Journal of Chromatography A, 1217 (2010) 34–39

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# A new non-synchronous preparative counter-current centrifuge—the next generation of dynamic extraction/chromatography devices with independent mixing and settling control, which offer a step change in efficiency

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ARTICLE INFO

*Article history:*
Received 14 August 2009
Received in revised form 14 October 2009
Accepted 22 October 2009
Available online 30 October 2009

*Keywords:*
Counter-current chromatography
Instrumentation
Centrifuge
Non-synchronous
Preparative

ABSTRACT

A new and significantly more robust design of non-synchronous coil planet centrifuge is introduced where the degree of mixing between two immiscible phases can be changed independently from the "g" field required to separate out the phases. A hypothesis that an optimum ratio between the speed of the bobbin and the speed of the rotor can be found to optimise the efficiency of the separation for a given force field is upheld for an intermediate polarity phase system. This paves the way for extensive further research to find the optimum non-synchronous conditions for a range of different phase systems that are desirable for the separation of large molecules, proteins and biologics but can tend to emulsify in the standard "J" type centrifuge systems currently available and routinely in use for aqueous organic phase systems. A step change of up to 30% in resolution and 90% in plate efficiency is demonstrated.



## 1. Introduction

The primary aim of this paper is to introduce a new non-synchronous coil planet centrifuge [1], which could revolutionise centrifugal liquid–liquid chromatography by enabling the separate and independent control of mixing and settling. This paper illustrates how the speed of the bobbin rotation (and hence mixing) can be controlled relative to the main rotor rotation, which determines the "g" field (and retention of the stationary liquid phase). Furthermore it hypothesises that for a given phase system, an optimum ratio between the bobbin rotation and the main rotor rotation can be found to give an optimum separation which will generally exceed the separation efficiency obtained using a standard "J" type centrifuge.

Ito's first paper in Nature demonstrated the potential of the non-synchronous coil planet centrifuge using a centrifuge with a closed column and no flying leads at all [2]. While attempts have been made since to build non-synchronous coil planet centrifuges with flying leads for continuous flow operation, with successful results using both physiological saline solution and aqueous two-phase systems for cell separations, they have required rotating seals to do so [3,4]. A non-synchronous coil planet centrifuge was designed and built at NIH by Ito in the early 1980s with no rotating seals [5] and was demonstrated to separate cells using a physiological saline solution [6]. However, the single column was small and the complexity of the flying lead mechanism meant that it would be rarely applied to high-speed CCC separations. Other designs also emerged but proved too complex to be reliable [7]. There have been various application papers in the literature using the non-synchronous coil planet centrifuge for the separation of erythrocytes [8] and mast cells [9] in their biologically intact form. In fact in a review on CCC in 1991, Ito predicted "future developments in CCC may be focussed on the improvement of the most intricate non-synchronous coil planet centrifuge scheme which has greater potential for the separation of biopolymers and cell particles" [10]. In the last few years, there has been further non-synchronous development in Japan by Shinomiya and his group for protein purifications [11–13] and for the separation of blood and mast cells in a single physiological saline solution [13,14]. However, it appears that the low number of publications using the non-synchronous coil planet centrifuge has more to do with engineering complexity and unreliability than the potential contribution to science.

The success of the Brunel team in understanding how to maintain stationary phase retention at flow rates required at larger scales has necessitated Dynamic Extractions Ltd. to understand and provide solutions to the engineering challenges of building larger coil planet centrifuges that are robust. This knowledge, together with a completely new and simplified approach to achieving a non-synchronous coil planet centrifuge using flying leads, is at the heart of this paper. The paper describes a new and elegant design for

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doi:10.1016/j.chroma.2009.10.055

