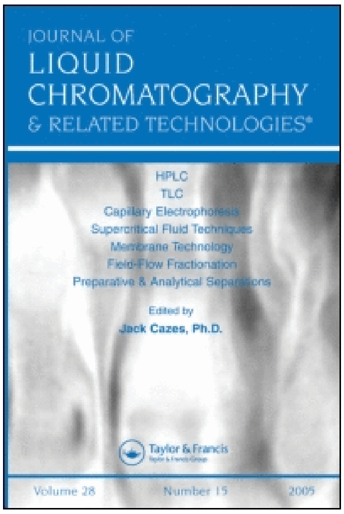

**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Organic Phase Retention in CCC: Effect of Temperature, Tubing Material, Tubing Bore, Mobile Phase Flow, and the Addition of an Extraction Agent

T. A. Maryutina[a]; S. N. Ignatova[a]; I. A. Sutherland[b]
[a] Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Moscow, Russia [b] Brunel Institute for Bioengineering, Brunel University, Uxbridge, UK

**To cite this Article** Maryutina, T. A. , Ignatova, S. N. and Sutherland, I. A.(2003) 'Organic Phase Retention in CCC: Effect of Temperature, Tubing Material, Tubing Bore, Mobile Phase Flow, and the Addition of an Extraction Agent', Journal of Liquid Chromatography & Related Technologies, 26: 9, 1537 — 1550

**To link to this Article: DOI:** 10.1081/JLC-120021265

**URL:** http://dx.doi.org/10.1081/JLC-120021265

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, Nos. 9 & 10, pp. 1537–1550, 2003

# Organic Phase Retention in CCC: Effect of Temperature, Tubing Material, Tubing Bore, Mobile Phase Flow, and the Addition of an Extraction Agent

**T. A. Maryutina,[1] S. N. Ignatova,[1] and I. A. Sutherland[2,*]**

[1]Vernadsky Institute of Geochemistry and Analytical Chemistry,
Russian Academy of Sciences, Moscow, Russia
[2]Brunel Institute for Bioengineering, Brunel University, Uxbridge, UK

## ABSTRACT

This paper describes an investigation of organic phase retention and how it is affected by changing certain variables that are in the control of the user. A binary approach is taken in order to perform a quick evaluation of how key factors, like the addition of an extraction agent or a change of temperature, flow, tubing material, and bore affect stationary phase retention. Several different phase systems were studied in this way (decane–water, chloroform–water, methylisobutylketone–water, and carbon tetrachloride–water). The results show that retention is higher and more stable for larger bore tubing, and that at the analytical scale, there

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[*] Correspondence: I. A. Sutherland, Brunel Institute for Bioengineering, Brunel University, Uxbridge, UB8 3PH, UK; E-mail: ian.sutherland@brunel.ac.uk.

DOI: 10.1081/JLC-120021265
1082-6076 (Print); 1520-572X (Online)

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are threshold affects at low rotational speeds, which can create significant differences in retention behavior.

*Key Words:* CCC; Phase retention; Solvents and reagents; J-type centrifuge; Milli-CCC®.

## INTRODUCTION

Successful CCC separation depends upon choosing a two-phase solvent system that provides the proper distribution ratio values for the compounds to be separated and satisfactory retention of the stationary phase. The number of potentially suitable CCC solvent systems can be so great that it may be difficult to select the most appropriate one.[1] The systems for inorganic separations are very different from those for organic separations, as in most cases, they contain a complexing (extracting) reagent (ligand) in the organic phase and mineral salts and/or acids or bases in the aqueous phase.[2] Thus, the complexation process, its rate, and the mass transfer rate, can play a significant role in the separation process.[3]

The stationary phase volume retained in a column is characterised by the $S_f$-factor (the ratio of the stationary phase volume $V_s$ to the total column volume $V_c$). The value of $S_f$ is dependent on two classes of the parameters or variables: active parameters (that can be changed during an experiment) and non-active parameters (that cannot be changed during an experiment). The active parameters are as follows: (1) the mobile phase flow-rate ($F$, mL/min) or the linear velocity ($u_m$, cm/s); (2) the rotation speed ($\omega$, rpm); (3) the temperature ($T_0$, °C); and (4) the physicochemical properties of the mobile phase. The non-active parameters are: (1) the geometrical parameters of the coil planetary centrifuge, such as the rotor radius ($R$, mm), $\beta$ value (where $\beta$ is the ratio of the planetary radius ($r$, mm) to the rotor radius; (2) the tubing diameter ($d_c$, mm I.D.); (3) tubing material; and (4) the physicochemical properties of the stationary phase (density and viscosity values of each phase and interfacial tension).

