sample can always be recovered from either the mobile phase or the stationary phase.
- *Less solvent usage.* The process uses significantly less solvent than Prep-HPLC so that more preparative separations can be carried out in the laboratory before needing to transfer the process to a hazard's laboratory.
- *High sample loading.* 70–90% of the column is stationary phase—a very large percentage compared to HPLC. This allows higher sample loading up to 10–30% of the column volume.
- *Crude extracts* (including *particles*) are acceptable—no pre-purification required—sample concentrations can be as high as 50% w/w.
- *Reverse or normal phase possible.* Can choose either phase as the mobile phase (normal or reverse phase operation) or even switch over during a run.
- *Predictable and reproducible runs* with no cross contamination or changes with time from solid supports. Optimization and loading studies can be performed at low scale and then the process can be predictably transferred to manufacturing scale with confidence that it will work.[2]
- *Regulation friendly.* The process is more attractive to industry from the regulatory point of view as it is more like liquid-liquid extraction.

Hydrodynamic versus Hydrostatic CCC

While both hydrodynamic and hydrostatic CCC instruments share the above advantages over solid phase chromatography, there are also some distinct differences between hydrodynamic and hydrostatic CCC which need to be mentioned. Hydrodynamic CCC stratifies two immiscible solvent phases along a continuous length of tubing, which is wound on a drum mounted on the planetary axis of a "J" type coil planet centrifuge. The advantage of this is that there are no rotating seals, the process is not pressure limited, it is easy to automate and easy to clean. The disadvantage is that it is sensitive to changes in interfacial tension between the phases and so is not as stable using aqueous two phase systems (APTS). Hydrostatic CCC, typically represented by Centrifugal Partition Chromatography, on the other hand has a series of chambers machined circumferentially round a rotor. The stationary phase is restrained physically by being trapped in each chamber, while the mobile phase flows through it in a cascading manner influenced by the Coriolis acceleration effect. The advantages of this method of retaining the stationary phase are that it is quiet and not so sensitive to changes in the physical properties of the phase systems. The disadvantages are that it is pressure limited like HPLC so there is a limit to the resolution achievable, by increasing the length of the column. There are rotating seals and the process is less efficient than hydrodynamic CCC, although the latest advances in mixing could have minimized this difference.

At the time of writing, there have been no direct comparisons made between preparative or industrial scale hydrodynamic and hydrostatic CCC, but the industrial scale-up of both technologies will greatly enhance the separation choices for industry, particularly as the molecular size of target compounds increases.

Hydrodynamic CCC

If the flow stops, the phases will redistribute to each end of the coil—the heavy phase gathers to the "Tail" and the light phase to the "Head" as defined by the Archimedes Rule. A high proportion of retained stationary phase is held in hydrodynamic equilibrium against the flow of the mobile phase and will depend on the value of this flow rate as defined by the "B" gradient.[3] Mass transfer is via wave mixing in each coil unit. The most common form is a continuous length of tubing rotated on a "J" type coil planet centrifuge. No rotating seals are required. The devices have two axes of rotation and a possibly noisy gear arrangement.

Hydrostatic CCC

If the flow stops, the stationary phase will remain in each discrete chamber. Stationary phase retention is hardly affected by changes in mobile phase flow rate. Mass transfer is from cascade mixing in each chamber. Hydrodynamic behavior can occur in each chamber, but the stationary phase is held fixed by the chamber geometry. The most common form is Centrifugal Partition Chromatography where there are a series of serially interconnected chambers located circumferentially round a disc which rotates. Rotating seals are required, but the devices have a single axis of rotation and run quietly.

Importance of High Resolution Liquid-liquid Extraction/Chromatography in the Future

High resolution manufacturing methods for viable biologics is going to become an important requirement in the future as bioactive compounds become increasingly larger and more complicated. These large scale liquid-liquid processes could become a serious alternative to production HPLC, as they can tolerate particulates and have high sample loading capacities.

