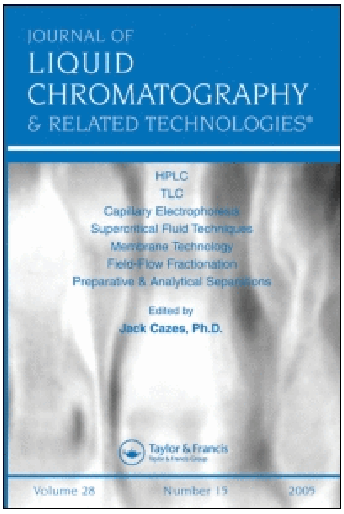

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

THE RELATIONSHIP BETWEEN RETENTION, LINEAR FLOW, AND DENSITY DIFFERENCE IN COUNTERCURRENT CHROMATOGRAPHY

I. A. Sutherland[a]; Q. Du[b]; P. Wood[a]
[a] Brunel Institute for Bioengineering, Brunel University, Uxbridge, UK [b] Department of Chemical Engineering, Zhejiang University, Hangzhou, Zhejiang, P. R. China

To cite this Article Sutherland, I. A. , Du, Q. and Wood, P.(2001) 'THE RELATIONSHIP BETWEEN RETENTION, LINEAR FLOW, AND DENSITY DIFFERENCE IN COUNTERCURRENT CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 24: 11, 1669 — 1683
To link to this Article: DOI: 10.1081/JLC-100104370
URL: http://dx.doi.org/10.1081/JLC-100104370

J. LIQ. CHROM. & REL. TECHNOL., 24(11&12), 1669–1683 (2001)

STUDIES OF LIQUID-LIQUID CIRCULATION

THE RELATIONSHIP BETWEEN RETENTION, LINEAR FLOW, AND DENSITY DIFFERENCE IN COUNTERCURRENT CHROMATOGRAPHY

I. A. Sutherland,[1,*] Q. Du,[2] and P. Wood[1]

[1]Brunel Institute for Bioengineering, Brunel University,
Uxbridge UB8 3PH, UK
[2] Department of Chemical Engineering, Zhejiang
University, Hangzhou, Zhejiang 310027, P. R. China

ABSTRACT

Countercurrent Chromatography is a high-resolution, liquid-liquid chromatography process which is generic in nature and can be used with a wide range of solvent systems. This paper shows there are simple relationships between the operational variables affecting CCC, which lead to a clearer understanding of how the process works. In the future, it will be easier to predict phase system behaviour from the physical characteristics of the phase system and the operating conditions of the coil planet centrifuge and, so, simplify the process of getting started with a new phase system.

---

*Corresponding author.

INTRODUCTION

Countercurrent Chromatography is a high-resolution, liquid-liquid chromatography process which is generic in nature and capable of use with an extremely wide variety of solvent phase systems. In CCC, there are two critical aspects that ensure its success as a high-resolution purification process: retention and resolution. It is essential that the solvent phase intended to be the stationary phase is "retained" relative to the mobile phase flow. If there is such "retention," then it is important that there is adequate mass transfer between these two phases so that a sample containing mixtures of components with differing partition coefficients can be adequately resolved.

This paper concentrates on retention, with the aim of establishing a relationship between retention behaviour and both the physical properties of the phase systems themselves and the operating characteristics/geometry of the coil planet centrifuge. Once such relationships are known, the CCC process will be less shrouded in mystery and easier to use for new chromatographers.

There has been some controversy in the literature about how the physical properties of the phase systems affect stationary phase retention (S_f). Early work by Berthod[1] suggested that the percentage retention of stationary phase was directly proportional to the density difference between the two phases, but later work by two groups has called this into question. Maryutina et al.[2] "have not managed to find any specific correlation between the densities of two liquid phases, their viscosities and the S_f factor." However, they were working at an extremely low speed (i.e. 450 rpm) where retention could be unreliable. They were also using a purpose built CPC with a R = 85 mm, r = 35 mm, PTFE tubing id = 1.5 mm, 0.75 mm wall, a speed of 350-500 rpm, and flow 1.1 mL/min. This considerably limited the "g" levels they could achieve, and suggests that they were working too near the critical point where retention can become unstable. Fedotov et al.,[3] using a Pharmatech CCC2000 instrument (R = 63.5 mm, d = 1.85 mm, V_c = 24mL, β = 0.38-0.53, flow 1 mL/min, and speed 400-1000 rpm), used a "n-decane : DBSA:water" system where they could change the interfacial tension of the phase system by changing the concentration of the surfactant while densities and viscosities remained substantially unchanged. The retentions they were measuring were between 15-50%, which suggests that they also were working at sub-optimal (quasi-unstable) conditions.

