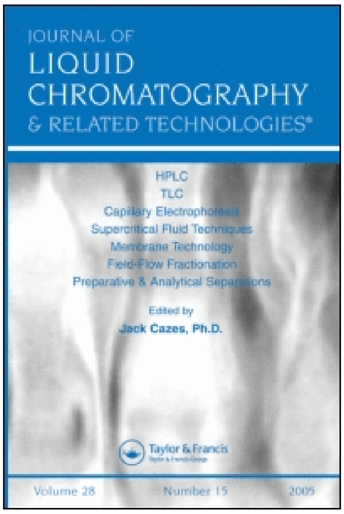

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Resolution in CCC: The Effect of Operating Conditions and Phase System Properties on Scale-Up

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Resolution in CCC: The Effect of Operating Conditions and Phase System Properties on Scale-Up

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ABSTRACT

Being able to accurately predict the eluted volume of a substance with a known distribution ratio will depend on accurately knowing the retention volume of the stationary phase. Sample resolution depends on a number of factors that are not so easy to predict: the properties of the phase systems, the number of mixing and settling cycles per unit time, the rate of mass transfer during mixing, and the quality of mixing between the phase systems. The extent of mixing between the phases will, in turn, depend on the flow rate of the mobile phase and the "g" field acting across the stratified phases within the coiled tubing. A systematic study is made of how sample resolution changes with the key operating variables associated with scale-up: the mobile phase flow, the bore of the tubing, and the rotational speed. It shows how the commonly accepted characteristic, good

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resolution at low flow and poor resolution at high flow, slowly changes as tubing bore increases to one of poor resolution at low flow rising to optimum resolution at high flow and a slow decline in resolution at very high flow. Furthermore, it goes on to show that, as phase system physical properties change when moving from hydrophobic phase systems to more polar hydrophilic ones, the optimum resolution remains in a similar speed and flow range. It also shows that the key variable for scale-up, the throughput of sample in kg/hour, increases significantly as mobile phase flow increases, provided the rotational speeds are high enough. Optimum throughput has not yet been reached, which is extremely promising for realising process scale CCC.

Key Words: Countercurrent chromatography; Resolution; Scaling-up; Throughput; Flow rate effects; Tubing bore effects.

INTRODUCTION

This paper builds on completed studies[1] that show that stationary phase retention can be predicted for a given phase system once the mobile phase viscosity, the density difference of the phase systems, the tubing bore, and the acceleration field are known. Wood[2] has shown that, for scale-up, as the tubing bore increases, the volume of the mobile phase remains constant. This leads to much higher stationary phase retention values for large bore tubing than for small bore tubing. How will this affect sample resolution? Will these high volume ratios in favor of the stationary phase lead to poor mass transfer between the phases and, hence, poor resolution as the process is scaled up?

This paper tries to answer these questions. It describes the systematic study of how the resolution between two known substances, with different distribution ratios, changes with the flow rate of the mobile phase and the speed of rotation of the coil planet centrifuge, in reverse phase mode, using three identical helical stainless steel coils differing only in their internal diameter or bore.

EXPERIMENTAL

Apparatus

A "J" type coil planet centrifuge and control system [Brunel CCC supplied by Romulus Technology (Space) Ltd] was used, with a rotor radius $R = 110\,\text{mm}$. This has been described in detail elsewhere,[3] but has since been upgraded with a quieter drive system capable of controlling rotational speed in the range 200–1200 rpm and a temperature control system controlling temperature between 25 and 35°C. In this study, the standard bobbins were removed from the rotor (Fig. 1) and replaced by two experimental bobbins. One bobbin contained two coils in a doubly wound helix (one between the other). The coils were 3.73 mm and 5.33 mm bore, and 4.76 mm and 6.35 mm outside diameter, respectively. The second bobbin had a single 7.73 mm bore coil of 9.53 mm outside diameter. All three coils were wound using stainless steel tubing of circular cross-section and had the same number of loops (10), the same pitch (11.5 mm), the same length ($L = 5.66$ m) and the same β value ($\beta = 0.82$). The first coil has a measured volume of 59.1 mL, the second 120.5 mL, and the third 259.5 mL. For notational purposes, the coils will be referred to as the 60, 120, and 260 mL coils. Their diameters were chosen so that the cross-sectional area approximately doubled with each incremental increase. In this way, it was possible to devise a simple flow scheme, as shown in Table 1.