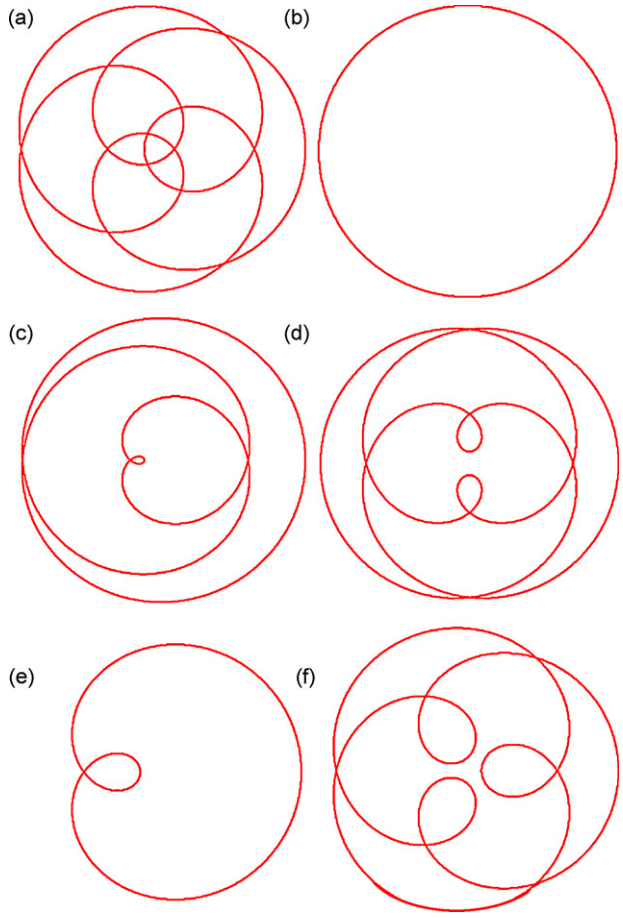


**Fig. 1.** The loci of a point on the column for (a) Pr = −0.60; (b) Pr = 0; (c) Pr = +0.33; (d) Pr = +0.67; (e) Pr = +1.0 and (f) Pr = + 1.5.

a non-synchronous coil planet centrifuge, which should achieve higher efficiencies in the separation of biologics and cells to allow non-synchronous centrifuges to become industrially competitive.

## 2. Non-synchronous theory

The general equations for the non-synchronous rotor and its accelerations are as follows [15,16]:

$$\text{Radial acceleration} = -R[\omega_R^2 \cos(\omega_r t) + \beta(\omega_R + \omega_r)^2]$$

$$\text{Tangential acceleration} = R\omega_R^2 \sin(\omega_r t)$$

where

$$\beta = \frac{r}{R}$$

The loci of a point on the column, as the ratio of bobbin to rotor speed (Pr) varies, is given in Fig. 1. For Pr = 0.33, the rotor rotates three times for every mixing cycle, whereas for Pr = 1.5 the rotor rotates only twice while there are three mixing cycles.

## 3. Non-synchronous design

The aim of this development was to design and construct a seal free, preparative-scale counter-current centrifuge where the rate and strength of mixing was decoupled from the settling forces.

A common feature of all CCC centrifuges that makes them ideal for bio-separations is a simple anti-twisting mechanism known as the "flying-lead" that allows external equipment to be connected to the spinning column. This device avoids problems such as over-heating and cross-contamination that can be associated with rotating/slipping seals.

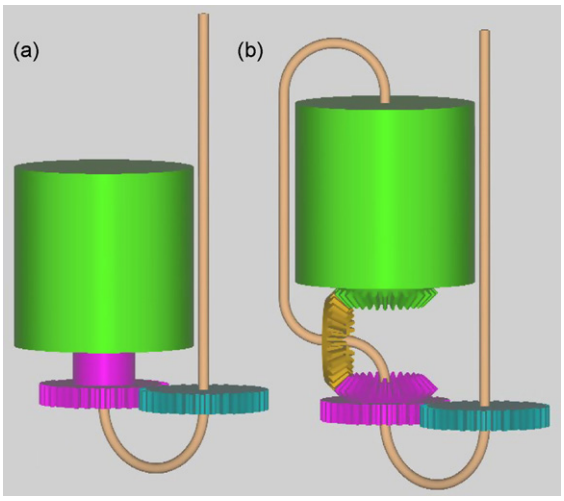


**Fig. 2.** Comparison of the (a) standard "J" type and the (b) new non-synchronous flying lead scheme with its "differential bobbin".

In order to create the accelerations in the fluids required to induce mixing and settling, the column (bobbin) of a counter-current centrifuge is spun eccentrically on a rotor which also rotates on its own axis. In order to connect the spinning column to the stationary outside world, a bundle of flexible tubes (the flying lead) runs from a fitting on the rotating axis of the column to a stationary fitting on the axis of the rotor (Fig. 2a).

In principle, the column can be rotated at any speed relative to the rotor but unfortunately; the current anti-twist mechanism requires that the column rotates at the same speed as the rotor. This means, for example, if high g-levels are required to separate similar density solvents then the rotor will have to rotate at high-speed and so will the column. This rapid rotation can cause aggressive mixing and settling which may damage delicate bio-molecules and even emulsify the solvent liquids rather than allowing them to mix and separate with each cycle. If the column did not rotate relative to the rotor, then each turn of the rotor would impart one turn of twist into the flying lead. In an "I" type machine, where the flying lead is effectively in-line, the column is rotated at the same speed as the rotor but in the reverse direction, thus exactly cancelling the imparted twist. In a "J" type machine the lead returns on itself in a "U", and so reverses the sense (or handedness) of the twist at the column end of the lead. Therefore, the column is also rotated at the same speed as the rotor but now in the same direction, so that it keeps up with the imparted twisting.

