Novel liquid-liquid scalable extraction/chromatography technology

December 3rd, 2008

Professor I.A. Sutherland

Delft, Utrecht, Rotterdam, BPP2005

- Introduction to industrial scale centrifugal liquid-liquid extraction and its *potential* for the purification and manufacture of proteins and biologics
- Reviewed Hydrostatic & Hydrodynamic Countercurrent Chromatography
- *Prospect* for true moving bed chromatography

Outline of Today's Talk

- Some advantages of working with liquid stationary phases
- Some additional not well known versatilities
- Quick review of high-resolution, hydrodynamic counter-current extraction
- Quick introduction to aqueous two-phase systems (ATPS)
- Focus on high-resolution, hydrostatic counter-current extraction using ATPS in isocratic mode
- Introduction to intermittent true moving bed extraction
- Other high resolution continuous extractions schemes in the offing

Key Advantages of a Liquid Stationary Phase

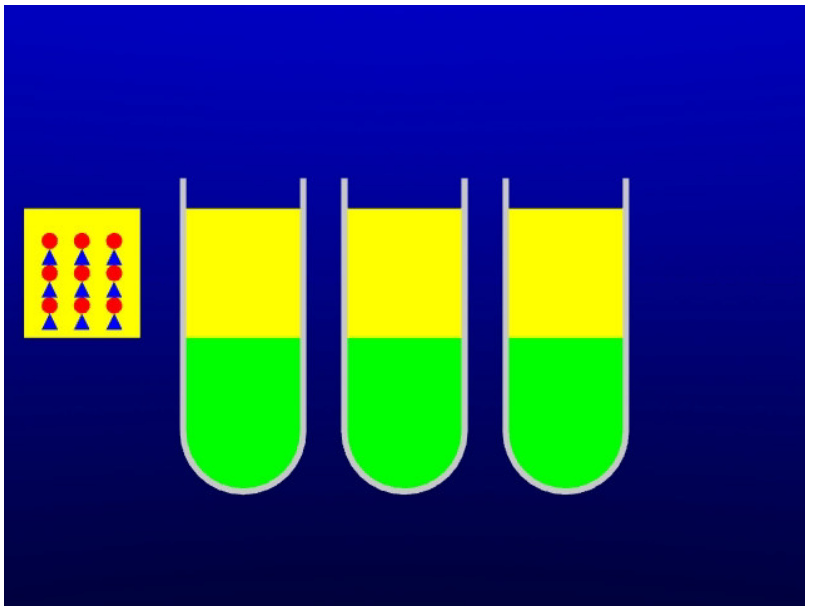
- **Total (100%) sample recovery –** liquid-liquid nature of the process means it is gentle and versatile and there is no irreversible adsorption to a solid support
- **Improved sample solubility** – both upper and lower phase available
- **Quick due to higher throughput** – High flow, low pressure system
- **Inexpensive & a Green Technology** compared to HPLC (significantly less solvent usage and no expensive solid supports)
- **Crude extracts,** including particulates can be handled reducing the number of separation steps in a process and reduced sample preparation requirements
- **High loading capacity** due to liquid nature of stationary phase
- **Easy & predictable scale-up** so that scale-down to scale-up philosophy can be applied
- **Low pressure system** – so columns can be lengthened to increase resolution and widened to increase capacity and throughput
- **Normal and reversed-phase operation** and the switching between them gives expanded operational capability – elution extrusion, dual mode, true moving bed

