# **Review of centrifugal liquid-liquid chromatography using aqueous two-phase solvent (ATPS) systems:** its scale-up and prospects for the future production of high value biologics.

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## Abstract

The future challenges in bioprocessing include developing new downstream processes for the purification and manufacture of the protein based medicines of the future to relieve the predicted bottleneck being produced by increasingly high titres from fermentation processes. This review looks at the recent developments in centrifugal liquid-liquid partition chromatography using aqueous two-phase solvent (ATPS) systems, a gentle host medium for biologics, and the prospect for scale-up and eventual manufacture of high value pharmaceutical products.

**Keywords:** Aqueous two-phase solvents, ATPS, aqueous-aqueous polymer phase systems, centrifugal partition chromatography, CPC, Countercurrent chromatography, CCC, HSCCC,

## Introduction

There have been significant and sustained advances in the development of both analytical and industrial scale liquid-liquid chromatography since the beginning of the millennium. In October 2006 I wrote a review article on recent progress on the industrial scale-up of counter-current chromatography (CCC) [1]. This charted the excellent progress being made by three companies: Dynamic Extractions Ltd in the UK with their 4.6 litre Maxi-DE centrifuge and Armen Instrument and Partus Technology in France with 12.5 litre and 25 litre Centrifugal Partition Chromatography (CPC) centrifuges respectively. Whereas Dynamic Extractions Ltd demonstrated a kilogram purification of glucoraphanin, a cancer chemotherapy agent required in large quantities for trials, the other two companies were unable to offer any separations that they could put in the public domain.

Margraff of Partus Technologies SA reported at CCC2004 in Tokyo how well their 25 litre CPC instrument retained both organic-organic and aqueous-aqueous phase systems [2]. They used naphthalene with a heptane-acetonitrile (1:1) phase system with an identical geometry reduced sized 5 litre rotor with 146 cells (instead of 766 for the 25 litre rotor) to demonstrate at 1300rpm and a flow of 200ml/min that they got 177 theoretical plates (ie more than the number of cells) with a back pressure of 43 bar and stationary phase volume retention of 84%. They attributed this high efficiency to the Multiknit ® tampons they inserted in each cell which increased their efficiency by 50% by increasing mixing and preventing streaming down the cell wall induced by the Coriolis effect [3,4]. They compared this efficiency with the standard cell design of the 5 litre Sanki LLI-7 which gave an equivalent efficiency of 0.38 theoretical plates (TP) per cell. When they scaled up to their 25 litre centrifuge they found that their efficiency only dropped to 610 theoretical plates with a stationary phase retention of 75% despite increasing the flow to 500ml/min and reducing the speed to 900rpm to keep the pressure manageable at 93 bar. They claimed that having 1.2 cells to achieve one theoretical plate was much better than classical CPC centrifuges that required between 3-5 cells per TP. They also tested their 25 litre CPC with aqueous two-phase solvent (ATPS) systems – PEG1000 and dibasic potassium phosphate (12.5%: 12.5% w/w) using Myoglobin as a test protein. They set up their stationary phase equilibrium at 700rpm and a flow rate of 200ml/min to give a stationary phase volume retention of 62.8% at 70 bar back pressure. They then increased the speed to 800rpm and the flow to 300ml/min (84 bar), injected 250ml of myoglobin (1% of column volume) in a 1:1 mixture of both phases at 2 g/L and eluted the myoglobin after 14 litres retention volume (45 minutes retention time) with a theoretical plate count of 580. They “temporarily” claimed [2] that the new cell design made the Partrition 25 CCC hydrostatic instrument especially suitable for protein purification and said that these preliminary conclusions will be firmly established as more experimental data becomes available, but I asked Magraff if there was more application information on this instrument and was told that there was none in the public domain.