In both cases, all that is required mechanically is that the bobbin is turned by a simple 1:1 toothed drive connecting the stationary case to the rotating column. The drive can be via gear or belt but it must be toothed so that it cannot slip.

The fact that there are two instances where a non-twisting connection can be reasonably simply achieved is serendipitous but it is important to recognise that the 1:1 ratio required by these flying lead systems is unlikely to be optimum for separation efficiency, hence the need for a non-synchronous machine.

In our proposed method (Fig. 2b) which uses a number of Midi standard parts provided by Dynamic Extractions Ltd. (DE), the anti-twist between the case and a column's planet gear is handled the same way as a standard DE HPCCC centrifuge but in addition, each gear has a simple differential mechanism that provides the anti-twist between itself and the spinning column. The column is driven simply by a second rotating sun gear as shown schematically

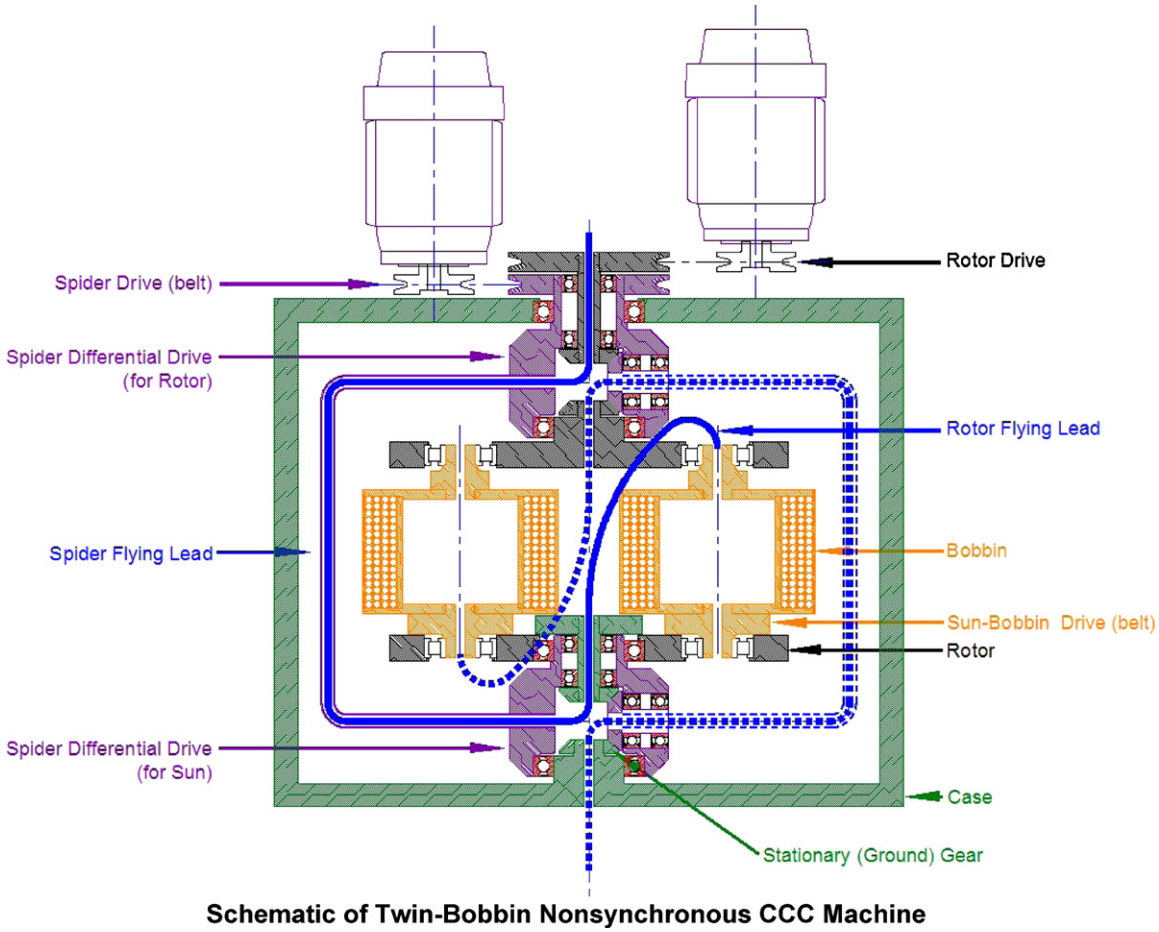


**Fig. 3.** Schematic of twin-bobbin non-synchronous CCC instrument.

for the synchronised and the new non-synchronous centrifuges in Fig. 2.