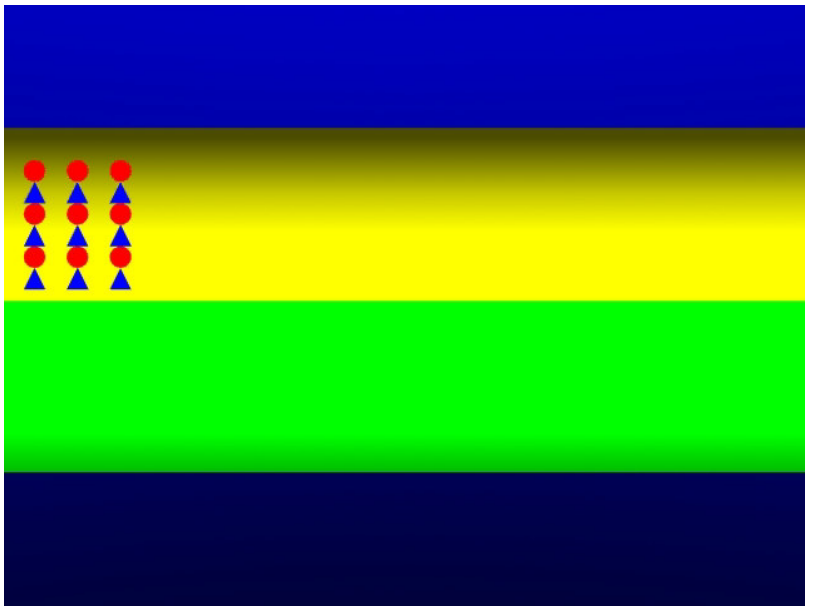
Other Little Known Advantages

- **Isocratic operation**
  - Either phase can be mobile (normal or reverse phase
  - The process can be switched half way through a run
  - The process can be stopped and the retained components pumped out (elution/extrusion – Berthod)
- **Co-current Chromatography/Extraction**