This paper builds on the observations made by Wood in his PhD thesis; that the coil planet centrifuge can be treated like a constant pressure head centrifugal pump[4] and the retention observations made by Du et al.,[5] who have shown that there is a linear relationship between the square root of the mobile phase flow rate and the stationary phase retention. Wood postulates that the piezometric pressures produced by the mobile phase flow balance are those produced by the natural hydrostatic and Archimedean action of the coil planet centrifuge,[6] leading to retention of the stationary phase. He emphasized that, for a given set of operating conditions, the pressure drop across the coils would remain constant, regardless of which phase was the mobile phase; and that increases in mobile phase flow would be compensated by increases in mobile phase cross-sectional area. He felt this could explain the observations of Du et al[5] who showed, for reverse phase operation where the lower phase is mobile, that the slope of the linear relationship is negative, i.e., as the mobile phase flow is increased the retention of stationary phase decreases. The equation of their observed relationships was as follows:

$$S_f = A - B\sqrt{F_m} \qquad (1)$$

where S_f is the stationary phase retention, F_m the mobile phase flow rate, A the intercept on the y axis, and B the gradient of the linear relationship.

This paper takes a closer look at the work of Du et al.,[5] and attempts to put their observations into a theoretical framework. The approach uses 3 phase systems of differing density difference, operating in reverse phase mode with the lower phase mobile at 3 levels of "g" field.

THEORY

If two non-miscible phases are introduced to opposite ends of a coil in countercurrent chromatography, they will distribute according the respective actions of Archimedean and Hydrostatic forces when the coil is subjected to coil planet centrifugation. It has been shown,[6] that the upper phase always moves to the head end of the coil and the heavy phase always moves toward the tail when the Archimedean and Hydrostatic forces are acting in the same direction, but this hydrodynamic behavior can be reversed for lower interfacial tension and low density difference phase systems when these forces oppose one another. Wood[4] further postulates that when the flow is introduced, the piezometric forces due to the flow of the mobile phase will balance the Archimedean and hydrostatic forces. He goes on to show that if the mobile phase is assumed to be laminar then the flow could be represented by the Hagen-Poiseuille equation for laminar flow as follows:

$$F_{21} = \frac{\pi {r_m}^4}{8\mu_m L}\left(P_2 - P_1\right) \qquad \text{(Hagen-Poiseuille)} \qquad (2)$$

where r_m = radius of circular area occupied by mobile phase

μ_m = Dynamic viscosity of the mobile phase

L = the length of tubing in the coil between positions 1 and 2

P_1 = the piezometric pressure at position 1 (Outlet end of coil)
P_2 = the piezometric pressure at position 2 (Inlet end of coil)
$F_{21} = F_m$ = the mobile flow rate from position 2 to position 1 as $P_2 > P_1$

Rearranging
$$F_m = Cr_m^{\ 4} \text{ or } \frac{F_m}{C} = r_m^{\ 4} \tag{3}$$

Where
$$C = \frac{\pi \Delta P}{8\mu_m L} \tag{4}$$

The Hagen-Poiseuille equation is normally used for laminar flow through pipes, where generally the pipe diameter, viscosity, and length are constant for a given experiment, and then the flow is proportional to pressure drop. The faster the flow, the higher the pressure drop. In this case, however, the coil planet centrifuge is acting like a constant pressure head pump where the pressure drop across the coils is set by the "g" field and the density difference between the phase systems (i.e., Archimedes & Hydrostatic Forces and not so much by the external mobile phase pump, which is pumping the phases in a direction they want to go in naturally) and, if these are constant, then it is assumed that the pressure drop will be constant. This is possible, because there is stratified flow where an increase in flow of the mobile phase is compensated by the change in cross-sectional area of the mobile phase flow. Hence, for a given geometry and a given phase system the pressure drop across the coils remains substantially constant as flow increases, regardless of which phase is the mobile phase.

In countercurrent chromatography, the flow rate (F_m) and the volume of the mobile phase (V_m) are easily measured. The coil system is initially filled with the phase intended to be the stationary phase - in reverse phase operation this will be the upper organic phase for this system. A measuring cylinder is then placed at the outlet and the coil planet centrifuge is set to rotate at a given speed (N) and the pump set to a given flow rate (F_m). The total volume collected in a given time will give the flow rate, while the amount of stationary phase displaced (after subtracting the volume of the inlet outlet leads) will give the volume of the mobile phase in the coil system. Knowing the coil length (L) from the manufacturer's information, the cross sectional area of the mobile phase (A_m), and hence, the linear flow of mobile phase (u_m) can be quickly calculated.