Meanwhile, the Armen 12.5 litre CPC – the Elite Continuum industrial scale instrument had been moved from the Institut Français du Pétrole (IFP) to Archimex in Vannes (Figure 1). I contacted Francois Couillard of Armen Instrument to ask if there was any more application information available on his 12.5 litre instrument and was told that it was still in the process of being installed at Archimex. I told him that my PhD student, Emma Bourton, was getting some interesting fractionations of Myoglobin and Lysozyme using their Laboratory Scale CPC instrument (Figure 2). We were in the process of doing some loading studies under our BBSRC Protein purification grant and would be interested to know if separations performed on the 1 litre CPC instrument would scale up to the 12.5 litre one. François Couillard agreed

that it would be nice to know too, so a short working visit was arranged for the last week of May 2007 and installation of the 12.5 litre instrument was completed just in time. The Brunel team (Emma Bourton, Peter Hewitson and Derek Fisher) completed the first phase of the optimisation and sample loading study and concluded that good resolution (Rs=1.3 between Myoglobin (N=283) and Lysozyme (N=223) could be achieved using a single rotor (500ml – 427ml cell volume) with a 10% column loading (40ml) of 2.2g/L of both Myoglobin and Lysozyme in a 1:1 mixture of upper and lower phase (Figure 3). The phase system was 12.5% w/w PEG1000 and 12.5% dibasic potassium phosphate (ie same as used by Partus) with the lower salt-rich phase the mobile phase. The 1 litre Armen Elite centrifuge was operated at 2000rpm (224g) and at a flow rate of 10 ml/min. Linear scale up to the 12.5 litre Armen Elite Continuum was planned which would require using just a single rotor (6 litre total volume) and a sample loading of 500ml at the same concentration with a mobile phase flow rate of 125ml/min. Sandwich injection (where the sample is injected just ahead of the mobile phase being pumped through the system) was employed for both runs. No optimisation was performed on the large industrial scale instrument. This is the first example [5] of rapid optimisation on a small lab/prep device being successfully scaled up to a pilot scale instrument with excellent peak resolution (Rs=1.88) between Myoglobin (N=163) and Lysozyme (N=276) – see Figure 4. It is even more impressive as optimisation results have been transferred from a different laboratory, with different operators and in a different country with different suppliers. The fact that this scale up was possible creates a benchmark for the large scale purification of biomolecules and makes a review of other protein purifications using ATPS and other applications using CPC, much more meaningful.

**Liquid-liquid chromatography columns.**

As a general rule, a liquid-liquid chromatography column can be treated just like a liquid-solid column and used with the same type of liquid chromatography setup (ie pumps, injection ports, spectrophotometers). Some users have a separate pump for the stationary phase and a valve to switch between the two, others have both phases equilibrated in a big bottle and move the suction lead from one liquid phase to the other when required. In liquid-liquid chromatography, there are a few simple rules that need to be known before starting, but otherwise the usual chromatography skills will be all that is required.

Having a liquid stationary phase can have a number of advantages [6] – there is no non-specific adsorption to a solid support; there is no risk of fouling (although with CPC and its fine interconnecting tubes some prefiltration is required); there is a much higher sample loading capacity as the volume normally taken up by the solid support is occupied by the liquid stationary phase which has capacity for solubilising your sample; hence higher throughputs and less solvent usage and tolerance of particulates. One disadvantage to having a liquid stationary phase is that you generally have to have a special mechanism to hold the stationary phase in place and make it possible for the mobile phase to flow through and past it in such a way that there is good mixing and mass transfer between the phases. However once this is accepted then the delights and advantages of working with liquid stationary phases can be realised: ease of scaling up using the same principle of purification is one of them and having such a large variety of phase systems to choose from is another. This latter advantage, once solvent selection methods are mastered can lead to considerable cost savings as there are no expensive solid supports. Liquid-liquid extraction columns [7] have been around for some time and are generally operated in unit gravity. These will not be considered here. Only the centrifugal methods of retaining the stationary phase will be reviewed as these are by far the

most common and the ones that are leading to high resolution, high performance industrial scale liquid-liquid chromatography.