## 4. Experimental

### 4.1. Reagents

Solvents used for the isocratic counter-current runs were of analytical grade and for HPLC analysis were HPLC grade from Fisher Chemicals (Loughborough, UK). Caffeine was supplied by Fisher Chemicals while ferulic acid and vanillin were purchased from Sigma–Aldrich (Gillingham, UK). HPLC grade water was purified from a Purite Select Fusion pure water system (Thame, UK).

### 4.2. Apparatus

The non-synchronous coil planet centrifuge (rotor and bobbins parts supplied by Dynamic Extractions,[1] Slough, UK) was fitted with two 4 mm i.d. preparative columns made of polyfluoroalkoxy tubing (PFA) each with volumes of 475 mL, though only one column was used for this study. The rotor and bobbins were identical to the standard Midi rotor and columns/bobbins produced by Dynamic Extractions Ltd. [17]. The layout is shown schematically in Fig. 3. When the spider drive is stationary, the sun gear is stationary and the centrifuge acts just like a "J" type centrifuge when the main rotor drive is activated. If the speed of the main rotor is $X$ rpm then the bobbin will rotate at $X$ rpm relative to the rotor. The effect of starting to rotate the spider at $Y$ rpm in the same direction is to reduce the speed of the column relative to the rotor to $(X - 2Y)$ rpm. Table 1 gives a range of different operating scenarios where the ratio between the bobbin speed relative to the rotor and the rotor speed (Pr) can be changed from −1 (I type centrifuge) through zero (toroidal coil centrifuge) to +1 (J type centrifuge) and beyond. The importance being that the control of settling ("g" field and the speed of the rotor) can be independent from the mixing process (the speed of the bobbin relative to the main force field).

Upper and lower phases were pumped using a preparative Knauer K-1800 HPLC pump (Berlin, Germany), the sample was injected through a sample loop (7.22 mL, 1.5% Vc) using a Knauer K-6 valve and a Gilson UV/VIS-151 spectrophotometer (Middleton, WI, USA) with a preparative flow cell, operating at 280 nm, used to monitor the eluant.

HPLC analysis was performed on a Waters Alliance 2695 separations module (Empower software) connected to a Waters 2996 photodiode array (DAD) detector (210–800 nm) using a Sunfire $C_{18}$ column (150 mm × 4.6 mm i.d., 5 μm, Waters, Milford, MA, USA).

### 4.3. Preparation of the two-phase solvent systems

The solvent system used consisting of n-heptane, ethyl acetate, methanol and water with volume ratios of 2:3:2:3 (HEMWat-15). The upper and lower phases were made up separately following procedures described by Berthod [18] and Garrard et al. [19].

[1] Although a high-performance rotor and bobbins were supplied by DE, the results given in this paper have been restricted to 800 rpm (i.e., HSCCC and not HPCCC specification).

**Table 1**
Relationship between the speed of the bobbin relative to the rotor and the speeds of the rotor and its spider.

| Rotor speed (rpm) | Bobbin speed (rpm) | Spider speed (rpm) | Spider direction (Fwd/Rev) | Bobbin/rotor speed (Pr) |
|---|---|---|---|---|
| 800 | 1200 | −200 | Rev | 1.50 |
| 800 | 800 | 0 | 0 | 1 ("J" type) |
| 800 | 533 | 133 | Fwd | 0.67 |
| 800 | 400 | 200 | Fwd | 0.50 |
| 800 | 267 | 267 | Fwd | 0.33 |
| 800 | 0 | 400 | Fwd | 0 (toroidal) |
| 800 | −490 | 645 | Fwd | −0.61 |
| 800 | −800 | 800 | Fwd | −1 ("I" type) |

### 4.4. Determination of distribution ratios or partition coefficients

Upper phase (2 mL) and lower phase (2 mL) were dispensed into a test tube. A sample mixture (2 mg) was added to the phase system. The test tube was shaken vigorously until equilibrium had been established in both phases. Equal volumes (1 mL) of upper and lower phases were pipetted into separate HPLC vials and evaporated to dryness under vacuum. Finally, the residues were diluted with methanol (1 mL) and analysed by HPLC. The distribution ratio/partition coefficient ($K_d$) of a particular compound in reverse phase mode was calculated as the ratio of peak area in the upper (stationary) phase to the peak area in the lower (mobile) phase.