To distinguish these experimental coils from others, they will be known as the IMI (EPSRC—Innovative Manufacturing Initiative) coils, which

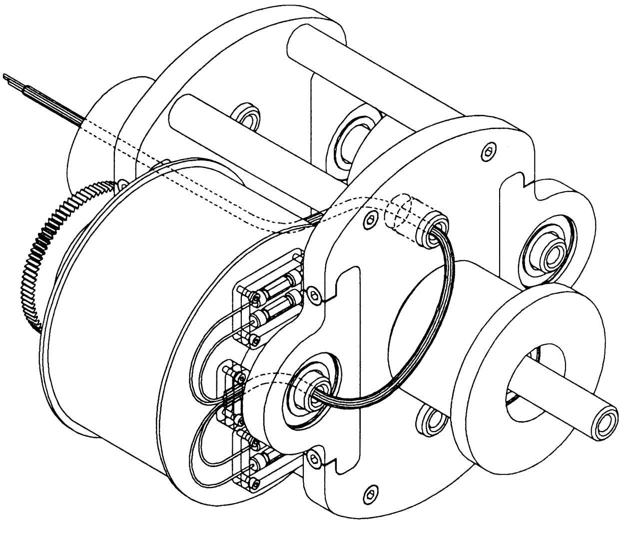

Figure 1. Detail of the two bobbin rotor ($R = 110$ mm) of the Brunel CCC (only one bobbin shown). The standard bobbins were replaced by two experimental bobbins both containing 10 loop fixed length ($L = 5.656$ m), fixed pitch (11.5 mm), stainless steel helical coils at a fixed β value of 0.82: one with two coils of 3.73 mm and 5.33 mm bore and the other with a single coil of 7.73 mm bore.

Table 1. The mobile phase flow scheme for the different bore tubing in order to maintain a normalised flow rate (F/A_c).

Bore (d_c, mm)	Area (A_c, mm^2)	Mobile phase flow (mL/min)			
3.73	11.0	5	10	20	40
5.33	22.3	10	20	40	80
7.73	46.9	20	40	80	160
F/A_c (approx.)		0.45	0.9	1.8	3.6

acknowledge their funding source. Normally, coils are wound in a multi-layer fashion on a bobbin where the helical pitch is dictated by the outside diameter of the tubing and the bobbin radius on which it is wound. Pitch, therefore, changes with β value. Great care has been taken in their manufacture to ensure, in this study, that the helical pitch and β value are constant.

Phase Systems

Three different heptane/ethyl acetate/methanol/water phase systems have been used, ranging from a hydrophobic phase system (4A), an intermediate (4B), through to a hydrophilic phase system (4C). The physical properties of these phase systems are listed in Table 2. All tests were conducted in the reverse phase mode with the aqueous phase mobile. Note that the mobile phase system viscosity remained constant as the phase system changed, but that density difference and interfacial tension reduced significantly as hydrophilicity increased.

Sample System

Uracil was used as a $K_D = 0$ marker peak. The chosen test system was benzyl alcohol (Sigma Cat. No. B-1042) and para-cresol (Aldrich Cat. No. C8, 575-1). Samples were made up in 100 mL volumetric flasks. A stock solution of uracil was prepared first in mobile phase to a concentration of 1.25 mg/mL. 500 mg of para-cresol was placed in the volumetric flask and dissolved in approximately 80 mL of mobile phase pumping from head to tail. Benzyl alcohol, 1 mL, was added to the flask followed by 4 mL of uracil stock solution. The solution was mixed thoroughly and then topped up to exactly 100 mL to give a final sample concentration of 0.05 mg/mL uracil, 5 mg/mL para-cresol, and 10.42 mg/mL benzyl alcohol. The sample was stored at room temperature in the dark. Twenty-mL aliquots were transferred to glass

Table 2. The physical properties of the heptane : ethyl acetate : methanol : water phase systems used at 30°C.

System	Composition H : EA : M : W	ρ_l (kg/m^3)	ρ_u (kg/m^3)	$\rho_l - \rho_u$ (kg/m^3)	μ_l Pa s (N s/m^2)	μ_u Pa s (N s/m^2)	τ_i (mNm)
4A	1.4 : 0.1 : 0.5 : 1.0	947	679	268	0.00136	0.00036	17.9
4B	1.4 : 0.6 : 1.0 : 1.0	938	708	230	0.00135	0.00035	6.2
4C	1.4 : 4.5 : 1.0 : 1.0	931	833	98	0.00135	0.00042	1.0

Note: l, lower phase; u, upper phase; ρ, density; μ, viscosity; τ_i, interfacial tension.

universals before use. Samples were injected using a Rheodyne injection port with a loop volume arranged to be 1% of the coil volume being used (V_c).

The above sample system was used for both the 4A and 4B phase systems of Table 2. The sample system for 4C was different, consisting of a mixture of 3,4-dihydroxy benzoic acid (10 mg/mL) and 3,4,5-trihydroxy benzoic acid (10 mg/mL) made up in mobile phase.