The influence of planetary centrifuge parameters and operation conditions on the stationary phase retention are well studied for simple two-phase liquid systems consisting of water and one or two organic solvents.[1,4–19] The stationary phase retention is known to increase with increasing the rotation speed and decreasing the mobile phase flow rate. Du et al.[16] have found an interesting correlation between the retention of stationary phase and the square root of mobile phase flow for 12 different phase systems. Sutherland[15,18] has shown that there is a linear relationship between the square of the mobile phase linear velocity and volume flow rate.

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The influence of physicochemical properties of two-phase liquid systems on the stationary phase retention has been under study by a number of different authors.[1,3–14,17,18,21–27] Parameters, such as the polarity of solvents, the density and viscosity differences of the two phases, the settling time, the interfacial tension, and capillary wavelength have been considered. A few types of classification of liquid systems on the basis of their physical properties have been proposed. Ito's classification[1,27] is most frequently used. It classified phase systems in three ways: hydrophobic, intermediate, and hydrophilic. Ito found, for 1.6 mm bore tubing, that the hydrophobic phase system had to be pumped from head to tail if the heavy phase was mobile and from tail to head if the light phase was mobile. For hydrophilic phase systems, the direction (i.e., head/tail) was reversed. The intermediate phase systems behaved like hydrophobic ones at high $\beta$ value and like hydrophilic phase systems at low $\beta$ value. One problem with this classification, as highlighted by Menet,[27] is that it is specific to 1.6 mm Teflon tubing at room temperature. Sutherland et al.[28] found, for large bore tubing, that all phases tested by Ito behaved like his hydrophobic phase systems (i.e., heavy phase had to be pumped from head to tail) providing the tail was arranged at the periphery, but that if the tail was at the centre the Ito classification would be valid.

The first attempt to correlate the stationary phase retention and physicochemical properties of liquid systems was made by Berthod and co-workers.[4,8,17] They reported that the value of $S_f$ increases linearly with an increase of the density difference between two phases ($\Delta\rho$). The authors found no reliable correlation between the densities of two liquid phases, their viscosities, and $S_f$-factor. Recent investigations by Sutherland on a 100 mL coil column with 1.6 mm bore tubing,[18] show that there are simple relationships between stationary phase retention, density difference, viscosity of mobile phase, mobile phase linear flow, and "g" field ($g = R\omega^2$, where $R$ is distance from the centre of the main rotor to the planetary axis, $\omega$—the angular speed of rotation) for the particular case of heptane–ethyl acetate–methanol–water solvent systems.

The addition of extracting reagents and mineral salts to a two-phase system (in the case of pH-zone separation and for inorganic separations) can strongly affect the physicochemical properties of liquid systems and, consequently, their hydrodynamic behavior and $S_f$ value. Varying concentrations of the system constituents used for inorganic separation allows selective changing of certain physicochemical parameters (interfacial tension $\gamma$, density difference between two liquid phases $\Delta\rho$, and viscosity of the organic stationary phase $\eta_{\mathrm{org}}$).[21–26] The type of the solvent may often have a great effect on the stationary phase retention, and, consequently, on the chromatographic process.

This work offers a simple, binary strategy for establishing the important critical variables affecting the $S_f$-factor, for a number of phase systems used in liquid–liquid extraction using extraction agents. The binary strategy used is as follows: temperature: 25°C or 30°C, tubing bore 0.8 mm or 5.3 mm, tubing material Teflon or stainless steel (SS), extracting agent 0 or 5% di-2-ethylhexylphosphoric acid (D2EHPA), and normalised flow rate ($F/A_c$ where $F$ is the volume flow rate and $A_c$ is the tubing cross-sectional area) 0.6/2.4 m/min depending on the tubing bore 0.8/5.3 mm of the CCC machine used.