The remaining part of this paper describes the scale-up of Hydrodynamic CCC in more detail, while the Partus Hydrostatic CCC is described elsewhere in this issue by Magraff.[1]

SCALE UP OF HYDRODYNAMIC CCC

How Planetary Motion Sets up Discrete Zones of Mixing and Settling Along the Column

The basic motion of the "J" type coil planet centrifuge is given in Figure 1a. The normal and tangential acceleration vectors are show in 50 freeze frame steps for one complete revolution of the main rotor and two of the planetary rotor. It can be seen that the acceleration vectors are much higher on the distal part of the cycle (A—using medical notation) and smaller in the proximal part (B). Note also, that the tangential acceleration vector changes direction each side of the cycle, which has the effect of enhancing mixing between the two layers of liquids (creating a swish/swash effect). Figure 1b shows a spiral coil added to the planetary rotor with two liquid phases held in hydrodynamic equilibrium—an upper phase (shaded light) and a lower phase (shaded dark). The phases are separated at the distal part of the rotation (A) and mixed (wave mixing) at the proximal part (B). The waves of mixing and settling are synchronized to their respective proximal and distal nodes and, so, move along the coil from the "Tail" (light spot) to the "Head" (dark spot) using the usual Archimedean screw notation. The coil can be considered as a continuous piece of tubing, as shown in the bottom part of the figure with the waves of mixing and settling moving like standing waves from left to right. Either

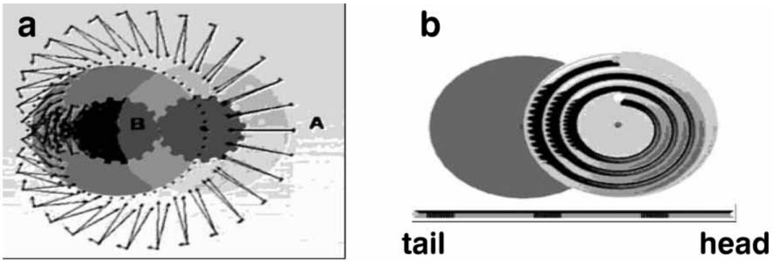

Figure 1. a) J-Type Coil\Planet Centrifuge showing radial and tangential accelerations for one rotation of the planetary rotor. Note high acceleration field at distal point mark "A" and low acceleration field at proximal point marked "B". b) A spiral coil was added to the planetary rotor to show how the two liquid phases separate at the distal side of the coil ("A" in Figure 1a) and mix on the distal side ("B" in Figure 1a). The waves of mixing and settling are synchronised to their respective proximal and distal nodes and so move along the coil from tail to head. The coil can be considered as a continuous piece of tubing as shown in the bottom part of the figure.

phase can be used as the mobile phase. In general, the upper (lighter) phase naturally moves towards the "Head" and the lower (heavier) phase moves toward the "Tail." Hydrodynamic equilibrium, therefore, is achieved by either flowing the upper phase from "Tail" to "Head" against the natural direction the stationary phase would like to go, or flowing the lower phase from head to tail against the natural direction the upper phase would like to go. This is a hydrodynamic equilibrium process which, if the flow stopped, would result in all of the upper phase in the coil moving to the "Head" end (dark spot) and the lower phase moving to the "Tail" (light spot).

A sample injected with the mobile phase will predictably elute at V_r according to its distribution ratio (D) as shown in Figure 2:

$$V_r = V_{ext} + V_m + DV_s \tag{1}$$

where V_r is the retention volume of a sample of distribution ratio (D), V_{ext} is the extra coil volume, V_m is the volume of mobile phase in the column and V_s is the volume of stationary phase in the column. An understanding of the volumes of stationary phase (V_s) and mobile phase (V_m) in the coil is essential if predictions are to be made.

Factors Affecting Hydrodynamic Equilibrium and the Retention of Stationary Phase in the Column

The volume retention ratio ($S_f = V_s/V_c$) of the stationary phase in a "J" type coil planet centrifuge has been shown to be linearly related to the square root of the mobile phase flow:[4]

$$S_f = A - B\ F^{0.5} \tag{2}$$

If allowance is made for the extra coil volume,[5] then, when S_f is expressed as a percentage, at no flow one would one expect 100% stationary phase retention as shown in Figure 3:

$$S_f = 100 - B\ F^{0.5} \tag{3}$$

Factors Affecting Scale-Up

The discovery of this linear relationship has led to the further discovery that the "B" gradient is inversely proportional to the tubing cross sectional area (or diameter squared), inversely proportional to the speed of rotation, inversely proportional to the square root of the density difference of the phase system, and proportional to the square root of the mobile phase viscosity.[6] This greatly speeds up the investigation of a new phase system for CCC as only one retention test need be performed at a given flow and speed and, then, retention for a range of other operating conditions can