  - Both phases can be pumped at the same time in the same direction
- **Counter-current Chromatography/Extraction**
  - Both phases are simultaneously flowed in opposite direction
  - Continuous processing of closely related binary compounds

Hydrodynamic Counter-current Extraction

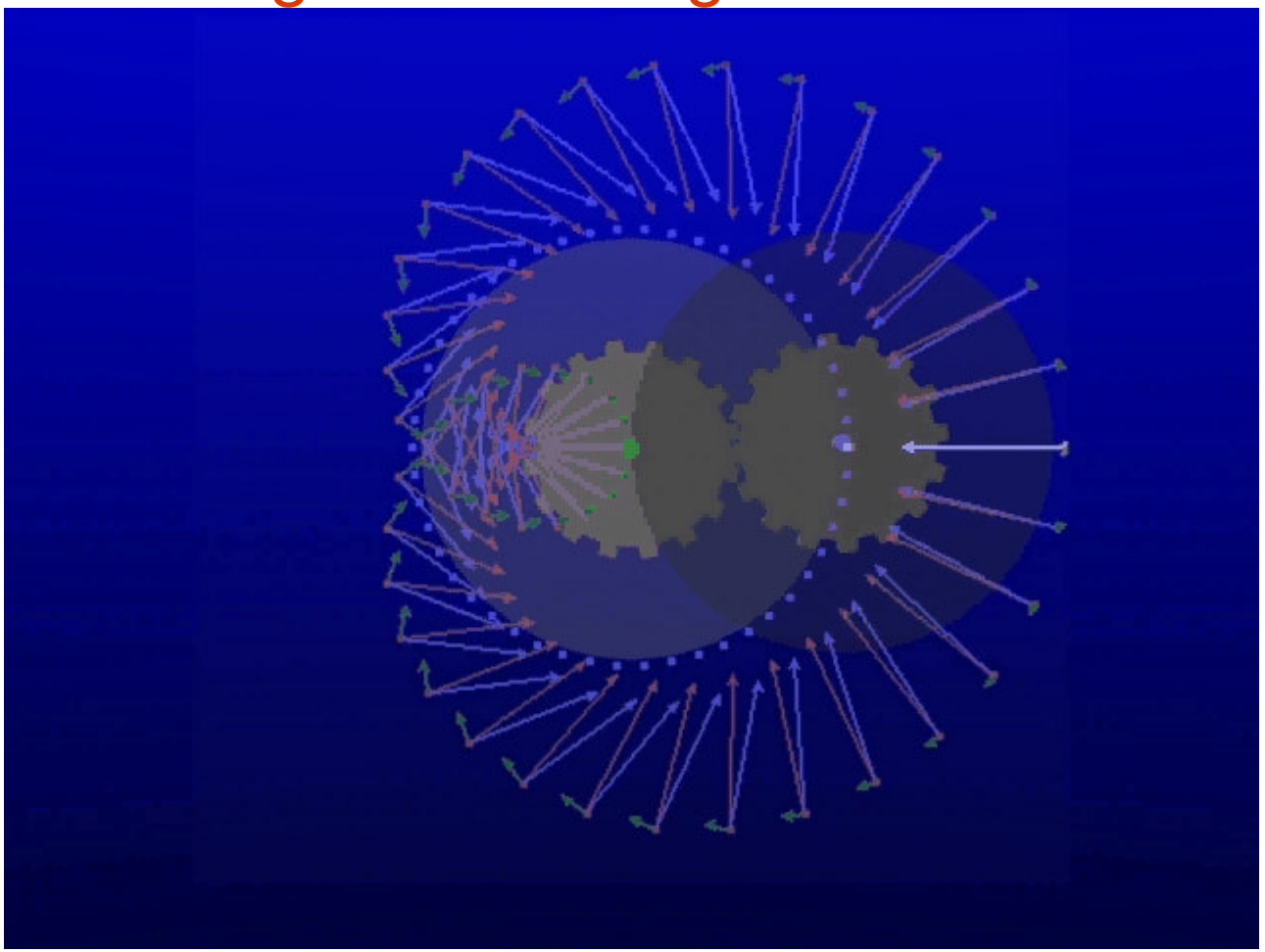
New Dynamic Extraction/Chromatography Technology