$$V_m = LA_m = L\pi r_m^{\ 2} = L\pi\sqrt{\frac{F_m}{C}} \tag{5}$$

$$u_m = \frac{F_m}{A_m} = \frac{F_m}{V_m}L \tag{6}$$

Substituting for V_m from (5) and squaring gives:

$$u_m^2 = \frac{CF_m}{\pi^2} = G_u F_m \qquad (7)$$

This gives a linear relationship[7] between the square of the mobile phase linear flow or velocity (u_m^2) and the mobile phase volume flow (F_m), the gradient of which (G_u) is related to the Hagen-Poiseuille relationship of equation (2) as follows:

$$G_u = \frac{C}{\pi^2} = \frac{\Delta P}{8\pi\mu_m L} \qquad (8)$$

Now, the retention of the stationary phase (S_f) can be easily calculated by subtracting the mobile phase volume (V_m) from the system volume (V_c) to give the volume of the stationary phase (V_s) and, hence, the % retention ($100V_s/V_c$). It can also be expressed as follows when substituting for V_m from equations (5):

$$S_f = \frac{V_s}{V_c} = 1 - \frac{V_m}{V_c} = 1 - \frac{L\pi}{V_c}\sqrt{\frac{F_m}{C}} = 1 - \frac{\pi}{A_c}\sqrt{\frac{F_m}{C}} \qquad (9)$$

where A_c is the cross-sectional area of the tubing of length L and total volume (V_c). This can be compared to Du et al's measured observations[5] summarised in equation (1), where constant A=1 and B= $\frac{\pi}{A_c\sqrt{C}}$.

It has been shown,[8] that for a given phase system and operating conditions, ΔP and L are constant and that the slope G_u is inversely proportional to the viscosity of the mobile phase. In this study, viscosity is constant as only the lower, more viscous, phase is mobile and the viscosity does not change significantly in the three phase systems studied. The only variable in this study will be ΔP, which is hypothesized to vary with the density difference of the two phases (ρ_l-ρ_u) and the magnitude of the gravitational field ($R\omega^2$), and it follows that the gradient G_u should vary proportionately. In practice, of course, the gravitational fields are fluctuating with the tangential component ($R\omega^2\sin\theta$) and the radial component $R\omega^2(4\beta \pm 1)$ and, in this study the $R\omega^2$ value only will be used. It should be noted, that the slope G_u is related to the gradient B of the Du retention/flow characteristic as follows:

$$B^2 G_u = \frac{1}{A_c^2} = \text{constant} \qquad (10)$$

where A_c is the cross-sectional area of the tubing.

EXPERIMENTAL

Experimental Apparatus

A standard preparative laboratory scale CCC[9] with 1.6mm bore PTFE tubing, β range of 0.87 to 0.90, and coil volume of 95.7 mL was supplied by Romulus Technology (Space) Ltd. as part of a BBSRC/DTI LINK study on the scale-up of countercurrent chromatography. The planetary radius was R=110mm with a variable speed of rotation. It was temperature controlled to operate at 30°C ± 1 in reverse phase mode, with the aqueous lower phase mobile, pumping from head (center) to tail (periphery). A Grant (Y6) water bath was used for preheating the mobile phase to 30°C.

A schematic layout of the retention test set-up is given in Figure 1. Flow was supplied by a Gilson HPLC pump (model 302) with a 100SC head. A Grant cooler (type RC1400G) supplied water/glycol coolant for the temperature control system.