## EXPERIMENTAL

### Apparatus

Investigations of stationary phase retention were made on two different J-type coil planet centrifuges: the Brunel-CCC® and the Milli-CCC®. They were both developed by Brunel Institute for Bioengineering, Brunel University (Uxbridge, UK). The main design and operation parameters are illustrated in Table 1. The Brunel CCC has two bobbins: the 5.53 mm (120 mL) has properties as indicated in Table 1, the other has a bore of 7.73 mm and capacity of 260 mL, but was only used for balancing purposes.

### Phase System Properties

Densities were estimated simply by weighing a known volume of each liquid phase. A glass capillary viscometer was used for measurements of

***Table 1.*** Technical characteristic of the CCC machines used.

| Parameters | Brunel-CCC | Milli-CCC® |
|---|---|---|
| Volume (mL) | 120 | 5 |
| Spool number | 1 | 1 |
| Tubing diameter (mm) | 5.33 | 0.76 |
| Planet radius ($r$, mm) | 90 | 34–39.5 |
| Rotor radius ($R$, mm) | 110 | 50 |
| $\beta$ Ratio | 0.82 | 0.68–0.79 |
| Rotation speed (rpm) | 200–1,200 | 300–2,100 |
| Flow rate (mL/min) | 10–100 | 0.25–2.0 |
| Pitch (mm) | 11.5 | 1.6 |
| Length ($L$, m) | 5.66 | 9.75 |
| Number of loops | 10 | 44 |
| Tubing material | Stainless steel (SS) | Teflon/SS |

viscosities of the solutions. A tensiometer (Lauda, Germany) with a thermostatic cell was used for measurements of interfacial tension of the systems under investigation by the ring method.[29]

## Reagents

Di-2-ethylhexylphosphoric acid (D2EHPA, Sigma) was used as extracting reagents. Chemically pure organic solvents were employed without additional treatment. The other chemicals were analytical grade reagents.

## CCC Test Procedure

Before the experiment began, the components of the two-phase liquid system were stirred and brought into equilibrium for mutual saturation of the phases. The two phases were separated shortly before use. The aqueous phase was used as the mobile phase and the organic one as the stationary phase. First, the coil column was filled with the organic phase while stationary. Once the column rotation was started, the aqueous phase was fed into its inlet. The mass force field, which arose during rotation, made it possible to retain a given volume of the stationary phase $V_s$ while the mobile phase was continuously pumped through. This retained volume of stationary phase would remain constant for a given rotational speed and flow. The test protocol used was to set the speed at its highest value (the one that would give the highest retention) to begin with. Once equilibrium was established and the amount of stationary phase displaced from the column measured, the rotational speed would be reduced by an increment and a new equilibrium established. Measuring the change in displaced volume, would allow a new stationary phase retention value to be calculated. This process was repeated until the speed was so low that all the stationary phase was displaced from the coil/column. Note the measurement accuracy was ±0.1 mL, giving ±2% accuracy for the $S_f$ factor.

## Comparative Studies with Different Solvents and Reagents

The range of four different organic solvents used for comparative studies is shown in Table 2, together with their respective densities and viscosities. Note, that the densities and viscosities of the mobile aqueous phases were approximately 1 g/mL and 1 cP, in all cases. The last row in Table 2 gives the density and viscosity of the extractive agent, D2EHPA. Note, that its viscosity is quite high.

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*Table 2.* Physicochemical parameters of solvents and extracting reagents.

| Solvent (extracting reagent) | $\rho$, $g/\mathrm{mL}$ | $\eta$, cP |
|---|---|---|
| *n*-Decane | 0.72 | 0.92 |
| Chloroform | 1.48 | 0.62 |
| Carbone tetrachloride | 1.58 | 0.97 |
| Methhylisobutylketone | 0.80 | 0.80 |
| D2EHPA | 0.97 | 45.6 |

## Comparative Studies Using Different J-Type Centrifuges

The behaviors of the organic phase in the different coil columns can be compared under same values of $u_m$ and $g$ factor, as was suggested by Sutherland.[15] The g field values for the two different centrifuges are presented in Table 3. Note, that normalised flow rates were used for the