Experimental Procedure for Obtaining a Chromatogram

The experimental set up is shown in Fig. 2. Solvent reservoirs are maintained at a controlled temperature of 30°C in a Grant water bath (Grant Instruments, Cambridge). The following auxiliary equipment is required: a constant flow pump for the mobile phase (Dynamax SD-1, supplied by Anachem, Luton, UK), a pump for the stationary phase (Gilson model 302, Anachem, Luton), a sample injection loop (Rheodyne, Cotati, California), upstream and down stream low dead volume switching valves (made under contract by Omnifit, Cambridge, UK), a whitespot nitrogen supply (BO High purity 052 Air Products Ltd, Southall, UK) for pumping out the coil contents, a chiller for the coil planet centrifuge temperature control system (Grant RC 1400G chiller, Grant Instruments, Cambridge), a flow through spectrophotometer (Gilson UV/VIS 151, supplied by Anachem, Luton, UK), two measuring cylinders of the appropriate volume, and a pressure release valve.

The coil system was initially filled with the organic stationary phase with the upstream switching valve switched to pump from the stationary phase reservoir. The coil was slowly rotated at 200 rpm with the head at the exit to screw any air bubbles to the exit. Then, with the upstream switching valve switched to pump mobile phase, the minimum flow corresponding to the minimum normalised flow rate for the coil being used (see Table 1) was set. The downstream switching valve was initially set to bypass the spectrophotometer. The pump was then switched on synchronously with a stopwatch. A short period was allowed to establish hydrodynamic equilibrium and to collect any displaced volume of stationary phase. The down stream switching valve was then switched to pass the outlet flow through the spectrophotometer and, via a back pressure restriction, on to a second measuring cylinder. The back pressure restriction was used to prevent air from coming out of solution in the mobile phase, which could interfere with the spectrophotometer reading. In the meantime, a sample would be loaded into the sample loop and injected at a noted time. Note that the optical density output from the spectrophotometer was connected, via a data logger (Picolog for Windows 32 bits, Pico Technology Limited), to a computer for electronic data capture. An event

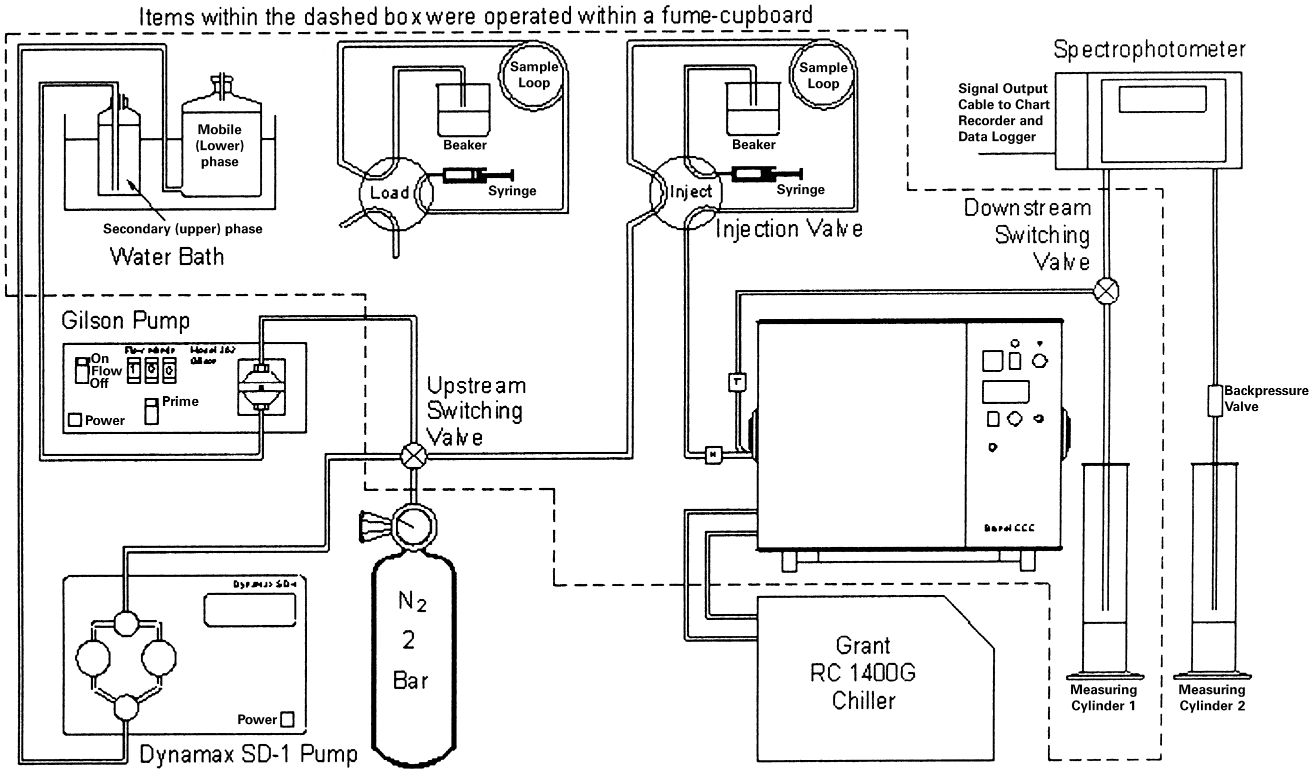

Figure 2. Schematic of experimental set up.

marker would be activated on the spectrophotometer to give a downward mark on the chromatogram at the point of injection.