There are two basic types of liquid-liquid chromatography columns: hydrodynamic and hydrostatic. I have described the difference between these in my last review [1] but I will still give a basic summary here for clarity. Both hydrodynamic and hydrostatic columns have two immiscible phases that form an upper (lighter) phase and a lower (heavier) phase. The column (total volume, $V_c$) is initially filled with the phase intended to be the stationary phase and the centrifuge switched on at a given rotational speed to retain this liquid phase once the mobile phase flow starts. Flow of the mobile phase will displace the stationary phase until hydrodynamic equilibrium has been reached between the two phases. Measuring the amount of displaced stationary phase ($V_d$) will give information on how much mobile phase ($V_m$) has been pumped into the column and hence, with knowledge of the volume of inlet and outlet leads ($V_{in} + V_{out}$), the volume of stationary phase ($V_s$) retained in the column will be known. This is often expressed as a retention factor (Sf=Vs/Vc). Sample injected with the mobile phase will then be predictably eluted according to its distribution ratio, $K_d$ (or partition coefficient). For example the volume retention of a $K_d$=0 substance will elute in the mobile phase volume ($V_m$) and the volume retention of a Kd=1 substance will be eluted in the system volume ($V_c = V_s+V_m$). The $K_d$=2 peak will elute at $2V_s+V_m$ and so on. The main difference between the two types of liquid-liquid chromatography is that when the flow stops in hydrodynamic CCC the heavy and light phases move to opposite ends of the column and in hydrostatic CCC they stay where they are. Also the mixing between the two phases is qualitatively different: a gentle form of wave mixing in hydrodynamic CCC and a more vigorous cascade mixing in hydrostatic CCC.

**The different varieties of hydrostatic and hydrodynamic columns**

***Hydrostatic Columns.*** In hydrostatic CCC there is a series of chambers mounted circumferentially round a disc interconnected by small connecting links from the bottom of one chamber to the top of the next (where the bottom is defined as furthest and the top as nearest from the centre of rotation on the centrifuge such that the heavy phase is thrown to the "bottom" or outer part of the chamber). The chambers (the active elements) and the interconnecting tubing (the passive elements) are initially filled with the phase intended to be the stationary phase. The disk is then rotated. The mobile phase is passed to the disc via a rotating seal. If the mobile phase is the upper phase it is flowed in "Ascending" mode so that it cascades up through the retained stationary lower phase in each chamber (up being toward the centre of rotation) until it elutes from the other end via a second rotating seal. If the lower phase is the mobile phase the flow is reversed and the operation is "Descending" mode where the lower phase cascades down through the retained upper phase in each chamber. If the flow is stopped for any reason, the stationary phase will remain trapped in each individual chamber. These hydrostatic centrifuges are often referred to as centrifugal partition chromatographs (CPC) and the most common form of construction is for the chambers to be etched or machined circumferentially round a disc and the discs stacked up one against the other to form a long rotor of serially interconnecting chambers. There can be as many as 1000 chambers connected in this way. A hydrostatic pressure will build up as the mobile phase slowly displaces the stationary phase and sets up a hydrostatic pressure difference in each chamber which is a function of the density difference between each phase, the distance from the inflow to the interface in each chamber and the "g" field times the number of chambers connected in series.

***Hydrodynamic Columns.*** In hydrodynamic CCC the liquid stationary phase is held in a stratified way along the length of a continuous piece of tubing. The mobile phase flows past this stationary phase and experiences a series of mixing and settling steps as it makes its way from one end of the tubing to the other. The sample is injected with the mobile phase (in either upper or lower phase) and elutes from the other end of the tubing at a time governed by how well it "partitions" between the mobile and stationary phases. If it is only soluble in the mobile phase ($K_d$=0) it will go through with the mobile phase, but if it is only soluble in the stationary phase (Kd=∞) it will stay in the column. However unlike solid phase chromatography the centrifuge can always be stopped and the stationary phase pumped out and retained substances recovered.

The most common (and simplest) form of hydrodynamic CCC column is the multilayer (J-Type) coil planet centrifuge, which consists of a planetary rotor which rotates in synchronised (1 to 1) planetary motion about the main axis of rotation. If you can imagine a gear meshing with and rotating around an identical gear then the rotating gear is the planetary one on which a bobbin is mounted and on which a continuous length of tubing can be wound. The tubing forms a multilayer helix which as it rotates causes the liquid phases to screw up to the head end of the coiled tube. But if the tube is closed then one phase displaces the other liquid phase to the opposite end, just as upper phase is displaced upwards in a test tube by the heavy liquid phase. In hydrodynamic CCC the general rule is that the heavy phase goes to the "Tail" while the lighter phase goes to the "Head". If the mobile phase is the lower phase then it is pumped from Head to Tail and if it is the upper phase it is pumped from Tail to Head.