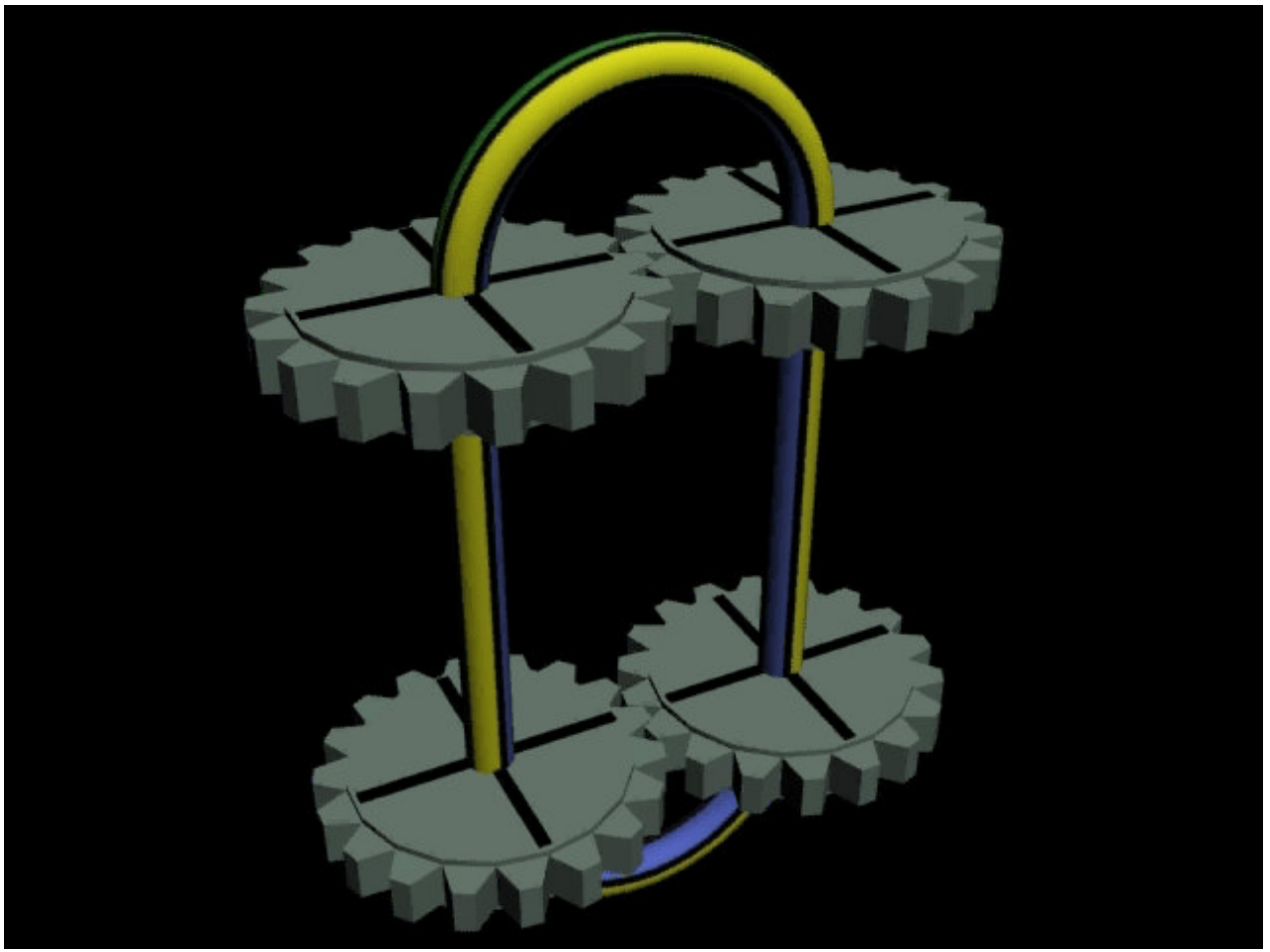


- **Developed now as a fast, robust, continuous process with separations in minutes as opposed to hours with previous technology**
- **Small molecules – Natural Products**
- **Potential for application to proteins, peptides, plasmids and other biologics**

Coil Planet Centrifugation producing mixing and settling zones along a continuous tube



Twist free connection to rotor



DE Centrifuges - Predictable Scale-up

CCC-Device	Bore	Area	B	Mean Flow	Flow at SP Retention (Sf) given below				
					92.5	90	85	80	70
Mini-DE	0.8	0.50	-37.01	0.50	0.04	0.07	0.16	0.29	0.66
Midi-DE1	1.6	2.01	-11.22	2.00	0.45	0.79	1.79	3.18	7.15
Midi-DE2	3.68	10.64	-3.13	10.00	5.74	10.21	22.97	40.83	91.87
Maxi-DE	10	78.54	-0.82	80.00	83.66	148.72	334.62	594.88	1338.49