### 4.5. Experimental procedure

#### 4.5.1. Establishing hydrodynamic equilibrium

Hydrodynamic equilibration was performed in reversed phase mode with the lower (aqueous) phase as the mobile phase and upper (organic) phase as the stationary phase. A single 475 mL column was initially filled with the HEMWat-15 upper phase at a flow rate of 200 mL/min, with the rotor and columns not rotating. The rotational speeds of the rotor and the spider were set to give either synchronous or non-synchronous operating modes in accordance with Table 1. The lower phase was flowed at 20 mL/min from head-centre to tail-periphery to equilibrate the system. Once break through had been observed and no more stripping of stationary phase was seen the volume of displaced stationary phase was recorded. All separations were carried out with the centrifuge and phase system temperature controlled at 30 °C.

#### 4.5.2. Sample preparation, separation and fraction collection

A sample solution containing three compounds from the GUESS mixture (1.0 mg/mL caffeine (C), $K_d$ = 0.14; 1.0 mg/mL ferulic acid (F), $K_d$ = 0.54 and 1.0 mg/mL vanillin (V), $K_d$ = 1.02) was made up in HEMWat-15 lower phase. The system was run in reversed phase mode (lower phase mobile at 20 mL/min), with the sample loaded from a 7.22 mL sample loop to give a total load of 21.7 mg of solids for each injection. The eluant was monitored by UV spectrophotometer and fractions were collected every minute for analysis by HPLC.

#### 4.5.3. HPLC analysis of fractions

The HPLC method, developed on a reversed phase Sunfire C18 column (150 mm × 4.6 mm i.d. 5 μm) thermostatted at 25 °C, used a 1:1 (v/v) mixture of 0.1% aqueous formic acid and acetonitrile as mobile phase in an isocratic mode with a flow rate of 1 mL/min and a run time of 3.5 min. Eluant was monitored using a PDA detector.

### 4.6. Measurement of resolution (Rs)

The resolution (*Rs*) between two peak of retention time $T_1$ and $T_2$ and baseline width of $w_1$ and $w_2$ is given by:

$$Rs = \frac{2(T_2 - T_1)}{(w_1 + w_2)}$$

## 5. Results and discussion

The variation of stationary phase volume retention (Sf) with the ratio of column to rotor speed (Pr) is shown in Fig. 4. It can be seen that for the Pr range from 0.33 to 1 Sf remains substantially constant with about a 5% increase for Pr = 0.5 and Pr = 0.66 compared to the standard "J" type mode (Pr = 1). For values of 0 < Pr < 0.33 and Pr > 1 the stationary phase retention drops below 50%. This would be expected for the Pr = 0 situation (the toroidal coil centrifuge) as this is a cascade mixing situation [20] where by definition Sf < 50%. Surprisingly as Pr values go negative (Pr = −0.66) retention increases again to 74%, but quickly drops to near zero for the "I" type centrifuge at Pr = −1.

The variation of resolution between caffeine/ferulic acid (Rs12) and caffeine/vanillin (Rs13) is plotted in Fig. 5a. It can be seen that there is up to a 30% increase in resolution at Pr = +0.66 compared to the standard "J" type centrifuge at Pr = +1. While a small part of this can be attributed to the 5% increase in retention of the stationary phase, the majority must be due to an increase in mixing efficiency and mass transfer deduced from the reduction in peak width in Fig. 5b. Resolution at Pr = 0 and Pr = +1.5 are about the same and as the stationary phase volume retentions were also similar it is assumed that mixing efficiencies are also equitable, the lower resolution being attributed to the lower Sf (Fig. 4). The surprising

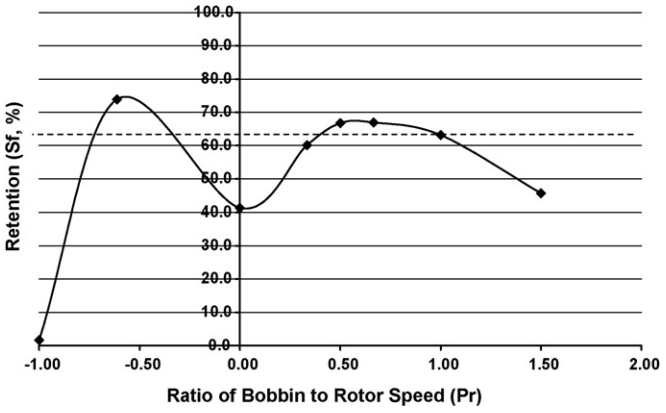


**Fig. 4.** Variation of stationary phase retention (Sf) with the ratio of bobbin to rotor speed (Pr). Run conditions: column volume—475 mL; volume inlet/outlet leads—10 mL; tubing bore—4 mm; rotor speed—800 rpm; bobbin speed—variable; flow rate—20 mL/min; phase system 15—H:E:M:W (2:3:2:3); mobile phase—lower phase; sample loading—7.2 mL at 1 mg/mL of each compound (caffeine, ferulic acid and vanillin).