The cross-axis coil planet centrifuge is similar to the J-type except the axis of rotation is displaced from the planetary axis to create a more complex motion which can enhance mixing for more viscous phase systems. This is known as the X-type coil planet centrifuge. There are others, which have been described by Ito [8], but it is not necessary to describe these here.

It is possible to create hybrid centrifuges where the tubing is wound in such a way the stationary phase can get trapped and the process becomes a hydrostatic one

**The toroidal coil centrifuge (TCC).**

This is one of the simplest forms of hydrostatic CCC where a continuous length of tubing is wound on a flexible nylon former and positioned circumferentially on a disc which is rotated to create a "g" field of between 70-200x unit gravity. While the first toroidal coil centrifuge was developed by Ito [9] the majority of early research using ATPS systems was performed by the author and Deborah Heywood Waddington at the National Institute for Medical Research in the 1980s [10-17]. More recently, Ito himself with co-workers [18,19] looked into the effect of the Coriolis force on mixing in a toroidal coil centrifuge and found that if the coils were wound in such a way as to align the cascade mixing to be "parallel" with the mixing zone in the tubing, much better resolution could be obtained with ATPS and could be sustained at much higher flow rates, compared to when it crosses the tube and runs down the side. He also showed that mass transfer rates reduce linearly with the log of the molecular weight of the protein, resulting in band broadening for larger molecular weight samples. He demonstrated this with some of the first separations of proteins using cytochrome C, myoglobin and lysozyme as a model system [19] with a 12.5% w/w PEG1000 and 12.5% dibasic potassium phosphate ATPS system using a 60m long tubing of 0.55mm diameter wound on a 1.5mm od nylon pipe to give 10,000 loops with total capacity 13ml. He achieved

resolutions between myoglobin and lysozyme of 1.5, 1.19 and 0.79 for flows of 0.05, 0.1 and 0.2 ml/min in 0.5mm tubing respectively, rotating at 1200rpm (270g).

**High Speed Counter- current Chromatography (HSCCC) – "J" Type**

Even as long ago as 1988, Ito and Oka [20] were demonstrating protein purifications of cytochrome C and lysozyme (Rs=2.6 in 6 hours) using ATPS (12.5% PEG 1000 & 12.5% anhydrous dibasic potassium phosphate) at 1ml/min and 800rpm with a 200ml column of 25mx1.6mm id. However these were not multilayer coils with wave mixing, but eccentrically mounted toroidally wound coils (on 4 x 12.5 cm long 1.25cm SS tubes) offset from the planetary centre by 3.5cm to give a hydrostatic cascade mixing. Similar coils were used for the semi-preparative purification of endogenous ligand for brain serotonin-2 receptors [21]. In 1991, Shibusawa & Ito [22] compared two different types of CCC instrument – a cross-axis coil planet centrifuge (X-type) and a horizontal coil planet centrifuge (J-Type). The Horizontal Coil Planet centrifuge had 8 eccentrically wound coils per bobbin of 1.6mm bore (beta=0.3) giving Rs=2.22 between cytochrome C and myoglobin and 0.43 between myoglobin and ovalbumin, but they were using Sigma ovalbumin that later turned out to have both monomer and dimmer forms [23,24]. Equivalent results on the cross axis instrument were better (Rs=2.26 and 0.82 respectively) but might not merit the extra complexity of the device. Successful separations have been performed using HSCCC "J" Type centrifuges on horseradish peroxidise [25], alpha amylase [26] and plasmids [27], however, recent research in my own laboratory by Hugh Guan [28] has shown excellent retention in multilayer coils using CCC, with wave mixing, but there is poor mass transfer due to poor mixing between the phases – hence the need for eccentrically mounted hydrostatic CCC coils to enhance mixing or a different approach like non-synchronous coil planet centrifugation.

**Non-Synchronous coil planet centrifuge**

The non-synchronous coil planet centrifuge allows the planetary coil to rotate at a slow speed (10rpm) while the main rotor rotates at a higher speed (800rpm) [29-31]. This allows the main rotor speed to determine the "g" field which control retention of the stationary phase and the planetary rotor to control the mixing. Shinomiya et al [29] have performed some excellent protein separations of Cytochrome C, Myoglobin and Lysozyme achieving a resolution between Myoglobin and Lysozyme of 1.83 at 0.2ml/min with the lower phase mobile with the speeds set in reverse mode at the above values. However, this was with co-axial multilayer coils. Eccentrically mounted coils were not so good reducing the resolution to 0.97.