Milli-CCC – R=50mm, Vc=5-17ml

Brunel-CCC – R=110mm, Vc=0.1-1 litre

Maxi-CCC – R=300mm, Vc=4.6 litre

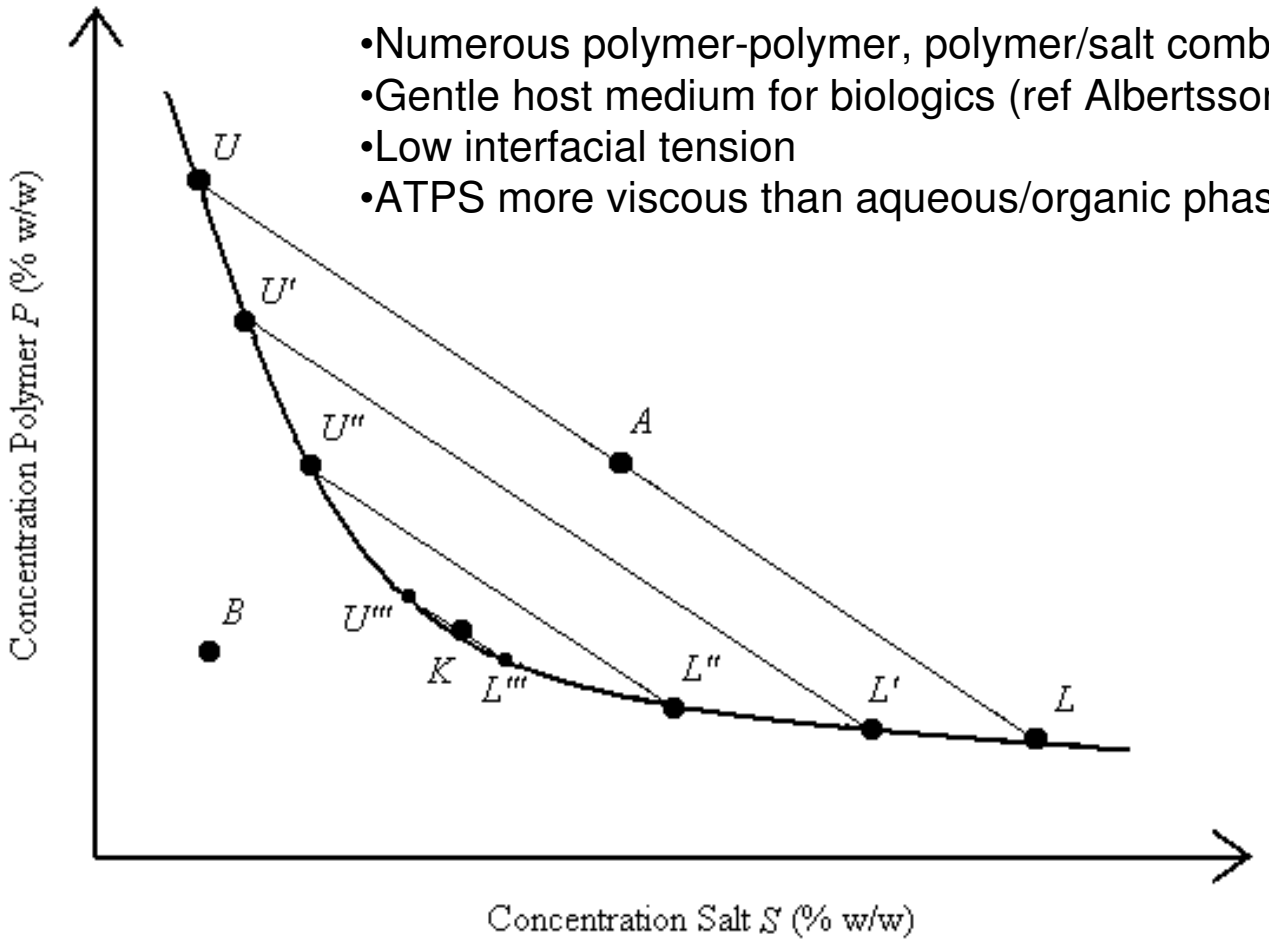
Hydrodynamic Counter-current Extraction

- **Continuous tube with wave mixing**
- **Stop the pump & liquid phases move to opposite ends of the tubing**
- **Ideal with low viscosity aqueous organic phases**
- **Hydrodynamic equilibrium between the mobile phase and the retained liquid stationary phase**
- ***Kg scale in days have been demonstrated with aqueous/organic phase systems***
- ***Feasibility of continuous countercurrent extraction has now been demonstrated at a pilot scale***

Aqueous Two-Phase Polymer Systems (ATPS)

Aqueous Two-Phase Systems (ATPS)

- Numerous polymer-polymer, polymer/salt combinations
- Gentle host medium for biologics (ref Albertsson, Kula, Hubbuch)
- Low interfacial tension
- ATPS more viscous than aqueous/organic phase systems



Cascade v. Wave Mixing

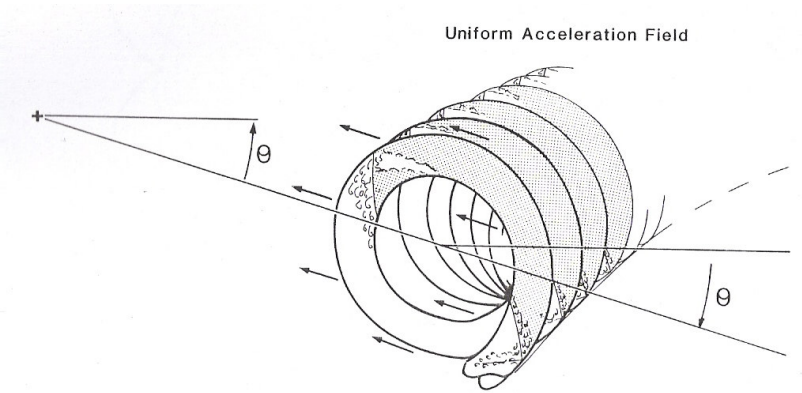


Fig. 1. Synchronous coil planet centrifuge—cascade mixing.