*Table 3.* g field values for different centrifuges (where $g = R\omega^2$).

| $\omega$, rpm | Brunel-CCC | Milli-CCC |
|---|---|---|
| 350 | 15.07 | 6.85 |
| 400 | 19.68 | 8.95 |
| 450 | 24.91 | 11.32 |
| 500 | 30.75 | 13.98 |
| 550 | 37.21 | 16.91 |
| 600 | 44.28 | 20.13 |
| 650 | 51.97 | 23.62 |
| 700 | 60.27 | 27.40 |
| 750 | 69.19 | 31.45 |
| 800 | 78.72 | 35.78 |
| 850 | 88.87 | 40.40 |
| 900 | 99.64 | 45.29 |
| 950 | 111.01 | 50.46 |
| 1,000 | 123.01 | 55.91 |
| 1,200 | 177.13 | 80.51 |
| 1,400 | 241.01 | 109.55 |
| 1,500 | | 125.80 |
| 1,800 | | 181.16 |
| 2,100 | | 246.57 |

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different sized tubing based on dividing the volume flow rate ($F$-mL/min) by the cross-sectional area of the tubing ($A_c$-mm$^2$). These were calculated as 0.27 mL/min and 1.1 mL/min for the two flows in the 0.76 mm bore tubing, and 14 mL/min and 54 mL/min for the two flows in the 5.33 mm bore tubing, giving mean linear flows of 0.6 and 2.4 m/min, respectively. Note, that the actual linear flow of the mobile phase is calculated by multiplying this value by the ratio of the coil volume to mobile phase volume $[S_f/(1 - S_f)]$.

## RESULTS AND DISCUSSION

### Milli-CCC®

The behavior of a two-phase system of various compositions was investigated using Milli-CCC® with either Teflon or stainless steel columns. The aqueous phase was the mobile phase in all cases.

A small addition (5%) of D2EHPA in *n*-decane (Fig. 1) leads to a significant increase in the $S_f$-factor for $F = 1.1$ mL/min (curves 1 and 3) and has, practically, no influence at $F = 0.27$ mL/min (not shown). The increasing

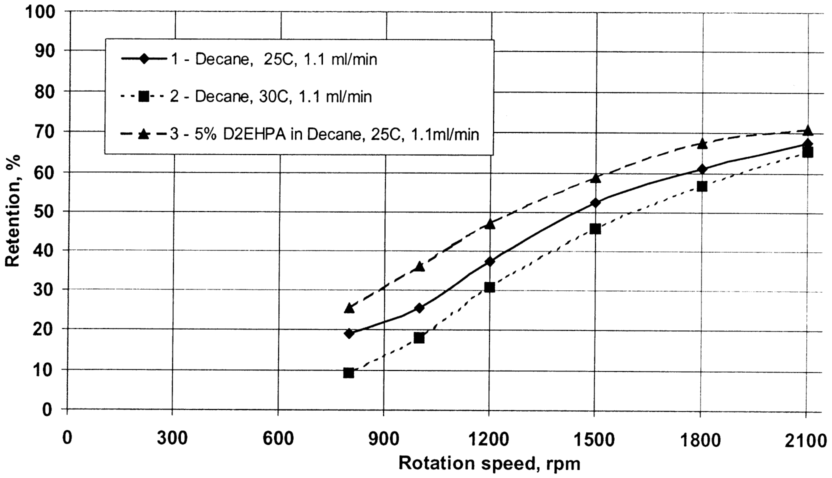

***Figure 1.*** Variation of stationary phase retention with the rotational speed for a decane/water phase system showing the effects of increasing temperature and adding 5% D2EHPA (Milli-CCC, Teflon column, mobile phase—water): 1—decane, temperature 25°C, $F = 1.1$ mL/min; 2—decane, temperature 30°C, $F = 1.1$ mL/min; 3—5% D2EHPA in decane, temperature 25°C, $F = 1.1$ mL/min.

of the temperature from 25°C to 30°C leads to a significant decrease in stationary phase retention (curves $1 \rightarrow 2$), but again, at $F = 0.27$ there is little difference.