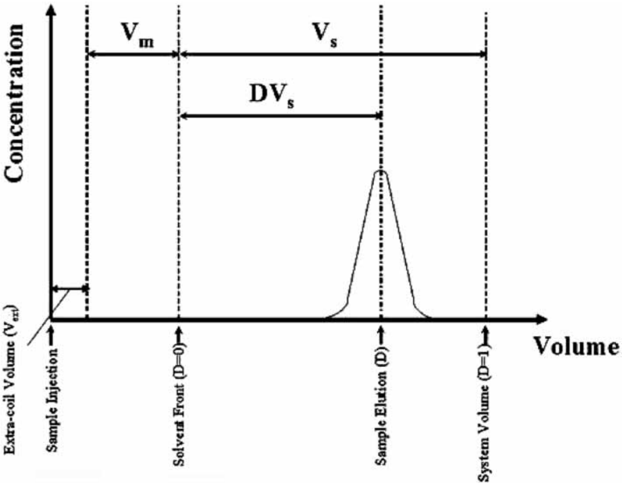

Figure 2. Elution profile of compound with a distribution ratio of D with mobile volume of V_m and stationary phase volume of V_s. Note the higher the stationary phase volume the wider the range between D = 0 and D = 1.

simply be computed. For example, in Figure 3, if a single retention study was performed at 25 mL/min, it would give a retention of about 85% (i.e., $V_s/V_c = 85\%$ and $V_m/V_c = 15\%$) and the "B" gradient could be calculated by drawing a line through (0, 100%) to give a "B" gradient value of 2.86. This could then be used to compute the retention values for any other flows or

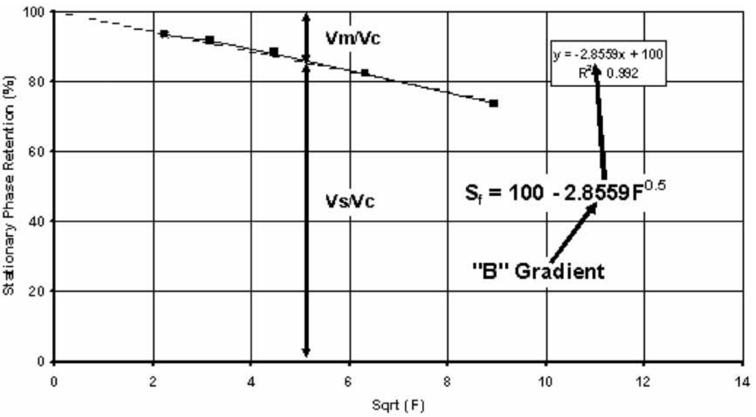

Figure 3. The variation of stationary phase retention plotted against the square root of mobile phase flow, showing how the volumes of mobile phase and stationary phase can be calculated for any flow value either from the graph or using the "B" gradient in the formula.

speeds based on the relationships described above. Figure 4 shows how the "B" gradient changes as scale-up proceeds by increasing the tubing bore from 3.7 mm (square points), through 5.3 mm (diamonds) to 7.7 mm (triangles). If these gradients were plotted against the reciprocal of the tubing cross-sectional area (1/A), they would fall on a straight line passing through the origin. Wood[6] observed that this relationship leads to the conclusion that, for a given flow (and, hence, pressure drop), the volume of stationary phase in the coil remains constant, as illustrated schematically in Figure 5. Table 1 contains the results and "B" gradients of 24 different retention experiments performed by Wood as part of his PhD.[7] These were all performed using a heptane-ethyl acetate-methanol-water (1.4:0.1:0.5:1.0 v/v/v/v) phase system and stainless steel coils of different diameters, but the same length. Figure 6 shows that, when the "B" gradient is plotted against $1/Nd_c^2$, where N is the rotational speed in rpm and d_c the tubing diameter in mm, the points fall on a straight line passing through the origin.

Factors Affecting Sample Processing Rates in CCC

Throughput (W_t) in CCC is a function of the product of sample volume (V_{sv}), and the sample concentration (C) divided by the cycle time (T_c):

$$W_t = V_{sv}C/T_c \tag{4}$$

The sample volume is proportional to the system volume. In practice, depending on the physical properties of the sample suspension, sample volumes can be as high as 30% of column volume, but typically they are 10%:

$$V_{sv} = \text{const} \times V_c \tag{5}$$

and the cycle time is also proportional to the system volume divided by the flow rate (F):

$$T_c = \text{const} \times V_c/F \tag{6}$$

Note the constant in Equation (6) will depend on the value of the distribution ratio (D).