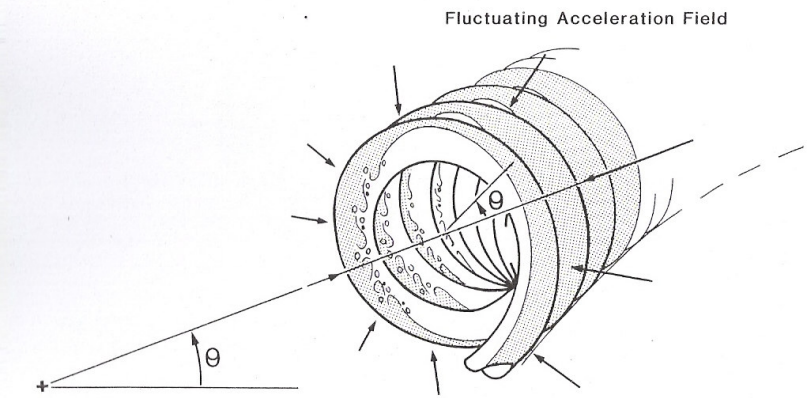


Fig. 2. Epicyclic coil planet centrifuge—wave mixing.

Cascade v. Wave Mixing

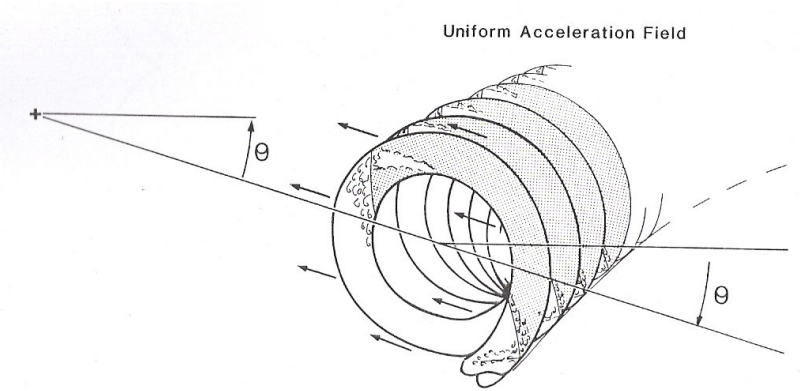


Fig. 1. Synchronous coil planet centrifuge—cascade mixing.

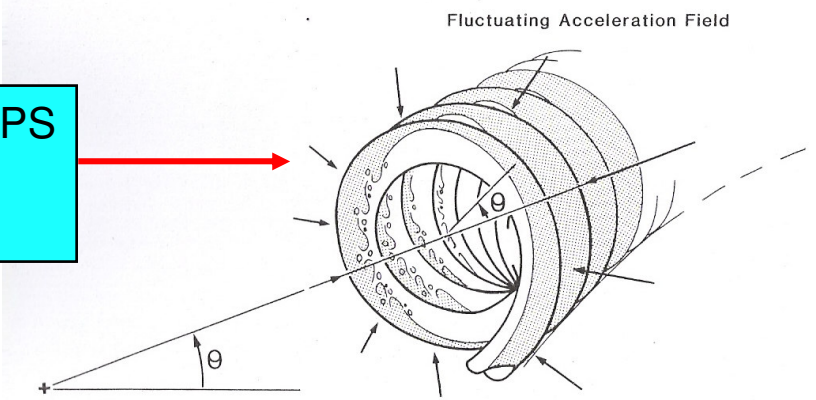


Fig. 2. Epicyclic coil planet centrifuge—wave mixing.