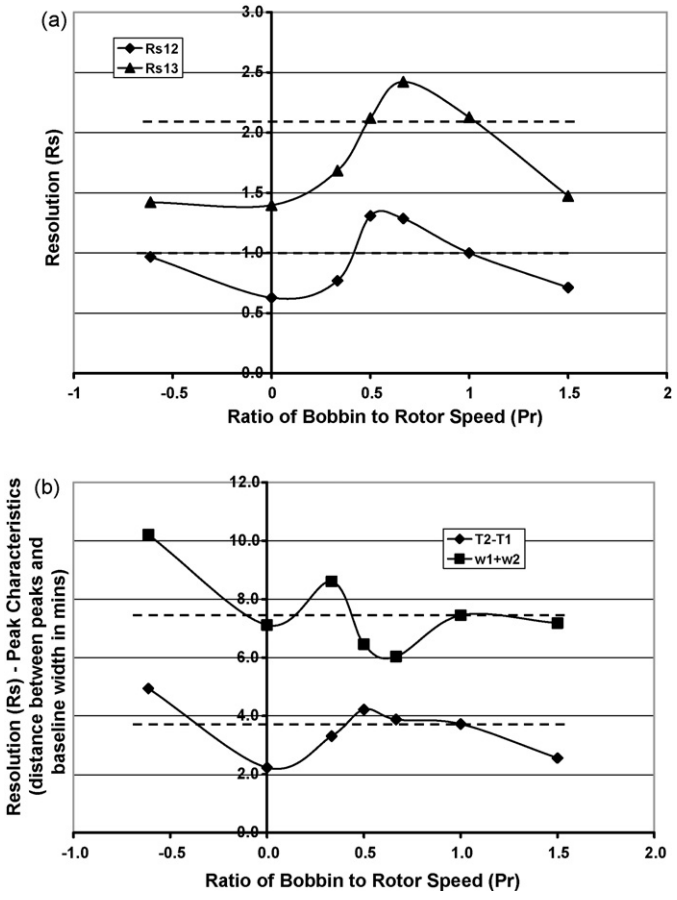


**Fig. 5.** The variation of (a) resolution (Rs) between caffeine and vanillin with ratio of bobbin to rotor speed (Pr) and (b) peak characteristics such as distance between peaks and baseline width. Run conditions as for Fig. 4.

result is that the increased stationary phase volume retention (Sf) at Pr = −0.66 did not translate into increased resolution. In fact, the resolution went down suggesting very poor phase mixing at this particular drive ratio (Pr).

In an attempt to understand the key elements affecting resolution: namely retention and phase mixing/mass transfer, the main components of the resolution formula $(T_2 - T_1)$ and $(w_1 + w_2)$ have been plotted separately in Fig. 5b. $(T_2 - T_1)$ should be totally predictable based on the retention of stationary phase (Sf). Comparison of the Sf curve in Fig. 4 with the $(T_2 - T_1)$ curve in Fig. 5b shows a qualitatively similar result. Plotting Sf values from Fig. 4 against $(T_2 - T_1)$ values from Fig. 5b gives a linear correlation ($R^2$) of 0.95. The width of a peak should represent the efficiency of mixing—the narrower the peak the more effective the mixing and mass transfer. It is well established that the "J" type centrifuge (Pr = +1) has a wave form of mixing, which is a gentle rather than violent mixing process. It is also well established [20] that the toroidal coil centrifuge has cascade mixing, which is more violent. What happens in between is a bit of a mystery, but clearly there are zones where, for this phase system at this "g" field there are zones of good mixing (∼Pr = +0.66 where peak width is a minimum) and zones of poor mixing (∼Pr = +0.33 where peak width is a maximum) and zones of extremely poor mixing (∼Pr = −0.66 where peak width is enormous).

Fig. 6 gives a comparison of the chromatograms obtained for each Pr value tested. The $K_d = 0$ point are indicated by an arrow based on the retention of stationary phase given in the legend. The $K_d = 1$ point (of course) is the same regardless of the retention (Sf) of each run at 24.3 min. What was surprising was that the point