Substituting for V_{sv} and T_c in Equation (4) gives:

$$W_t = \text{const} \times CF \tag{7}$$

The essential factor illustrated here is that, in hydrodynamic CCC, the throughput (W_t) is purely a function of the flow rate and loading concentration and is independent of the length of the column and its volume. For example, doubling the length of the column will increase the resolution by $\sqrt{2}$, but also doubles the cycle time. However this is offset by the fact that twice as much sample can be loaded onto the column. The importance of this is that throughput is essentially independent of column volume and, therefore, the column

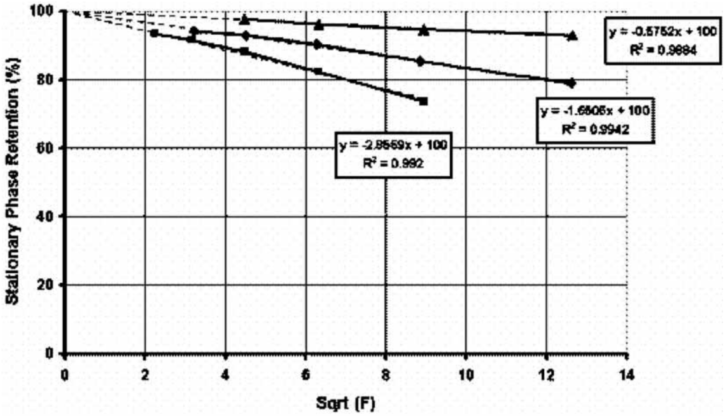

Figure 4. The variation of stationary phase retention plotted against the square root of mobile phase flow, showing how the "B" gradient changes as tubing bore is increased from 3.7 mm (square point), through 5.3 mm (diamonds) to 7.7 mm (triangles).

capacity and length can be chosen to give the appropriate resolution for a given target compound.

Scaling from Analytical through to Process Scale

Table 2 lists the "B" gradients for a range of different "J" type coil planet centrifuges ranging from analytical instruments (PharmaTech—$R = 38$ mm, $d_c = 0.8$ mm) to process scale instruments (Maxi-DE—$R = 300$ mm, $d_c = 10$ mm). Again, the "B" gradients can be plotted against the reciprocal

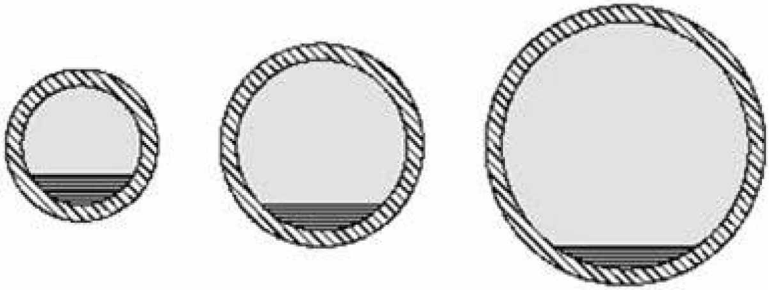

Figure 5. As tubing bore is increased from 3.7 mm (left) 5.3 mm (middle) to 7.7 mm (right) the cross-sectional area of the mobile phase (shaded) and, hence, volume (if the coil lengths are the same) is constant for a given flow.

Table 1. Data from a series of experiments performed by Wood[7] using 3 different coils of the same length and □ value but differing in bore and speed of operation. All experiments were performed in normal phase mode with a heptane-ethyl acetate-methanol-water phase system (1.4:0.1:0.5:1.0, v/v/v/v)