The elution of the sample could be monitored on the computer screen and second and third injections of the sample made. It was found that repeat chromatograms gave such reproducible results that repeat injections were only made if, for any reason, there had been a poor chromatogram.

Once a chromatogram had been recorded for a given flow rate, then the mobile flow rate would be doubled at a noted time and the above procedure repeated. A typical chromatogram, taken during this procedure, is shown in Fig. 3. This process of flow doubling would then be repeated until the maximum normalised flow of $F/A_c = 3.6$ was reached (see Table 1). Note that the normalised flow has dimensions of m/min and represents the mobile phase flow rate for zero retention of stationary phase. The actual linear flow rate (v) can be calculated by multiplying by $1/(1 - S_f)$ where S_f is the retention of stationary phase expressed as a proportion of coil volume ($S_f = V_s/V_c$):

$$v = \frac{F}{A_c(1 - S_f)} \tag{1}$$

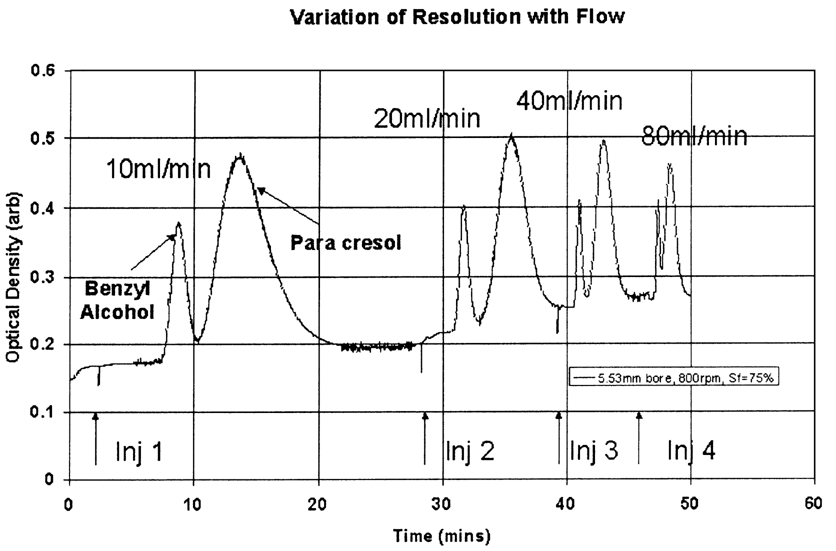

Figure 3. Typical chromatogram taken at $\omega = 800$ rpm for the $d_c = 5.53$ mm bore coil of volume $V_c = 120$ mL showing how resolution changes during the flow doubling protocol.

Experimental Procedure for Measuring Retention and Resolution from Chromatograms

A typical chromatogram showing the uracil marker peak is shown in Fig. 4. Injection of the sample mixture is indicated by a downward marker on the trace (arrowed). The uracil peak elutes first, followed by the benzyl alcohol ($K_D = 0.33$) and para-cresol ($K_D = 1.13$) peaks. The elution times (t_0, t_1, and t_2) and baseline peak widths (w_0, w_1, and w_2) of all the peaks are measured from the trace and entered on a spreadsheet from which retention, resolution, and distribution ratio data can be calculated as follows:

$$\mathrm{Rs}_{12} = \frac{2(t_2 - t_1)}{w_1 + w_2} \tag{2}$$

$$V_{\mathrm{dis}} = \frac{t_0}{F} \tag{3}$$

where t_0 is the elution time of the uracil solvent front marker peak, F the mobile phase flow, and V_{dis} is the calculated displaced volume.