The influence of the type tubing material (SS and Teflon) on the retention of the stationary phase was investigated using phase systems based on chloroform (Fig. 2). The curves (Fig. 2) characterising the chloroform–water and 5% D2EHPA in chloroform–water systems in each type of column are very similar. Adding of 5% D2EHPA to chloroform leads to a slight increase in the retention factors for both types of the column. For the SS, column retention of the stationary organic phase with and without D2EHPA can be maintained for lower rotational speeds (curves 1 and 3), compared to using Teflon tubing (curves 2 and 4). The effect of adding D2EHPA has little effect at high speed for the stainless steel tubing, but extends the operating range to lower rotational speeds, but for Teflon tubing, there is no change at the low speed cut-off point but a slight increase at high speeds. This can lead to significant differences in retention at critical speeds of rotation (800–1200 rpm or 35–80 *g*). For example, at 900 rpm there is 70% retention with SS and no retention at all with Teflon. Note, that for systems based on chloroform at a mobile phase flow rate of 1.1 mL/min, the threshold effect, where retention of

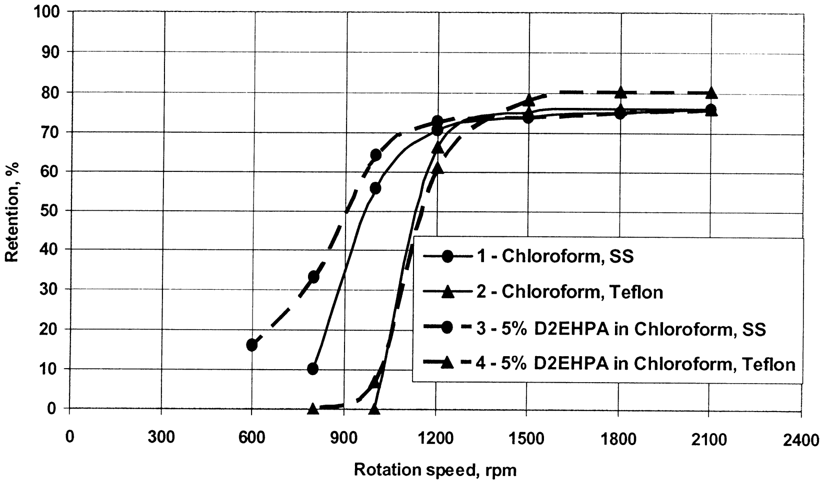

***Figure 2.*** Variation of stationary phase retention with the rotational speed for a chloroform/water phase system showing the effect of tubing material and adding 5% D2EHPA (Milli-CCC, $F = 0.27$ mL/min, temperature 25°C, mobile phase—water): 1—chloroform (SS column); 2—chloroform (Teflon column); 3—5% D2EHPA in chloroform (SS column); 4—5% D2EHPA in chloroform (Teflon column).

the stationary phase suddenly becomes possible, increases to a speed of rotation of 1500 rpm.

Adding 5% D2EHPA to the MIBK–water phase system (Fig. 3), leads to a decrease of the retention factor in SS column. A similar result was found with the Moscow CCC[24] using a Teflon column. It should be mentioned, that in Milli-CCC, there was no significant different between Teflon and SS for MIBK/water, but the 5% D2EHPA in MIBK–water system is significantly better retained in the Teflon column. The difference in the behavior of both the chloroform and MIBK systems in different types of column material, probably can be explained by the different wetting characteristics between the phase systems and Teflon or SS. For example, it has been noticed that phase systems with a higher interfacial tension, all other parameters remaining constant, have a higher threshold effect and, therefore, require a higher rotational speed or "g" field for retention of the stationary to take place.