Experiment code	Coil volume (mL)	Speed N (rpm)	"g"	Bore dc (cm)	$1/Nd^2$ $(rpm)^{-1}$ $(cm)^2$	"B" Gradient % $(min/mL)^{0.5}$	R^2 fit	Intercept
4A IMI SS120CoilNM-01	120.5	605	45.01	0.533	0.58182	2.446	0.9950	100
4A IMI SS120CoilNM-02	120.5	603	44.71	0.533	0.58375	2.366	0.9932	101.34
4A IMI SS120CoilNM-03	120.5	803	79.29	0.533	0.43836	1.6506	0.9942	100
4A IMI SS120CoilNM-04	120.5	1005	124.20	0.533	0.35025	1.3195	0.9993	100
4A IMI SS120CoilNM-05	120.5	1206	178.84	0.533	0.29188	1.1536	0.9979	100
4A IMI SS120CoilNM-06	120.5	1420	247.96	0.533	0.24789	0.8864	0.9963	100
4A IMI SS120CoilNM-11	120.5	802	79.09	0.533	0.43691	1.607	0.9977	100
4A IMI SS120CoilNM-13	120.5	802	79.09	0.533	0.43891	1.6113	0.9874	100
4A IMI SS120CoilNM-14	120.5	799	78.50	0.533	0.44055	1.6426	0.9738	100
4A IMI SS120CoilNM-15	120.5	803	79.29	0.533	0.43836	1.6694	0.9785	100
4A IMI SS120CoilNM-16	120.5	1403	242.05	0.533	0.25089	0.8496	0.9893	100
4A IMI SS260CoilNM-17	259.5	604	44.86	0.773	0.27708	0.8929	0.9995	100
4A IMI SS260CoilNM-18	259.5	801	78.89	0.773	0.20893	0.5752	0.9884	100
4A IMI SS260CoilNM-19	259.5	500	30.74	0.773	0.33471	1.154	0.9905	100
4A IMI SS260CoilNM-20	259.5	400	19.67	0.773	0.41839	1.4385	0.9887	100
4A IMI SS260CoilNM-21	259.5	300	11.07	0.773	0.55785	1.6818	0.9963	100
4A IMI SS60CoilNM-22	59.1	601	44.42	0.373	1.19594	3.991	0.9971	100
4A IMI SS60CoilNM-23	59.1	800	78.70	0.373	0.69845	3.0598	0.9978	100
4A IMI SS60CoilNM-24	59.1	1000	122.96	0.373	0.71876	2.0563	0.9985	100
4A IMI SS60CoilNM-25	59.1	1200	177.07	0.373	0.59896	1.9928	0.9961	100
4A IMI SS60CoilNM-26	59.1	1404	242.39	0.373	0.51194	1.6409	0.9930	100
4A IMI SS60CoilNM-27	59.1	1403	242.05	0.373	0.51230	1.7854	0.9994	100
4A IMI SS60CoilNM-30	59.1	1200	177.07	0.373	0.59896	1.8052	0.9955	100
4A IMI SS60CoilNM-31	59.1	1200	177.07	0.373	0.59896	1.7004	0.9868	100

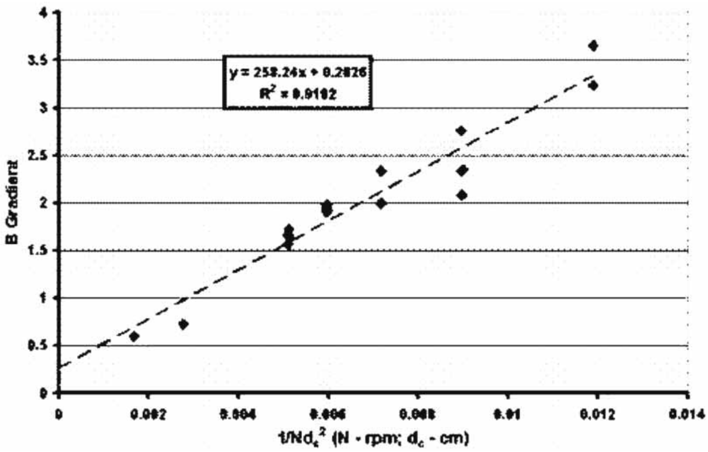

Figure 6. Variation of B gradient for the 24 retention results listed in Table 1 for three different coil diameters operating at a range of different speeds using a Heptane:Ethyl acetate:Methanol:Water phase system (1.4:0.1:0.5:1.0 v/v/v/v).

of the cross-sectional area (1/A) to give a linear relationship at a constant "g" field. A straight line was obtained with the equation:

$$B = 18.22^{*}(1/A) + 0.903 \quad n = 6, \quad r^2 = 0.997 \tag{8}$$

This means that predictions can be made using an analytical scale centrifuge on how process scale centrifuges will perform. For example, sample loading studies will be able to be performed at the analytical scale, greatly reducing the cost and risk of setting up a large scale process.