**Cross-axis Coil Planet Centrifuge – "X" Type)**

In 1998 Shinomiya et al [32], using a 12.5% PEG1000: 12.5% dibasic potassium phosphate ATPS system, found eccentric coils (0.85mm) slightly better than the toroidal ones, but it is not clear how they coped with the toroidal effect in these X-axis coils with such a complicated force field. Later using 1mm coils they compared eccentrically with toroidally wound coils [33]. Lower phase mobile was better than upper phase mobile and T(C) > H(P) was slightly better than H(C) > T(P) (Rs= 1.19 and 1.23 for eccentric and 1.19 and 1.67 for toroidal for H>T and T>H respectively).

Later in 2000 they used the cross axis CPC with PEG-Dextran Phase systems [34] – with the eccentric coils above they got fractionations of myoglobin and lysozyme at various salt concentrations that were not as good as they had got with PEG-salt but they did demonstrate that it was possible to run with PEG-Dextran systems which opened up possibilities of working with a wider range of proteins that partition well in such systems.

Sets of spiral column assemblies [35] did not seem to be any better than the toroidal or eccentrically mounted ones tested earlier, but could enable spiral discs to be machined from solid and stacked.

Other applications using cross-axis coil planet centrifuges were purification of single stranded DNA [36] and a one step purification of histone deacetylase [37], both from *Escherichia coli* lysate.

A new small-scale cross-axis coil planet centrifuge was reported in 2006 [38] which was about half the size of previously reported centrifuges with a capacity of only 54ml, but rotating at 2000rpm with a "g" field of 537g. Some excellent fractions of cytochrome C, myoglobin and Lysozyme were demonstrated with the 12.5% PEG1000: 12.5% dibasic potassium phosphate ATPS system.

**Centrifugal Partition Chromatography (CPC)**

In 1990, Foucault and Nakanishi published a comparison of several aqueous two-phase solvent systems (ATPS) for the fractionation of biopolymers by CPC [39]. Surprisingly there are only a few applications of using ATPS in the CPC [40-42] and only one for proteins giving not very high resolution separations of BSA and lysozyme [41]. A recent paper describes a rather novel CPC device made using toroidally wound tubing mounted circumferentially (eccentrically) round a disk that is rotated in a centrifugal field [43]. This would create cascade mixing much like a toroidal coil centrifuge (CCC). They demonstrate separations with a wide range of phase systems including ATPS, but their Myoglobin/Lysozyme resolutions are not as good as Shinomiya's using his cross-axis coil planet centrifuge [23-24, 32-35].

Since CPC was first introduced in 1982 [44], early use focussed on determining octanol-water partition coefficients [45-47]. Solvent systems [48-51], temperature [52], mass transfer [53] and gradient elution [54] were important areas of study. Applications were various, mainly using aqueous organic phase systems, focussing on heavy metals [55-61] and natural products [62-83]. Preparative isolations became more possible once ion-exchange displacement centrifugal partition chromatography was developed [84-87]. Chiral applications were another important preparative development [88-90].

However, the most important advances in the technology have come from research into the hydrodynamics and kinetics of mixing. Starting with van Buel as early as 1988 [91] with his stroboscopic studies, and later the recognition of the importance of the Coriolis effect [92-93] culminated in a very detailed and definitive study by Luc Marchal et al [94] that has defined the recent breakthrough in cell (chamber) design paving the way for the scale-up and increased efficiency of the process. In addition, a very thorough and readable review of the history of centrifugal partition chromatography was published by Marchal, Legrand and Foucault in 2003 [3], but did not mention applications or potential applications with ATPS.

However, there are a couple of reviews on the use of ATPS in chromatography. One is by Menet [96] and the other by Shibusawa [96]. Kula reviewed the trends and potential industrial application of ATPS as long ago as 1990 [97], when ATPS systems were being used in large industrial scale countercurrent extraction columns [98]. With the development of today's new industrial scale CCC equipment these papers may well be worth revisiting.