$$V_m = V_{\mathrm{dis}} - V_{\mathrm{ext}} \tag{4}$$

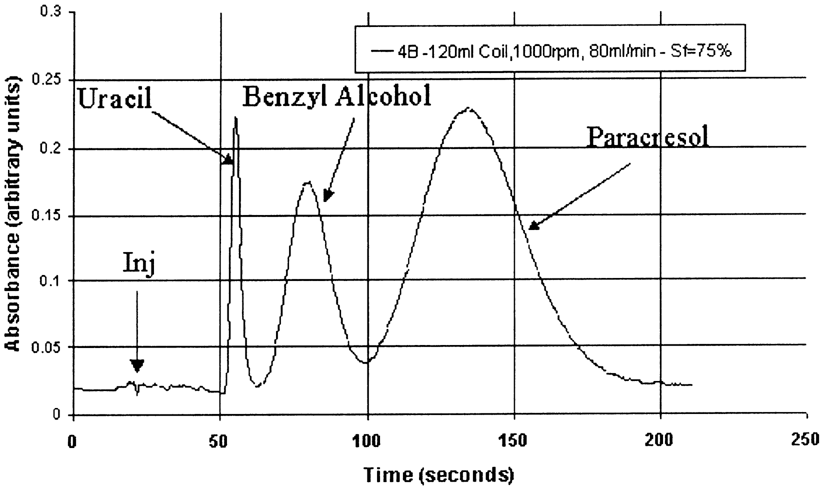

Figure 4. Typical chromatogram taken at $\omega = 1000$ rpm and $F = 80$ mL/min for the $d_c = 5.53$ mm bore coil of volume $V_c = 120$ mL, showing point of injection, the uracil marker peak and the elution of benzyl alcohol and para-cresol used for measuring resolution.

where V_{ext} is the extra-coil volume.[4] Stationary phase volume (V_s) and percentage stationary phase retention (S_f) can then be obtained as follows:

$$V_s = V_c - V_m \tag{5}$$

$$S_f = 100\left(\frac{V_s}{V_c}\right) \tag{6}$$

The percentage stationary phase retention for the phase systems tests can be predicted theoretically and has been extensively reported elsewhere.[1]

$$S_f = 100 - BF^{1/2} \tag{7}$$

where the slope B has been shown to be inversely proportional to the bore of the tubing.[1]

RESULTS AND DISCUSSION

The variation of resolution with mobile phase flow for the 3.73 mm bore coil is given in Fig. 5 for four different rotational speeds ranging from 600–1200 rpm. At the lowest speed, when the retention of the stationary

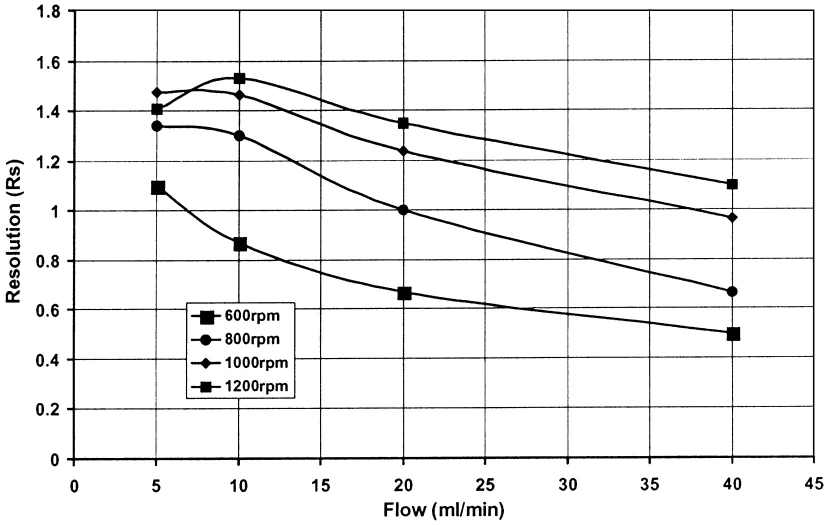

Figure 5. Variation of resolution (Rs) with mobile phase flow for the 60 mL coil ($d_c = 3.73$ mm) and 4A phase system for a range of rotational speeds.

phases is lowest,[1] the resolution follows the classical form of analytical CCC, where the resolution is highest at low flow and gradually declines as flow is increased. As the rotational speed increases, the retention of the stationary phase becomes higher,[1] and the mixing and settling rate (synchronous with rotational speed) becomes higher. This results in increased resolution in the higher speed range, but it can be seen that, at the lowest flow (5 mL/min), and at the lowest rotational speed (600 rpm), the resolution actually begins to fall.

This trend continues as the bore of the tubing increases. For the 5.53 mm bore tubing (Fig. 6), resolution at all speeds, ranging from 600 to 1200 rpm, is lower at low flow, increases to a maximum, and then starts to fall off again. The optimum resolution moves to a higher mobile phase flow rate as rotational speed increases (18 mL/min at 600 rpm, 21 mL/min at 800 rpm, 38 mL/min at 1000 rpm, and 47 mL/min at 1200 rpm). For the 7.73 mm bore tubing (Fig. 7), a similar pattern is seen with even lower resolutions at low mobile phase flow rates. The optimum resolutions also move to higher flow rates as the speed of rotation increases (24 mL/min at 600 rpm, 44 mL/min at 800 rpm, 70 mL/min at 1000 rpm, and 88 mL/min at 1200 rpm). Plotting resolution against rotational speed (Fig. 8) clearly shows how at low flow resolution decreases as rotational speed (and, hence, stationary phase retention) increases and how, at high flow, the opposite is true.