Hydrodynamic systems retain ATPS but wave mixing is too gentle for large proteins

Cascade v. Wave Mixing

Large scale toroidal hydrostatic systems being constructed but not yet tested

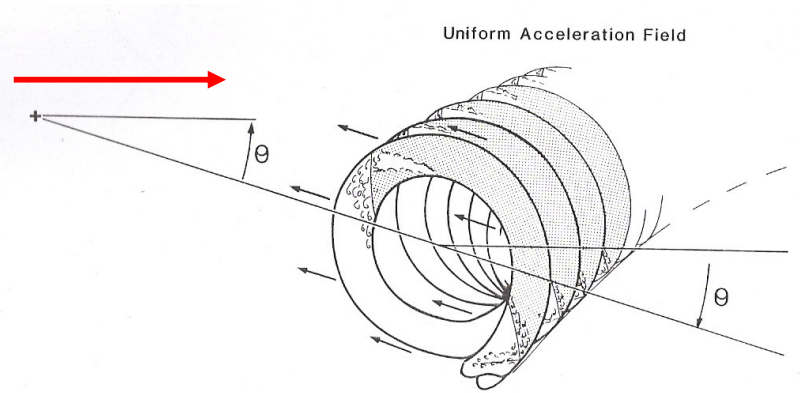


Fig. 1. Synchronous coil planet centrifuge—cascade mixing.

Hydrodynamic systems retain ATPS but wave mixing is too gentle for large proteins

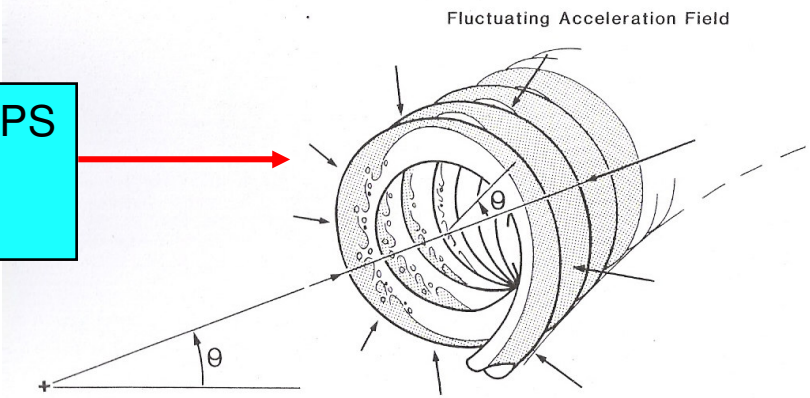


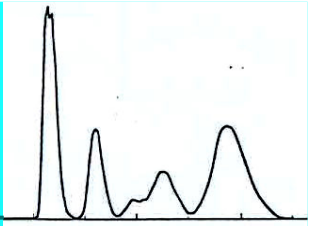
Fig. 2. Epicyclic coil planet centrifuge—wave mixing.