**Comparison of different centrifuge designs used for fractionating proteins.**

Table 1 lists the operational conditions and resolutions obtained using 1) Cytochrome C or BSA, 2) Myoglobin and 3) Lysosyme as test samples. It should be noted that all CCC instruments used were working in some form of hydrostatic mode with cascade mixing – even using HSCCC, which normally uses multilayer coils, eccentrically mounted toroidal coils were necessary to achieve the appropriate mixing between ATPS systems [20,22]. It is difficult to make direct comparisons when the operating conditions vary so much, but all separations in this Table 1 use the same 12.5%PEG1000: 12.5% dibasic potassium phosphate ATPS system with lower phase mobile and some combination of cytochrome C, Myoglobin and Lysozyme, apart from the Sanki CPC [41] which used BSA and Lysozyme in a PEG6000 and mixture of dibasic and monobasic potassium phosphate. However the BSA (like Cytochrome C) tends to elute with the solvent front, so is comparable.

The general conclusion from this table is that there is not too much to choose between the different techniques at the analytical scale except that the non-synchronous coil planet centrifuge seems to give the best resolution, possibly because mixing can be controlled independently from phase retention. However there have been no attempts to scale up any of these techniques and so there is an opportunity still waiting to happen.

While the Sanki CPC [41] separation of BSA and Lysozyme in comparison with the other CCC technique's separations of Cytochrome C and Lysozyme is of a similar order, the important advance with the new cell (chamber) designs is that it is now possible to get higher efficiency separations at much higher throughputs and with much higher sample loadings [5].

**Conclusion.**

The increasingly high titres being predicted from fermentation processes are highlighting a bottleneck in down stream processing capability. There is an urgent need for new approaches which can address the processing requirements for the production of high molecular weight biologics. Liquid-liquid chromatography using aqueous two-phase solvent (ATPS) systems is one approach and this review can conclude that promising steps are being made on scale-up of centrifugal partition chromatography to a level where it could be extremely valuable for high value-added products. Currently the continuous tube approaches using CCC have not yet been scaled up but offer advantages if they are. One limitation is still the limited solubility which for ATPS is in the order of a few g/L compared to two orders of magnitude higher with aqueous-organic phase systems. But research on new two-phase systems is underway [99] with two-phase liquid systems based on ionic liquids having significant promise and reverse micelle approaches which also offer potential for higher throughput [100]. The advances in the scale-up of liquid-liquid chromatography plus new research effort on phase system development to exploit such technology offers the potential for significant advances in the field that could be as significant as the emergence of preparative and industrial scale chromatography in the early 1990s.

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| Centrifuge | Vc (ml) | Sample (%CV) | d (mm) | L (m) | N (rpm) | F (ml/min) | Rs12 | Rs23 | Rs13 | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| TCC | 13 | | 0.55 | 60 | 1200 | 0.05 | 1.56 | 1.50 | 2.58 | [19] |
| | 13 | | 0.55 | 60 | 1200 | 0.10 | 0.97 | 1.19 | 1.97 | [19] |
| | 13 | | 0.55 | 60 | 1200 | 0.20 | 0.77 | 0.79 | 1.39 | [19] |
| HSCCC | 200 | | 1.6 | 100 | 800 | 1.00 | | | 2.60 | [20] |
| | 220 | | 1.6 | 109 | 800 | 0.50 | 2.22 | | | [22] |
| | 220 | | 1.6 | 109 | 800 | 0.70 | 1.43 | | | [22] |
| | 220 | | 1.6 | 109 | 800 | 1.10 | 0.73 | | | [22] |
| Non-Synch CPC - coaxial | 39 | 2.6 | 0.8 | 78 | 800/10 | 0.20 | 1.62 | 1.83 | 3.18 | [29] |
| -eccentric | 20 | 5.0 | 0.8 | 40 | 800/10 | 0.20 | 0.53 | 0.97 | 1.51 | [29] |
| X-axis CPC -Eccentric | 28 | 3.6 | 1 | 36 | 800 | 0.20 | 0.91 | | | [24] |
| Eccentric - T>H Outward | 27 | 3.7 | 1 | 34 | 800 | 0.20 | | 1.21 | | [33] |
| Toroidal T>H Outward | 29 | 3.4 | 1 | 37 | 800 | 0.20 | | 1.70 | | [33] |
| CPC - 230ml | 230 | 0.4 | | | 1200 | 0.80 | 1.73 | | | [41] |
| CPC - 1 litre | 500 | 2 | | | 2000 | 5.00 | | 3.25 | | [5] |
| | 500 | 2 | | | 2000 | 5.00 | | 2.26 | | [5] |
| | 500 | 10 | | | 2000 | 5.00 | | 1.28 | | [5] |
| CPC - 12.5 litre | 6250 | 10 | | | 1293 | 125.00 | | 1.88 | | [5] |