The increasing of the mobile phase rate leads to a decrease $S_f$ value of the $CCl_4$ organic phase (Fig. 4). It was found that adding 5% D2EHPA to the organic phase had practically no influence on the $S_f$ at a flow rate of $F = 0.27$ mL/min, but led to a significant decrease of $S_f$ values at $F = 1.1$ mL/min and $\omega > 1000$ rpm. Note, that the addition of 5% D2EHPA had absolutely no effect on the threshold cut-off in retention, but increasing

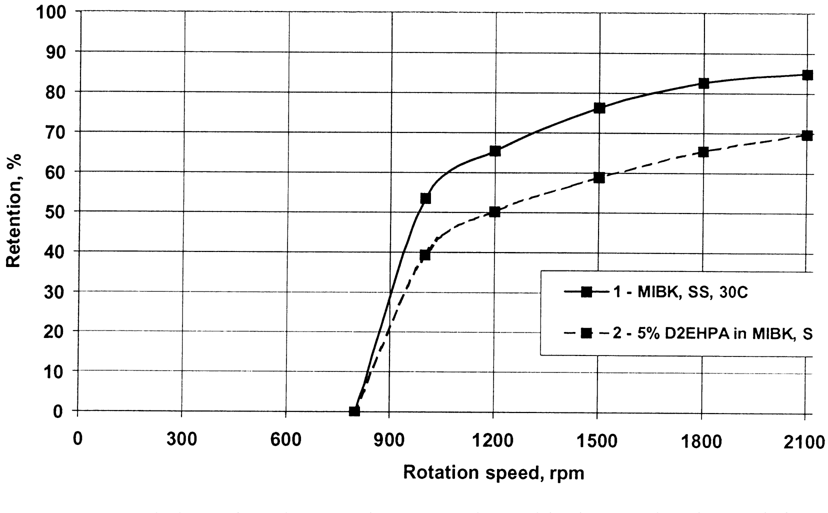

*Figure 3.* Variation of stationary phase retention with the rotational speed for a MIBK/water phase system showing the effect of adding 5% D2EHPA (Milli-CCC, $F = 0.45$ mL/min, temperature 30°C, mobile phase—water): 1—MIBK (SS column); 2—5% D2EHPA in MIBK (SS column).

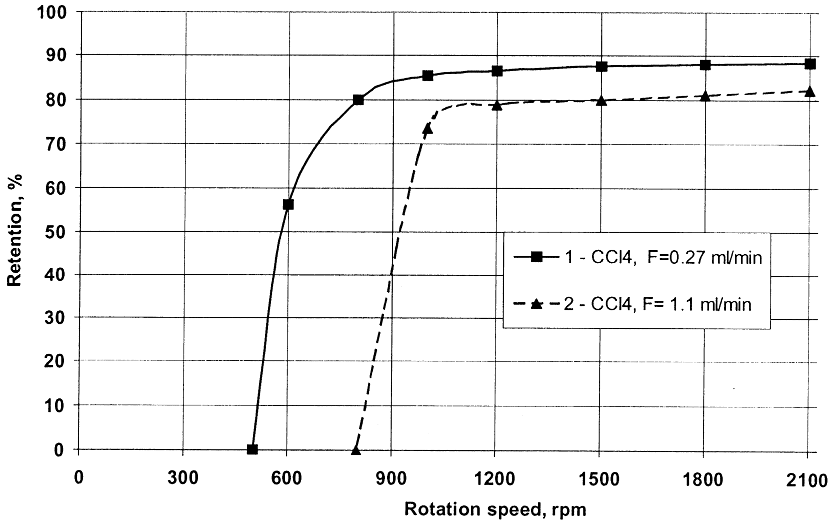

***Figure 4.*** Variation of stationary phase retention with the rotational speed for a $CCl_4$/water phase system showing the effect of increasing mobile phase flow (Milli-CCC, SS column, temperature 30°C, mobile phase—water): 1—$CCl_4$ ($F = 0.27$ mL/min); 2—$CCl_4$ ($F = 1.1$ mL/min).

the flow rate from 0.27 to 1.1 increased the threshold cut off from 500 rpm to 800 rpm.

Comparing data on the retention factor ($S_f$) for decane and chloroform phase systems undergoing the same experimental conditions, it is observed that under high speed of rotation (when *g* value was more then 120) the composition of the phase system does not have a dramatic influence on the $S_f$ value, and above 180 *g*, the retention was almost constant.

## Brunel CCC®

The addition of 5% D2EHPA to the chloroform–water phase system had very little effect at high speeds of rotation (200–1400 rpm).