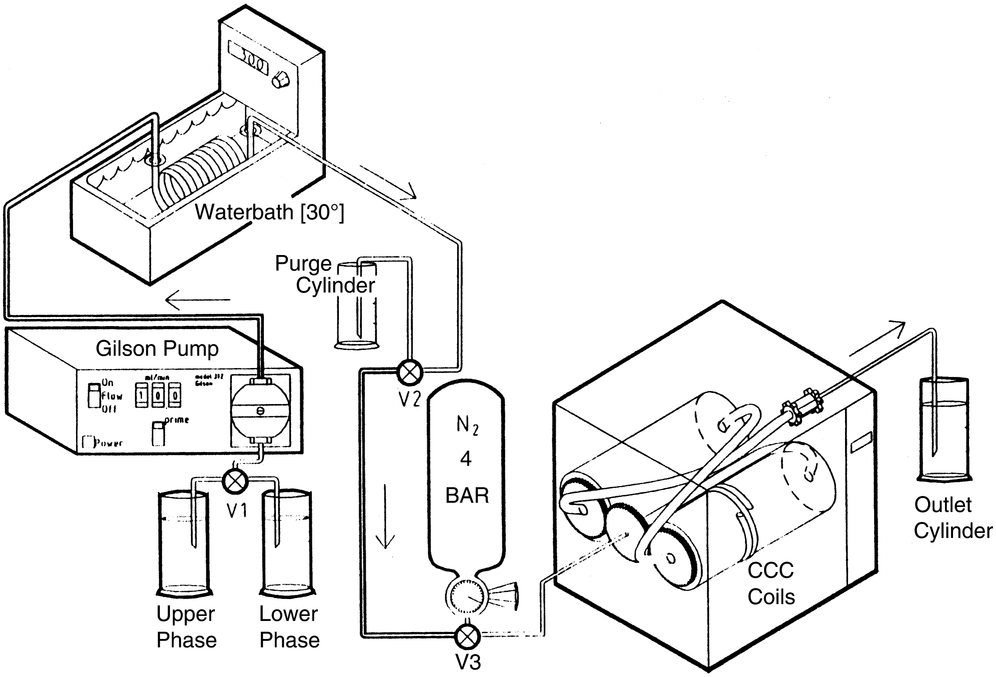

Figure 1. Schematic layout of retention test set-up.

Table 1. The Physical Properties of the Heptane Phase Systems

System	Composition H:EA:M:W	ρ_l kg/m^3	ρ_u kg/m^3	ρ_l-ρ_u kg/m^3	μ_l Pas (Ns/m^2)	μ_u Pas (Ns/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

Phase Systems

Three Heptane/Ethyl Acetate/Methanol/Water phase systems with constant mobile phase viscosity and varying density difference were chosen for this study. Their composition and physical properties are listed in Table 1. The solvents were supplied by Merck BDH. 2 litres were prepared, mixed, and allowed to equilibrate for 24 hours in advance of a run. The equilibrated phase system was out gassed by sonication for 20 minutes using a Jencon Soniclean.

Retention Tests

The retention test procedure, with reference to Figure 1, is as follows: assuming the CCC coils were already purged with nitrogen, the Gilson pump and the water bath preheating coil would first be primed with upper phase (the intended stationary phase), with valve (V1) switched to "upper" and with the control valve (V2) switched to "prime". Once the system was purged in this way, the control valve (V2) would be switched to "run," the outlet tube placed in a measuring cylinder, and the pump activated to fill the CCC coils with upper phase at 10 mL/min.

The Gilson pump and the water bath preheating coil were then primed with lower phase, with valve (V1) switched to lower and the control valve (V2) switched to "prime." Once primed, the control valve (V2) would then be switched to "run" and the outlet tube taped in place in a fresh 25 mL or 100 mL outlet measuring cylinder. The rotor speed would then be set in the reverse direction to the desired speed, the control temperature set to 30°C,and the pump set to 1 mL/min. The pump and stop watch were then activated simultaneously.

Initially, the upper (stationary) phase elutes from the outlet tube. When the lower (mobile) phase breaks through, the lower phase would continue to be pumped until the volume of upper phase displaced became constant - the system would then be in equilibrium. At a convenient time, the pump and stopwatch were stopped simultaneously and the total & lower phase volumes noted. Subtraction of the two gave the volume of stationary phase eluted (V_e) from which the volume of mobile in the coil (V_m) could be calculated by subtracting the volume of the inlet and outlet leads. Note, that stopping the pump while the rotor is still rotating will redistribute the phases in the coil (the heavier (lower) phase moving to the "tail" and the lighter (upper) phase moving to the "head"). A new hydrodynamic equilibrium can now be established at a higher flow, even without stopping the flow, by switching the pump setting from 1 to 2 mL/min. After a short initial period of mobile phase elution, more stationary phase would be eluted until the new hydrodynamic equilibrium was reached. Again, the pump and stop watch were stopped and the new total and lower phase levels noted, from which the new stationary phase volume and retention could be calculated. A similar procedure was used for 4 mL/min and 8 mL/min, although, fresh measuring cylinders may be needed after each flow setting. Once this retention variation with flow test is complete, the rotor, pump, and stopwatch would be switched off simultaneously. Once the rotor was stationary, a nitrogen line would be connected to the inlet, the pressure set on the regulator to 4bar, and the contents pumped out of the coils into a 100 mL measuring cylinder. In this way, the volume of lower phase retained in the coil can be checked against the retention calculated for the last flow setting. From the results of this test procedure, it is possible to calculate the retention (S_f), mobile phase volume (V_m), mean linear velocity (u_m) for each flow rate (F_m), and plot the S_f versus $\sqrt{F}$ plot[5] and the u_m^2 versus F_m plot.[6]

RESULTS

A typical Du plot[5] of retention (S_f) against the square root of flow ($\sqrt{F}$) is given in Figure 2. Figure 3 gives the variation of the square of mean mobile phase linear velocity (u_m^2) with flow (F). The linearity of Figure 3 and the replotting of the Du plots in this form,[7] support the Wood hypothesis[4] that the pressure drop across the coils are constant for a given set of operating conditions. Note, that in all Figures, the symbol (F) has been used for flow expressed in mL/min, the units commonly used in practice, whereas F_m,used in the Theory section, has units of m^3/sec. The linear regressions are based on an intercept of 100% in Figure 2 and zero in Figure 3. The results of 14 retention tests performed on the Quattro over a period of 6 months by two different operators for three different phase systems of varying density difference and operating at three different values of "g" field, are given in Table 2. All linear regressions are 5-point, including intercepts at 100% for "B" and zero for "G_u". The one exception is a 4-point and is asterisked in Table 2. Five of these results (Q3, Q4, Q15, Q21 & Q23) were the same test conditions repeated at different times for the 4A phase system operating at 800rpm. The Du slope (B) for these repeat results, based on the notation of