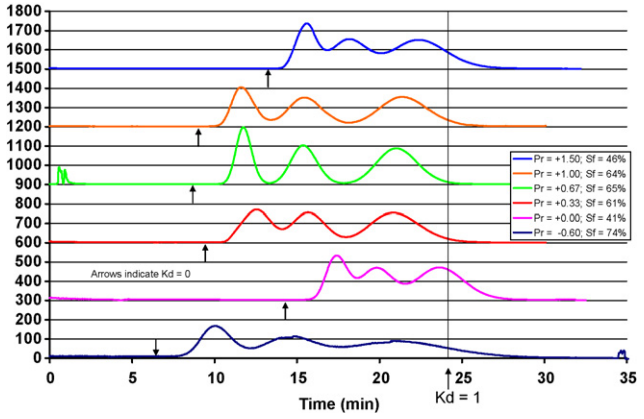


**Fig. 6.** Chromatograms from the separation of caffeine, ferulic acid and vanillin for different ratios of bobbin to rotor speed (Pr). Arrows make the $K_d = 0$ point of each chromatogram.

of elution of each peak did not occur exactly where retention theory would predict. This may be attributed in some case to poor mixing, or perhaps more correctly to good mixing in part of the coil and "dead zones" with no mixing in other parts. Much more systematic research is required before this is established. It should be noted that the vanillin peak ($K_d = 1.02$) in general eluted earlier than expected (mean $K_d$ from chromatograms 0.89), but slight variations of this kind are common [21]. Table 2 gives the variation of stationary phase volume retention (Sf), resolution (Rs) and chromatographic efficiency ($N$) as the ratio between bobbin and rotor speed is varied. Table 2 illustrates that chromatographic efficiency ($N$) is not the best measure of efficiency in CCC, which is why actual resolution between two compounds is used here. The highest number of theoretical plates (>500) is for poor resolution where Sf is low. At high resolution and high Sf, chromatographic efficiency can be much lower. It should be emphasised that this is because CCC is more of a multistage extraction process with a large volume of stationary phase than a chromatography process. Selectivity and the number of mixing and settling cycles in CCC are more important than the number of theoretical plates.

Clearly for this phase system at this acceleration field (800 rpm–76 g) the optimum resolution occurs at Pr = 0.66 and there is a change in resolution which is up to 30% higher than for the standard "J" type centrifuge (up to 90% more efficient in terms of theoretical plates—Table 2). At an enhanced "g" field the optimum Pr may be different. For different phase systems, we would hypothesise that there would be a different optimum Pr. Phase systems with a low density difference (like aqueous two-phase systems—ATPS) may require higher "g" to separate them, but a lower column rotation or Pr value to create gentle mixing as the phase system has such a low interfacial tension. The permutations and combinations are quite extensive and will require a lot of further research to understand fully. This may require a consortium of laboratories to conduct the necessary research. Nevertheless,

**Table 2**
Variation of stationary phase volume retention (Sf), resolution (Rs) and chromatographic efficiency ($N$) with the ratio of bobbin to rotor speed (Pr).

| Pr | Sf | Rs12 | Rs23 | Rs13 | N1 | N2 | N3 |
|---|---|---|---|---|---|---|---|
| 1.50 | 45.7 | 0.71 | 0.78 | 1.47 | 516 | 286 | 208 |
| 1.00 | 63.8 | 1.00 | 1.13 | 2.13 | 260 | 198 | 201 |
| 0.67 | 65.1 | 1.29 | 1.23 | 2.42 | 400 | 310 | 258 |
| 0.50 | 66.7 | 1.31 | 1.08 | 2.12 | 233 | 384 | 262 |
| 0.33 | 61.5 | 0.77 | 0.95 | 1.68 | 194 | 219 | 222 |
| 0.00 | 41.3 | 0.63 | 0.81 | 1.40 | 527 | 417 | 295 |
| −0.61 | 73.9 | 0.97 | 0.70 | 1.42 | 98 | 99 | 55 |

the reward for such effort could be step changes in efficiency of counter-current chromatography and an extension of the technology and its application to the separation of biologics either using tried and tested phase systems like ATPS [22] or the new phase systems involving ionic liquids [23]. For the latter the density difference is larger requiring lower "g", and hence slower rotor speeds are feasible, but the phases are more viscous calling for more mixing energy. In this case, as an extreme opposite to ATPS, maybe Pr ratios > 1.5 will be required.

## 6. Conclusions

The major objective was to design, construct and perform feasibility studies in preparation for a new generation of seal free dynamic extraction/chromatography devices that independently control mixing and settling, and this objective has been met. The new machine is a standard preparative twin column rotor built within a secondary anti-twist framework that allows the normally stationary sun gear to rotate and thus add or subtract to the independent rotation of the columns.

This new prototype allows the mixing between the two immiscible liquid phases to be varied independently from the settling process, thus enabling a radical new process technology for the efficient recovery and purification of the next generation of biologics based therapeutics. In the course of further work, the prototype will be used:

(1) For fundamental studies on the partitioning of these sensitive bioparticles.
(2) For more preparative studies (this study used sample loadings which were about 200× less than is feasible).
(3) For higher performance at higher speed (up to 1400 rpm—compared to 800 rpm used in this study).