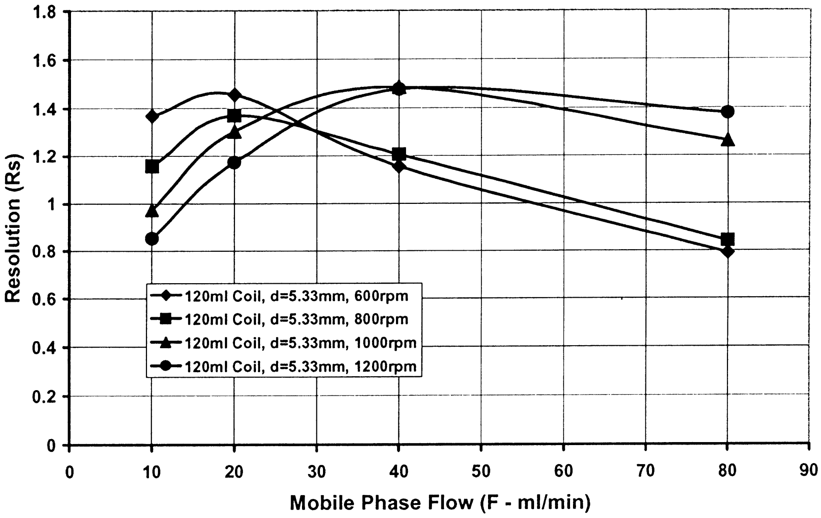

Figure 6. Variation of resolution (Rs) with mobile phase flow for the 120 mL coil ($d_c = 5.33$ mm) and 4A phase system for a range of rotational speeds.

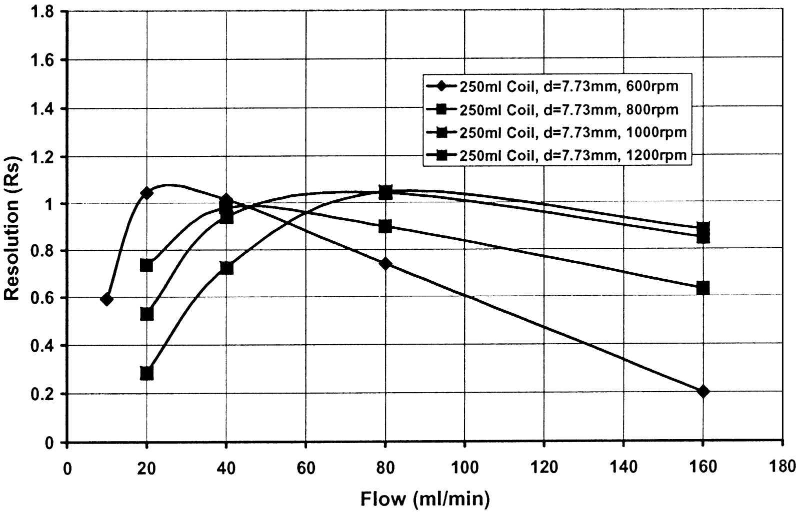

Figure 7. Variation of resolution (Rs) with mobile phase flow for the 260 mL coil ($d_c = 7.73$ mm) and 4A phase system for a range of rotational speeds.

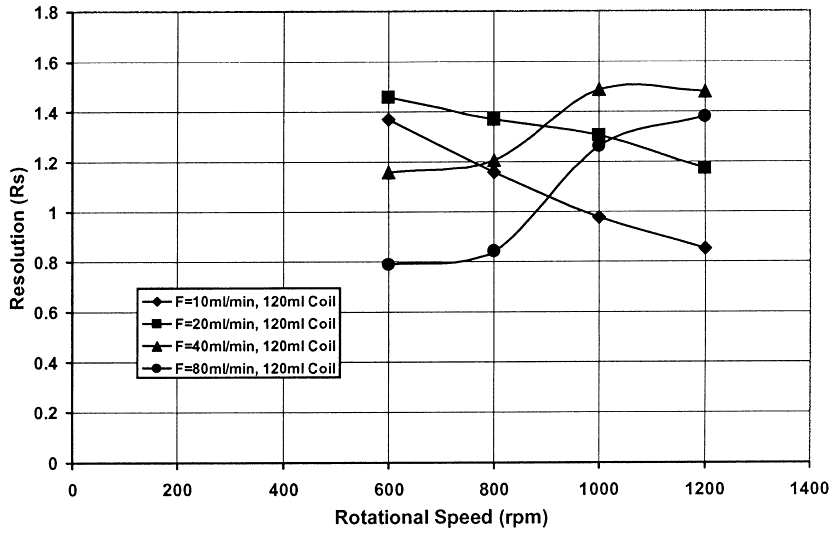

Figure 8. Variation of resolution (Rs) with rotational speed for the 120 mL coil ($d_c = 5.53$ mm) and 4A phase system for a range of mobile phase flow rates.