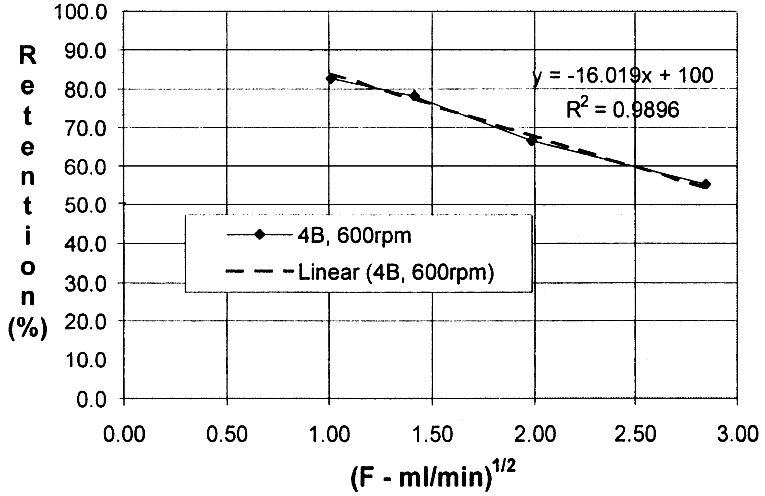

Figure 2. Typical plot of stationary phase retention against the square root of flow for Q11 in Table 2: the 4B phase system at 600 rpm.

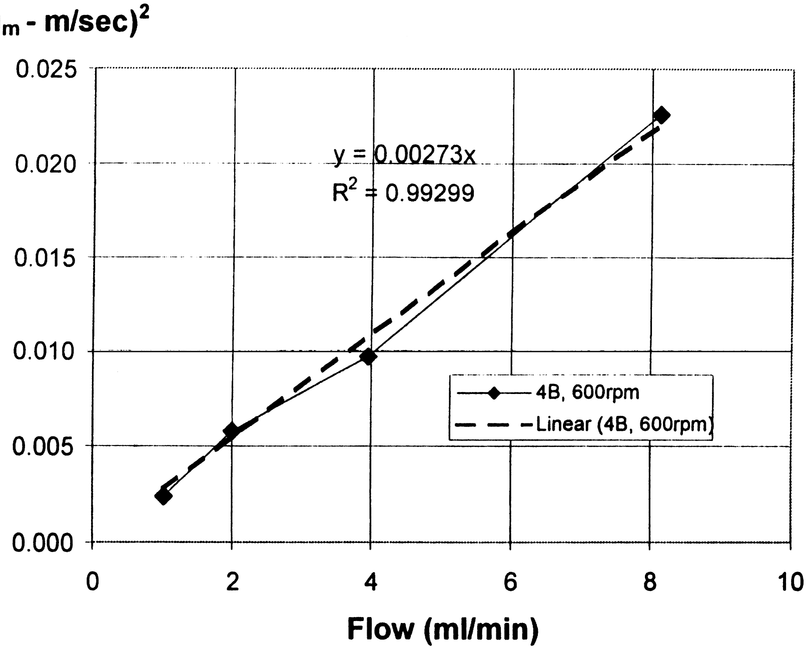

Figure 3. The variation of the square of linear velocity of the mobile phase with mobile phase flow rate for the plot given in Figure 2.

Table 2. Summary of Quattro Tests with Linear Regression Results

Run	Sys	$\Delta\rho$ (kg/m^3)	N (rpm)	$R\omega^2$ (m/s^2)	$B^{(1)}$	Corr. r^2	$G_u^{(2)}$	Corr. r^2
Q3	4A	268	800	772	−11.329	0.961	0.00535	0.960
Q4	4A	268	800	772	−11.381	0.968	0.00529	0.970
Q6	4A	268	700	591	−13.418	0.951	0.00356	0.871
Q7	4A	268	600	434	−14.306	0.942	0.00321	0.892
Q9	4B	230	800	772	−13.071	0.987	0.00398	0.990
Q10	4B	230	700	591	−14.419	0.987	0.00334	0.996
Q11	4B	230	600	434	−16.019	0.990	0.00273	0.993
Q12	4C	98	800	772	−23.019	0.978	0.00135	0.994
Q13	4C	98	700	591	−24.670	0.979	0.00117	0.996
Q14	4C	98	600	434	−27.478	0.955	0.00096	0.989
Q15	4A	268	800	772	−12.026	0.923	0.00500	0.984
Q21	4A	268	800	772	−11.542	0.971	0.00505	0.954
Q23	4A	268	800	772	−11.413	0.972	0.00507	0.938
Q27	4B	230	800	772	−12.221	0.982	0.00468	0.996

(1) Units as slope of Figure 2.
(2) Units as slope of Figure 3.