The prototype centrifuge design was passed to the industrial collaborator, Dynamic Extractions Ltd. and is now the subject of a PCT Patent application GB2446129 "Non-synchronous Drive for centrifuges used in counter-current chromatography" [1]. The test results show that the new non-synchronous design can be up to 30% more efficient, in terms of increase in resolution and more than 90% more efficient in terms of theoretical plates, than conventional synchronous designs when the rotation of the column is slowed relative to that of the rotor. The test system uses a relative low interfacial tension phase system suggesting that column rotary speed controls mixing and hence can be adjusted for very low interfacial tension phase systems. Thus allowing the separation of very hydrophilic compounds and biologics by the tuning of this non-synchronous prototype. The results are so encouraging that a new EPSRC/BBSRC grant will be submitted for a more detailed study of all the variables involved with a much larger range of phase systems.

An unexpected bonus, in the early stages of this research work, was the use of toothed belts making the operation of the prototype significantly quieter. Since then toothed belt drives have been refined and improved making modern high g-level CCC instruments extremely quiet.

## Acknowledgements

We would like to express our thanks to Dr. Ito, Christopher Folkard, Tony Bunce and Dynamic Extractions Ltd. for their invaluable insight and assistance. The authors would like to gratefully acknowledge financial support from the UK BBSRC-Tools and Resources Programme Grant Ref. No. BB/E012949/1.

## References

[1] D. Hawes, GB Patent No. 2446129A, filed on February 2nd, 2007 and published on August 6th, 2008.
[2] Y. Ito, M. Weinstein, I. Aoki, R. Harada, E. Kimura, K. Nunogaki, Nature 212 (1966) 985.
[3] Y. Ito, P. Carmeci, I.A. Sutherland, Anal. Biochem. 94 (1979) 240.
[4] I.A. Sutherland, Y. Ito, Anal. Biochem. 108 (1980) 367.
[5] Y. Ito, G.T. Bramblett, R. Bhatnagar, M. Huberman, L.L. Leive, L.M. Cullinane, W. Groves, Sep. Sci. Technol. 18 (1983) 33.
[6] Y. Ito, P. Carmeci, R. Bhatnagar, S.B. Leighton, R. Seldon, Sep. Sci. Technol. 15 (1980) 1589.
[7] I.A. Sutherland, Rotating coil centrifuge, U.K. Patent Application No. 42034/78 (1978).
[8] J.M. Van Alstine, R.S. Snyder, L.J. Karr, J.M. Harris, J. Liq. Chromatogr. 8 (1985) 2293.
[9] T. Okada, D.D. Metcalfe, Y. Ito, Int. Arch. Allergy Immunol. 109 (1996) 376.
[10] Y. Ito, J. Chromatogr. 538 (1991) 3.
[11] K. Shinomiya, Y. Kabasawa, K. Yanagidaira, H. Sasaki, M. Muto, T. Okada, Y. Ito, J. Chromatogr. A 1005 (2003) 103.
[12] K. Shinomiya, Y. Ito, J. Liq. Chromatogr. Rel. Technol. 27 (2004) 3243.
[13] K. Shinomiya, K. Kobayashi, H. Oshima, T. Okada, K. Yanagidaira, Y. Ito, J. Liq. Chromatogr. Rel. Technol. 30 (2007) 2681.
[14] K. Shinomiya, T. Okada, Y. Ito, J. Liq. Chromatogr. Rel. Technol. 28 (2005) 835.
[15] K. Kobayashi, H. Oshima, K. Shinomiya, Y. Ito, J. Liq. Chromatogr. Rel. Technol. 28 (2005) 1839.
[16] P.L. Wood, J. Chromatogr. A. (accepted for publication).
[17] Dynamic Extractions Ltd., www.dynamicextractions.com.
[18] A. Berthod, Countercurrent Chromatography, The Support-Free Liquid Stationary Phase: Comprehensive Analytical Chemistry, vol. 38, Elsevier, Amsterdam, 2002.
[19] I.J. Garrard, L. Janaway, D. Fisher, J. Liq. Chromatogr. Rel. Technol. 30 (2007) 151.
[20] I.A. Sutherland, D. Heywood-Waddington, Y. Ito, J. Chromatogr. 384 (1987) 197.
[21] J. Friesen, G. Pauli, J. Chromatogr. A 1216 (2009) 4225.
[22] I.A. Sutherland, Curr. Opin. Drug Discov. Dev. 10 (2007) 540.
[23] A. Berthod, S. Carda-Broch, Anal. Bioanal. Chem. 380 (2004) 168.