Wood et al.[1] has shown that the coil planet centrifuge behaves like a constant pressure pump and that the volume of mobile phase remains constant[2] despite the tubing bore increasing. The effect of this is that stationary phase retention increases significantly with tubing bore. Wood et al.[1] and Wood[2] has quantified this for normal phase flow (Table 3). He showed that the slope (*B*) of the stationary phase retention/square root of flow curve [Eq. (7)] was proportional to the inverse of the cross-sectional area of the tubing. Table 3 summarises his results for normal phase flow at 800 rpm, showing that the slope (*B*) significantly decreases as the bore increases. The stationary phase retention, volume of mobile phase, and linear flow are also given for the specific flow rate of 40 mL/min. It can be seen that, as bore increases, the percentage of stationary phase increases. The most striking observation made by Wood[2] was that the volume of mobile phase for a given flow remained approximately constant. This supported his hypothesis that the coil planet centrifuge was working like a constant pressure pump[1] and that the linear velocity of the mobile phase for a given flow rate also remained approximately constant. As mobile phase flow increases, the stationary phase volume decreases (with a proportional increase in mobile phase volume); this has two effects: (i) a reduction in time for mass transfer to take place and (ii) an increase in surface area leading to an increase in mass transfer. The fact that resolution increases at high mobile phase flow and high rotational speed must mean that the increased flow induces even more efficient mixing, which is more than able to compensate for the faster transit times. This, of course, is very good news for scaling-up of the process. The resolution results plotted in Figs. 6 and 8 have been used to calculate run times. These run times have been adjusted, assuming resolution is proportional to the square root of the length of the tubing[6] and daily throughput calculated to give a resolution of $Rs = 1.5$. The results are plotted in Fig. 9 and show, for low speeds, that there is an optimum

Table 3. The variation of slope B [Eq. (6)] with tubing bore/area showing in addition, for a specific flow rate of 40 mL/min, how the retention of the stationary phase (S_f) increases with tubing bore (*d*) whole mobile phase volume (V_m) and mobile phase linear flow (*v*) remain substantially constant (4A Phase system at 800 rpm after Wood et al.[1]).

Bore (*d*, mm)	Area (A, mm^2)	Slope (min/mL)$^{1/2}$	Corr. coeff. (R^2)	Retention S_f (%)	Mobile vol. V_m (mL)	Linear velocity *v* (m/sec)
3.73	10.93	2.856	0.992	82.2	11.2	0.34
5.33	22.31	1.651	0.994	90.3	11.7	0.31
7.73	46.93	0.575	0.988	96.2	10.0	0.37

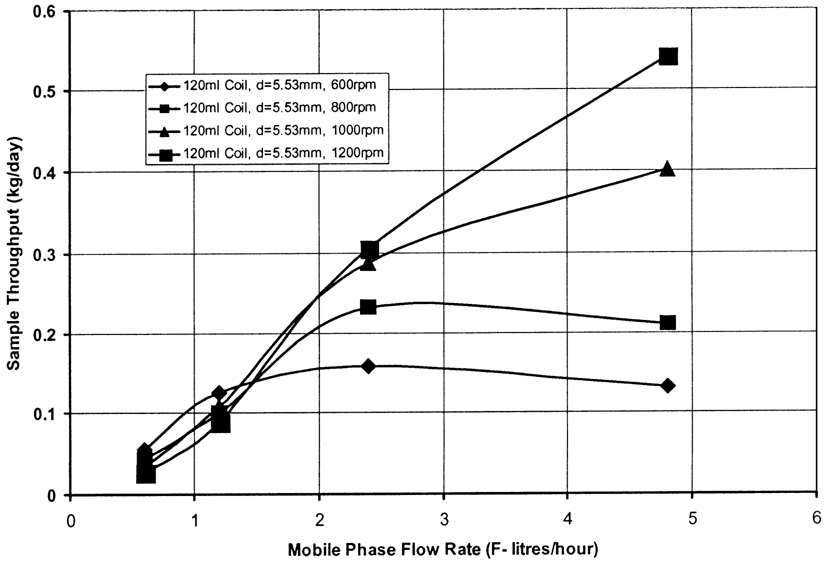

Figure 9. Variation of sample throughput with mobile phase flow rate normalised to give a resolution (Rs = 1.5).

mobile phase flow of 2.4 L/hour for 600 rpm and 2.8 L/hour for 800 rpm. The optimum for both 1000 rpm and 1200 rpm has not yet been reached and may be considerably greater than 4.8 L/hour, particularly at 1200 rpm.

The above results, for the 4A phase system, are very encouraging for scale-up to the pilot scale and beyond. But, can lower interfacial tension phase systems be operated in the "J" type coil planet centrifuge with the same degree of confidence? Figure 10 is a plot of the variation of resolution with mobile phase flow for an intermediate 4B phase system (see Table 2). It can be seen that there are quantitatively similar results, with poor resolution at low flow increasing to a maximum as flow increases and slowly decreasing at high flow. For a low interfacial tension phase system (4C—Table 2) with a different model system, there is also a similar characteristic (Fig. 11). Surprisingly, at 1000 rpm, the optimum flow for all three phase systems is between 40 and 50 mL/min.