Figure 2, is - 11.538 ± 0.284 and the slope of the linear velocity squared/flow plot, based on the notation and units used in Figure 3, is 0.00520 ± 0.00016 (G_u = 3.091×10^5 $m^{-1}s^{-1}$ ± 0.094×10^5 using the units of equation (8), which gives experimental errors in the order of ± 3%.

The variations of the u_m^2/F_m slope G_u with density difference and acceleration field are plotted in Figures 4 and 5, respectively. In both cases, it can be seen that the variation is a linear one. From equation (8), it can be seen that G_u is proportional to the pressure drop across the coils (ΔP) and inversely proportional to the viscosity (μ_m) of the mobile phase. The results of Figures 4 & 5 indicate that the density difference (ρ_L-ρ_U) and acceleration field ($R\omega^2$) are proportional to the pressure drop and, so, plotting the u_m^2/F_m slope G_u against $(\rho_L-\rho_U)R\omega^2/\mu_m$ should give a linear result. This is shown to be the case in Figure 6 with a linear regression correlation coefficient (R^2 = 0.968). When repeat experiments are removed, the 10 point regression (including the origin) is R^2=0.959.

CONCLUSION

This study has shown that there is a good correlation between the slope (G_u) of the square of the linear velocity flow characteristic and $(\rho_L-\rho_U)R\omega^2/\mu_m$. As these terms have the same dimensions ($m^{-1}s^{-1}$), it follows that the slope (Su) is a numeric or non-dimensional number:

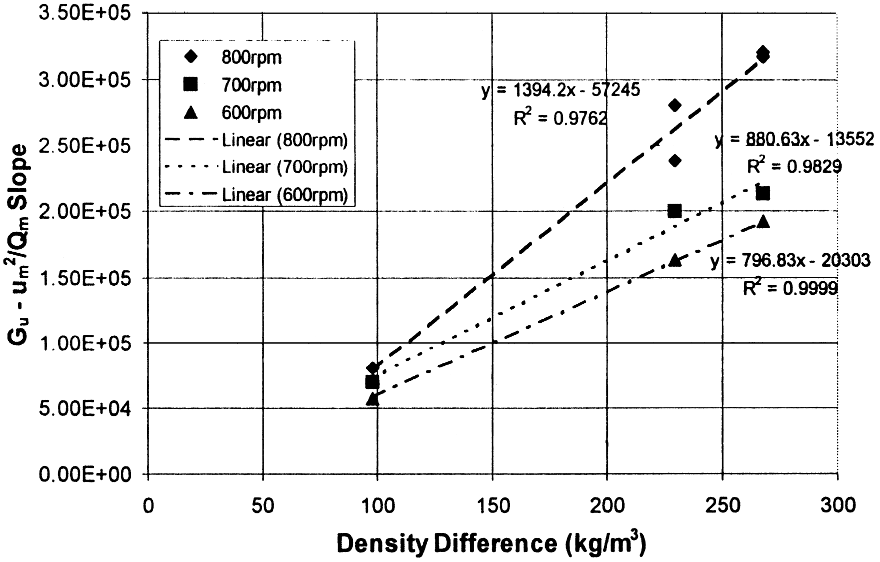

Figure 4. The variations of the u_m^2/F_m slope G_u with density difference.