Table 3 gives the "B" gradients for 4 different scale centrifuges produced by Dynamic Extractions Ltd (DE): Mini-DE (Figure 7a—R = 50 mm, $d_c = 0.8$ mm), Midi-DE (Figure 7b—R = 110 mm, $d_c = 1.6$ mm & 3.7 mm) and the Maxi-DE (Figure 7c—R = 300 mm, $d_c = 10$ mm). Based on linear scale up of cross-sectional area (i.e., with flows of 0.5, 2, 10, and 80 mL/min for the four centrifuge configurations listed in Table 3), stationary

Table 2. The variation of "B" gradient for a range of different centrifuges at different scale and operating at a similar "g" level. The phase system was a heptane-ethyl acetate-methanol-water (1.4:0.1:0.5:1.0, v/v/v/v)

CCC device	Bore	Area	B	Linear scale-up	Extra	Total
Mini-DE	0.8	0.50	−37.01	1×	1×	1×
Midi-DE1	1.6	2.01	−11.22	4×	2.7×	11×
Midi-DE2	3.68	10.64	−3.13	20×	7×	140×
Maxi-DE	10	78.54	−0.82	160×	12.7×	2037×

Table 3. "B" gradients for the Brunel Mini-DE, Midi-DE and Maxi-DE centrifuges showing the linear scale-up based on cross-sectional area and the extra scale-up possible to achieve the same stationary phase retention of 70% column volume at each scale

CCC-device	V_c (mL)	Bore (mm)	A (mm^2)	1/A (mm^{-2})	$1/d^2$ (mm^{-2})	d^2 (mm^2)	R (mm)	N (rpm)	G	B $(mL/min)^{0.5}$	1/B $(mL/min)^{0.5}$
PharmaTech3000	31.2	0.8	0.50	1.989	1.563	0.64	38	1360	79	37.01	0.027
Mini-SERI	21.5	1.6	2.01	0.497	0.391	2.56	50	1224	84	91.5	0.109
Brunel-CCC	95.7	1.6	2.01	0.497	0.391	2.56	110	800	78	11.22	0.089
Midi-DE	928	3.68	10.64	0.094	0.074	13.54	110	800	78	3.13	0.319
Midi-IMI-1	60	3.73	10.93	0.092	0.072	13.91	110	800	78	2.86	0.350
Midi-IMI-2	120	5.3	22.06	0.045	0.036	28.09	110	800	78	1.65	0.606
Midi-IMI-3	260	7.7	46.57	0.021	0.017	59.29	110	800	78	0.58	1.724
Maxi-DE	4750	10	78.54	0.013	0.010	100.00	110	484	78	0.82	1.220

phase volume retentions of 70%, 85%, 90%, and 92.5% were obtained, respectively. Under these circumstances, the linear scale-up based on flow is 1x, 4x, 20x, and 160x, respectively, as shown in Table 3. If, in each centrifuge, the flow is increased to maintain a stationary phase retention of 70%, then much higher flows are theoretically possible with scale-up factors of 1x, 11x, 140x, and 2037x, respectively.

Validation of these predictions has so far been limited to only a 600x scale-up from Mini-DE to Maxi-DE, as shown in Figure 8. This limitation in scale was simply restricted by the flow rates of the pumps available at the time.