**Table 1:** *Comparison of the different types of hydrodynamic and hydrostatic centrifuges when fractionating 1) Cytochrome C or BSA, 2) Myoglobin and 3) Lysozyme.*

**Figure 1:** *The 1 litre Centrifugal Partition Chromatography set up in the Advanced Bioprocessing Centre at Brunel Institute for Bioengineering, Uxbridge, UK*

**Figure 2:** *The Armen 12.5 litre Centrifugal Partition Chromatography system at Archimex in Vannes, France.*

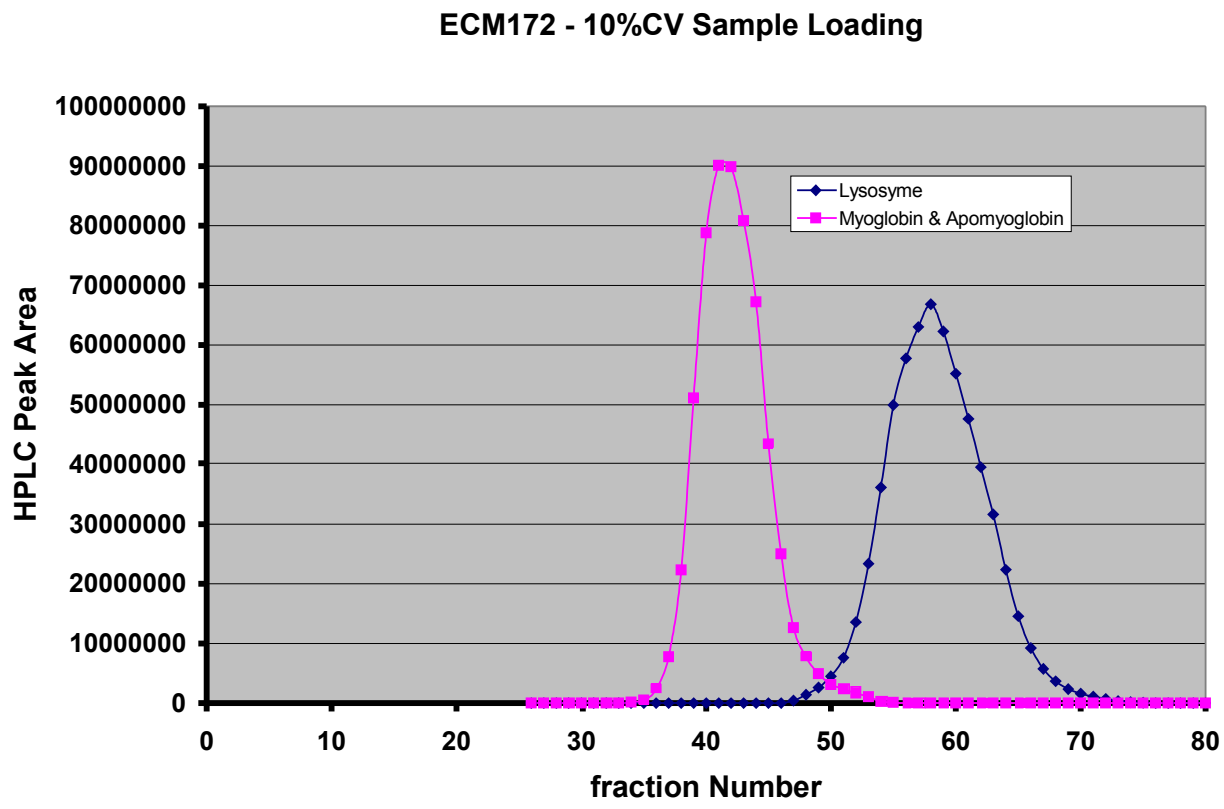


**Figure 3:** *Fractionation of Myoglobin and Lysozyme using the 1 litre Centrifugal Partition Chromatograph Unit. Experimental set up: Total column volume (single rotor): 500ml (429ml active; 71ml passive); Phase system: 12.5%PEG1000:12.5% biphasic potassium