The variation of stationary phase retention with "g" field is shown for both the Milli-CCC (0.76 mm bore, 5 mL capacity) and the Brunel-CCC (5.33 mm bore and 120 mL capacity). It can be seen,that the stationary phase retention is much higher for the larger bore tubing, and the threshold affect moves from approximately 50*g* for the Milli-CCC to virtual zero for Brunel-CCC with 5.33 mm bore tubing.

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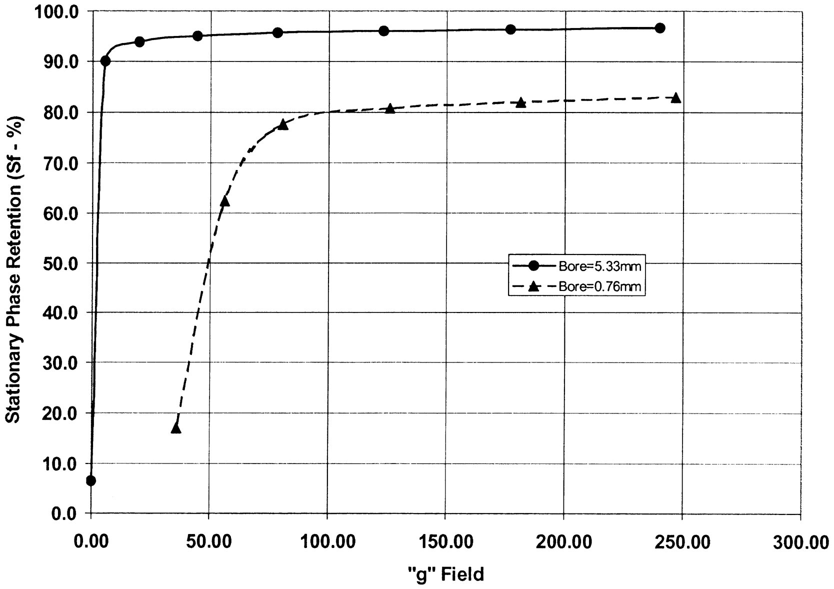

*Figure 5.* Variation of stationary phase retention with "g" field a chloroform/water phase system showing the effect of increasing bore 1—chloroform—Brunel CCC, SS column, tubing bore 5.33 mm, column volume 120 mL, $F = 14$ mL/min, mobile phase—water, temperature 25°C. 2—chloroform—Milli-CCC, SS column, tubing 0.76 mm bore, column volume 4.94 mL, $F = 0.27$ mL/min, mobile phase—water, temperature 25°C.

## CONCLUSIONS

For reproducible results, it is very important to pay attention to critical variables that can change during an experiment, such as temperature. If trying to reproduce someone elses experimental results on a different CCC device, it is important to note any difference in tubing bore, tubing material, and "g" field.

There appears to be a threshold effect where, below a certain speed (or "g" field), stationary phase retention no longer becomes possible. This clearly is influenced by a number of factors:

The tubing bore—using small bore tubing requires a higher threshold *g* value for the retention of the stationary phase.

Temperature—increasing temperature (and, hence, reducing the viscosity) can influence the retention of the stationary phase.

Tubing material—the type of tubing material (i.e., SS or Teflon) can influence the onset of retention for some phase systems

The composition of the phase system does not have a dramatic influence on the $S_f$ value under high speed of rotation (when $g$ value is more then 120), and above $180\,g$, the retention is almost constant.

The use of large bore tubing (more then 1.6 mm) helps to minimize the influence of rotational speed on the retention of stationary phase.

This preliminary research has highlighted stationary phase retention threshold effects that occur at critical "g" levels. They appear to be influenced by flow, the physical properties of the phase system, and "g" level. It is hypothesised, that these threshold effects are a function of the surface wetting characteristics between the phase system and the tubing material that will be more enhanced for small-bore tubing where the surface area to fluid volume ratio is higher. It is important for analytical-CCC, which uses small bore tubing, that more research is performed to characterise and understand this phenomenon, which is not explained by the hydrodynamic hypothesis proposed by Wood et al.[30]

## ACKNOWLEDGMENT

This work was supported by INTAS Grant, Reference 00-0782.

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Received June 25, 2002
Accepted December 15, 2002
Manuscript 6044M

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