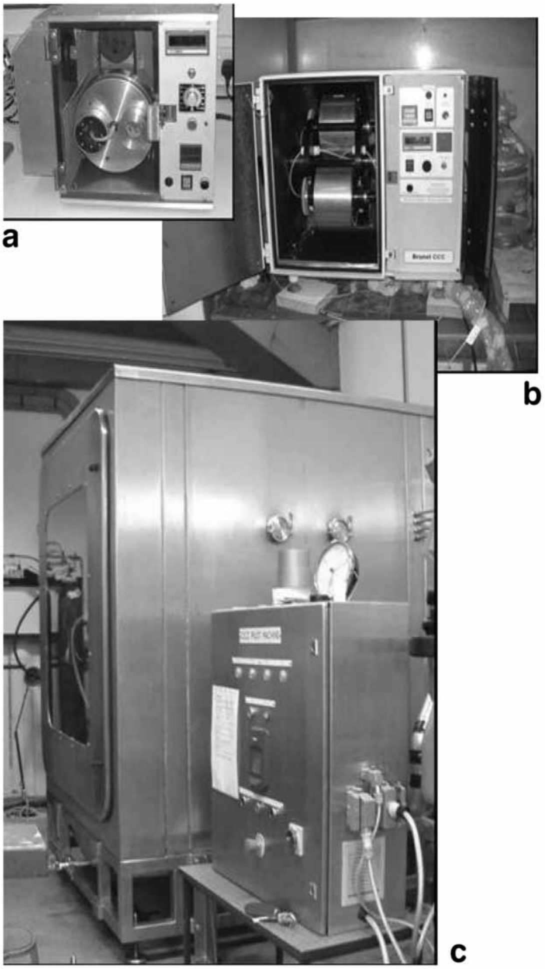

Figure 7. a) the Mini-DE (5–17 mL capacity—mg scale—W35xH35xD42 cm); b) the Midi-DE (0.1–1.2 liter—g scale—W65xH51xD170 cm) and c) the Maxi-DE (5–25 liter—kg scale—W150xH190xD170 cm).[8]

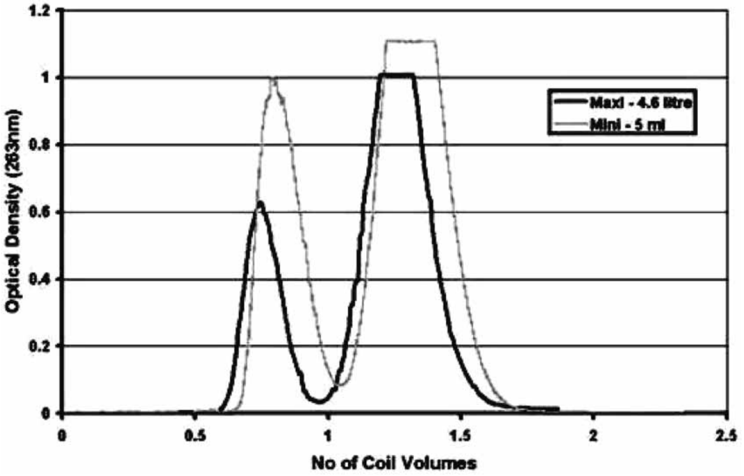

Figure 8. Chromatograms illustrating the scale-up from the Mini-DE centrifuge ($d_c = 0.8$ mm, $V_c = 5$ mL, R = 50 mm, F = 1 mL/min, 26 mg benzyl alcohol and 12 mg p-cresol injected) to the Maxi-DE centrifuge ($d_c = 10$ mm, $V_c = 4.6$ liters, R = 300 mm, F = 600 mL/min, 24000 mg benzyl alcohol and 11500 mg p-cresol injected). Sample mixture: 52.5 mg/mL benzyl alcohol and 25 mg/mL p-cresol. Sample loading 10% column volume. Phase system: heptane-ethyl acetate-methanol-water (1.4:0.1:0.5:1.0, v/v/v/v).

The chromatograms shown in Figure 8 are plotted against the number of coil volumes to get them onto a comparable scale. While it can be seen that the chromatograms are similar, the Maxi-DE gives a higher resolution (Rs = 1.46 compared to Rs = 1.37 for Mini-DE), showing that the Maxi-DE flow could be increased still further to achieve comparable resolution.

CONCLUSION

This research has shown that, based on stationary phase volume retention alone, scale-up factors can be achieved that are greater than linear scale-up, based on tubing cross-sectional area. Results have been presented that illustrate that a scale-up of 600x can be achieved when the linear scale-up based on cross-sectional area is only 160x. Theoretically, scale-up factors of 2700x are feasible between the Mini-DE and Maxi-DE. While sample loading studies have been performed on a number of target compounds at analytical scale and translated to process scale, the results have been retained by the industries concerned. Sample loading studies are currently being performed using the model separation system shown in Figure 9 and these results will be published shortly.

While the chromatogram results given in this paper are extremely promising, they are still preliminary. Further research is required to verify that the same sample loading proportions as described in Equation (5) are feasible as column length is increased. If this is verified, then the scale-up of CCC will become very powerful as it is not pressure limited and resolution will be able to be tailor-made to each separation system.

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