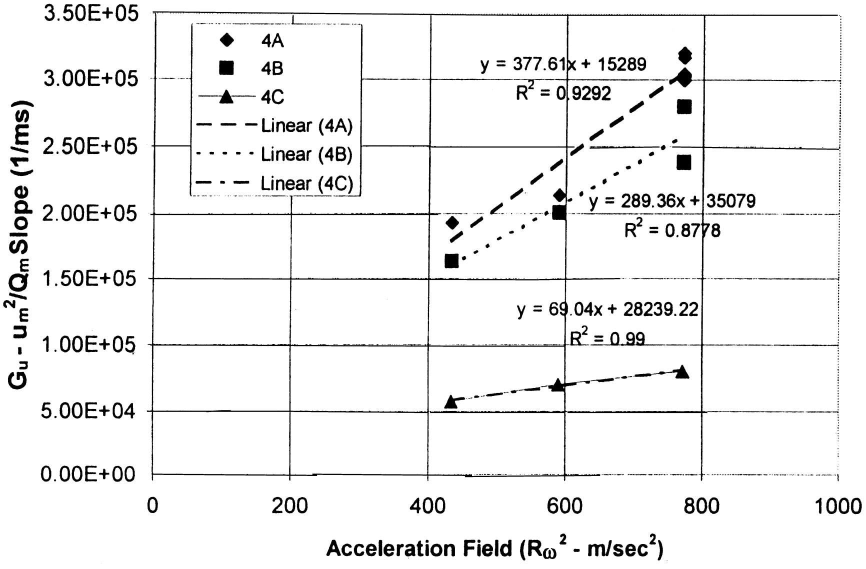

Figure 5. The variations of the u_m^2/F_m slope G_u with acceleration field ($R\omega^2$).

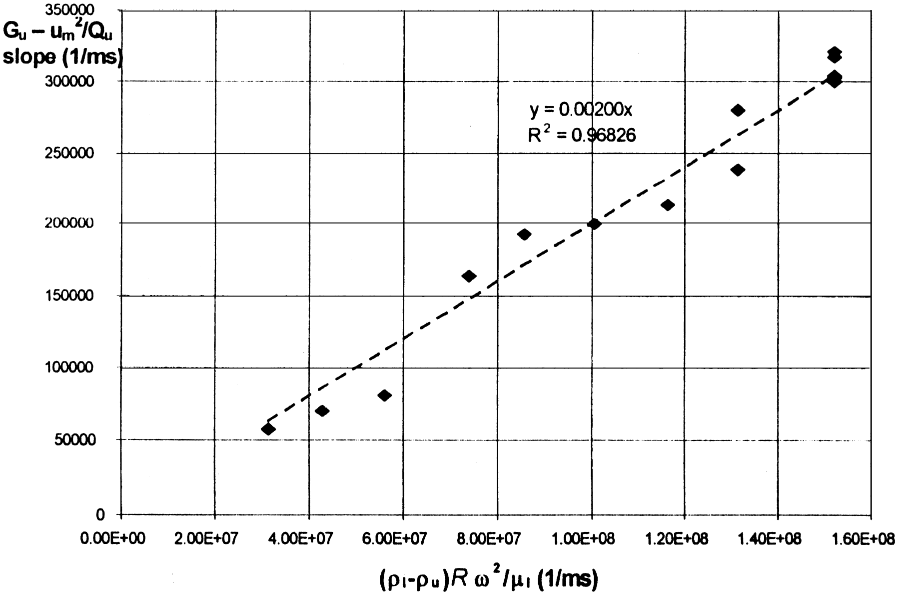

Figure 6. The variation of u_m^2/F_m slope G_u against $(\rho_L\text{-}\rho_U)R\omega^2/\mu_l$.

$$S_u = \frac{u_m^2\mu_m}{F_m\left(\rho_L - \rho_U\right)R\omega^2} = 0.002 \tag{11}$$

This can be expressed in terms of the Du slope (B) or the square of the mobile phase linear velocity/flow slope (Gu) as follows:

$$S_u = \frac{G_u\mu_m}{\left(\rho_L - \rho_U\right)R\omega^2} = \frac{\mu_m}{B^2A_c^2\left(\rho_L - \rho_U\right)R\omega^2} = 0.002 \tag{12}$$

Or, expressed another way:

$$G_u = \frac{S_u\left(\rho_L - \rho_U\right)R\omega^2}{\mu_m} \tag{13}$$

$$B = \frac{1}{A_c\omega}\sqrt{\frac{\mu_m}{S_u\left(\rho_L - \rho_U\right)R}} \tag{14}$$

It clarifies the relationship between retention & mean mobile phase linear flow (velocity), showing how increases in speed and density difference and reductions in viscosity reduce the Du slope (B) to improve the retention flow characteristics. It also shows how the same changes in these variables directly increase G_u, which will result in a faster throughput for a given volume flow and offers the potential for more efficient results.

While all these results suggest that Hagen-Poiseuille can be applied in this special case of stratified flow, it should be noted that Hagen-Poiseuille normally applies to laminar single-phase flow. It can be argued, that when the flow approaches zero, the phases will still move relative to one another (to the opposite ends of the tubing) and, therefore, the velocity at zero flow is not zero. An alternative way of looking at this is, that as flow approaches zero, the retention of stationary phase approaches 100%. Therefore, there is no second phase to redistribute as the flow approaches zero. So, Hagen-Poiseuille may be able to be applied after all, provided the flow is laminar. Reynold's numbers currently observed in CCC are less than 1000, which suggest laminar flow, but it should be noted that the cyclical tangential acceleration[10] could set up linear flow fluctuations, which could pass the laminar turbulent transition point. This theory, therefore, has to be treated cautiously and is only valid for flow slowing increasing from zero and displacing the stationary phase as it does so. It is likely to be less valid at high retention and low linear flow (velocity). This is due to the area of the mobile phase volume occupying a segment of the tubing, which is assigned a mean hydraulic depth,[11] and this approximation can become less accurate as the area approximates to a thin line, as opposed to a more substantial segment.