CONCLUSIONS

Previous work has shown[2] that stationary phase volume increases with scale-up and increasing the bore of the tubing. However, it was not known

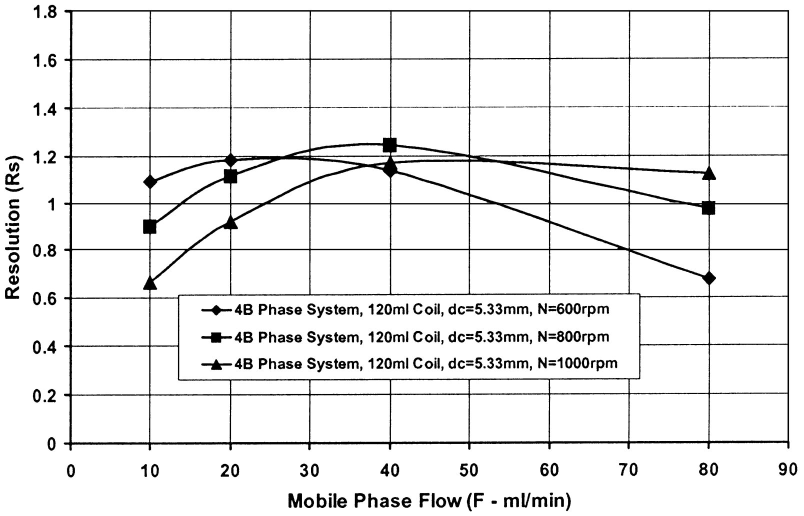

Figure 10. Variation of resolution (Rs) with mobile phase flow for the 120 mL coil ($d_c = 5.33$ mm) and 4B phase system for a range of rotational speeds.

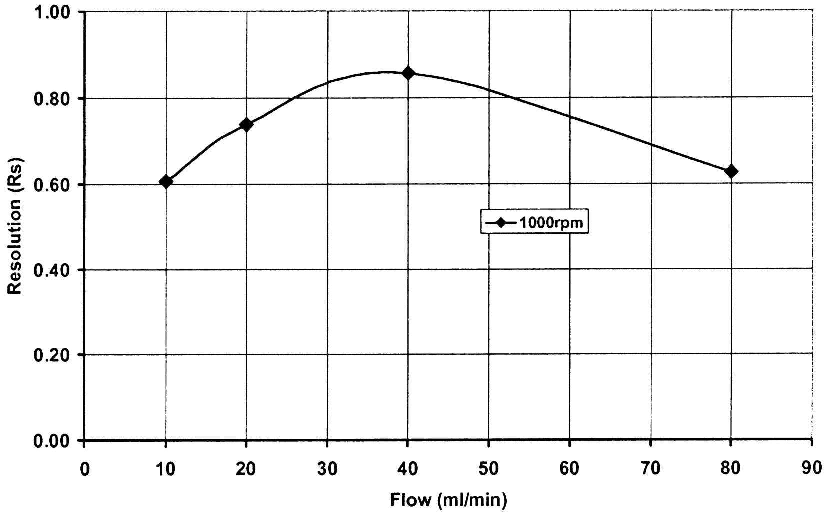

Figure 11. Variation of resolution (Rs) with mobile phase flow for the 120 mL coil ($d_c = 5.33$ mm) and 4C phase system at 1000 rpm.

what would happen to resolution. It was anticipated that resolution could become worse as the tubing bore increased, due to the ratio of the bulk volume to interfacial area increasing. This was found to happen at low flow, but the reverse was true at high flow. The resolution of the process actually increased with mobile phase flow until an optimum occurred and then there was a steady decrease. It also increased with rotational speed at high flows, but not at low flows. Furthermore, this observation was not restricted to high interfacial tension phase systems. The same behavior was also true for intermediate and low interfacial tension phase systems.

However, stationary phase retention has been found to behave quite predictably and can be simply modelled.[2,7] This may not be true of the mixing and settling behaviors that determine the resolution. It is interesting to note the sigmoid shapes of the resolution-speed curves at high mobile phase flow rates (Fig. 8). They show that there appears to be a flow transition between 800 and 1000 rpm, above which there is significantly better mass transfer and mixing.

These results were performed with variable retention. In other words, the coils were initially filled with the phase intended to be the stationary phase and each mobile phase flow was allowed to establish its own stationary phase equilibrium and displace the appropriate amount of stationary phase. This means that the stationary phase retention is a variable, which will also influence the resolution (higher stationary phase retention leading to higher resolution). As these results are so promising for scale-up, it is intended to repeat selected tests at a constant retention of stationary phase. Resolution will then give a direct measure of mixing efficiency.

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