phosphate ATPS; Speed – 2000rpm (224g); Flow of lower mobile phase: 10ml/min; Sample loading: 90mg lysozyme, 90mg myoglobin in 40ml (~10% CV) 50:50 ATPS mix; Stationary phase retention: breakthrough – 52%; end – 19%.*

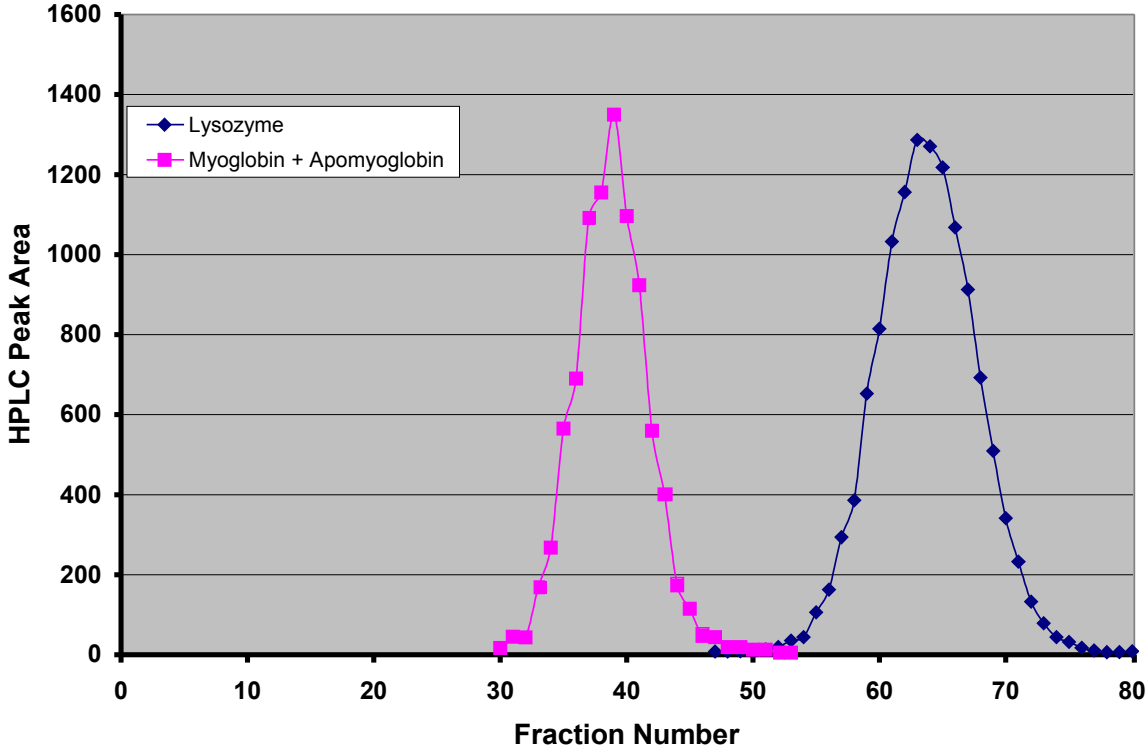


**Figure 4:** *Fractionation of Myoglobin and Lysozyme using the 12.5 litre Centrifugal Partition Chromatograph Unit. Experimental set up: Total column volume (single rotor): 6.0 litres (4.412L active; 1.588L passive); Phase system: 12.5%PEG1000:12.5% biphasic potassium phosphate ATPS; Speed – 1293rpm (224g); Flow of lower mobile phase: 125ml/min; Sample loading: 1.1g lysozyme, 1.1g myoglobin in 500ml (~10% CV) 50:50 ATPS mix; Stationary phase retention: breakthrough – 63%; end – 22%.*