More work is now required on measuring pressure drop as a function of density difference, viscosity, and acceleration field, and seeing how this changes with tubing bore. It appears that there is an extremely good prospect of being able to predict retention from physical characteristics of the solvent phase system and the operating conditions, which will simplify using CCC on new phase systems in the future.

NOMENCLATURE

Symbols Used

β	The ratio r/R
ΔP	Pressure difference (P_2 - P_1) between the outlet and inlet ends of the coil
μ_m	Viscosity of the mobile phase
ρ_U, ρ_L	Density of upper phase & lower phases
ω	Angular velocity of rotor

A_c	Cross-sectional area of tubing
A_m	Cross-sectional area of mobile phase in tubing
F_m	Mobile phase flow rate
g	Earth's gravitational field
L	Total length of tubing in coil system
N	Rotational speed
r	Distance from the planetary axis to a given point on the planetary rotor (bobbin)
r_m	Radius of circular area occupied by the mobile phase
n	Scaling factor for "g" field
P_1	The piezometric pressure at position 1 (Outlet end of coil)
P_2	The piezometric pressure at position 2 (Inlet end of coil)
R	Distance from centre of main rotor to the planetary axis
S_f	Retention of stationary phase
u_m	Mean linear velocity of mobile phase
V_c	Coil system volume
V_m	Volume of mobile phase in coil system
V_s	Volume of stationary phase in coil system

REFERENCES

1. Berthod A; Schmitt, N. Water-Organic Solvent Systems in Countercurrent Chromatography: Liquid Stationary Phase Retention and Solvent Polarity. Talanta **1993**, *40* (10), 1489-1498.
2. Maryutina T.A.; Ignatova, S.N.; Fedotov, P.S.; Spivakov, B.Y.; Thiebaut D. Influence of Composition and Some Physico-Chemical Properties of Two Phase Liquid Systems on the Stationary Phase Retention in a Coil Planet Centrifuge. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21* (1-2) 19-37.
3. Fedotov, P.S.; Thiebaut, D. Retention of the Stationary Phase in a Coil Planet Centrifuge: Effects of Interfacial Tension, Density Difference, and Viscosities of Liquid Phases. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21* (1-2) 39-51.
4. Wood, P. *Hydrodynamics of Countercurrent Chromatography*; Ph.D. thesis, Brunel University: Uxbridge, UK, 2001.
5. Du, Q.; Wu, C.; Qian, G.; Wu, P.; Ito, Y. Relationship Between the Flow Rate of the Mobile Phase and Retention of the Stationary Phase in Countercurrent Chromatography. J. Chromatogr. A **1999**, *835*, 231-235.
6. Sutherland, I.A.; Muytjens, J.; Prins, M.; Wood, P.A. New Hypothesis On Phase Distribution In Countercurrent Chromatography. J. Liq. Chromatogr. & Rel. Technol. **2000**, *23* in press.

7. Sutherland, I.A. The Relationship Between Retention, Linear Velocity and Flow in Countercurrent Chromatography. J. Chromatog. A **2000**, *886* (1-2), 283-287.
8. Sutherland, I.A.; Kidwell, H.; Wood, P. *Comparing Normal and Reverse Phase CCC: The Importance of the Viscosity of the Mobile Phase*, Pittsburgh Conference, New Orleans, March 6-9th, 2001; Abstract Submission No 1074.
9. Sutherland, I.A.; Brown, L.; Forbes, S.; Games, D.; Hawes, D.; Hostettmann, K.; McKerrell, E.H.; Marston, A.; Wheatley, D.; Wood, P. Countercurrent Chromatography (CCC) and its Versatile Application as an Industrial Purification & Production Process. J. Liq. Chromatog. & Rel. Technol. **1998**, *21* (3), 279-298.
10. Wood, P.; Sutherland, I.A. Mixing, Settling and the Movement of the Interface Between the Mobile and Stationary Phases in CCC. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24* (11/12) .
11. Sutherland, I.A.; Booth A.; Brown L.; Kemp B.; Kidwell, H.; Games, D.; Graham, A.S.; Guillon, G.G.; Hawes, D.; Hayes, M.; Janaway, L.; Lye, G.; Massey, P.; Preston, C.; Shering, P.; Shoulder, T.; Strawson, C.; Wood, P. Industrial Scale-up of Countercurrent Chromatography. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24* (11/12).

Received September 8, 2000
Author's Revision October 16, 2000
Accepted December 3, 2000
Manuscript 5479