Hydrostatic Counter-current Extraction

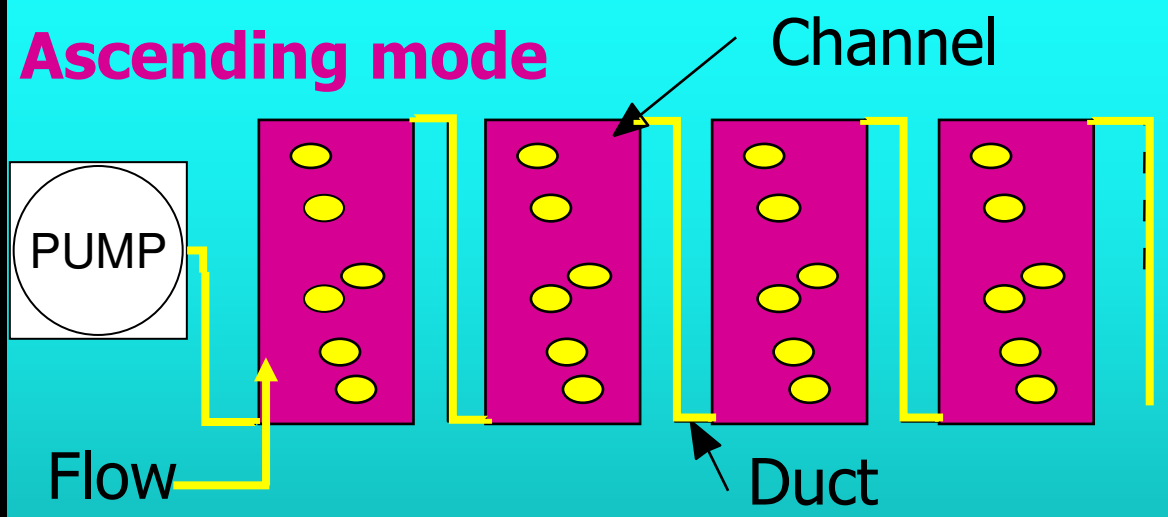
Hydrostatic Counter-current Extraction

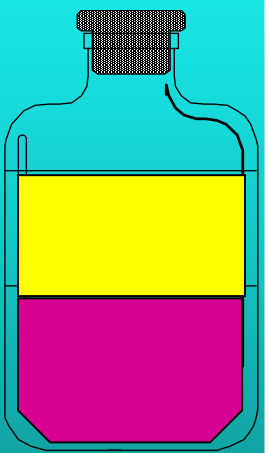
- Often discrete chambers with narrow bore interconnecting tubes
- Cascade mixing (like waterfalls)
- Stop the pump and the stationary phase stays where it is – trapped in the chambers
- The focus today - Centrifugal Partition Chromatography (CPC)

CPC: ... Without solid support ...

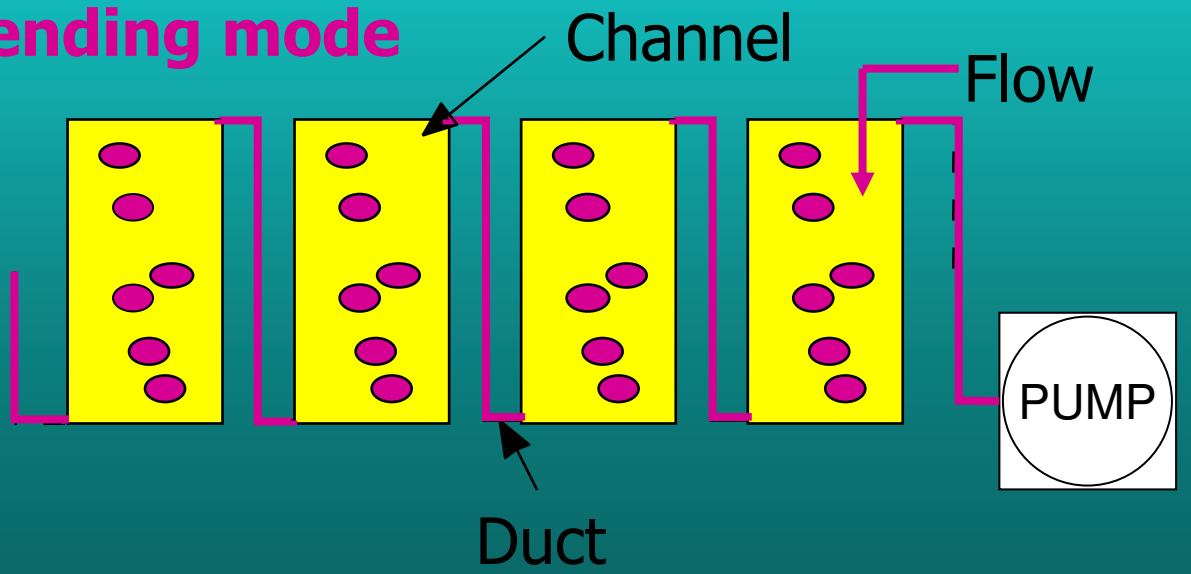


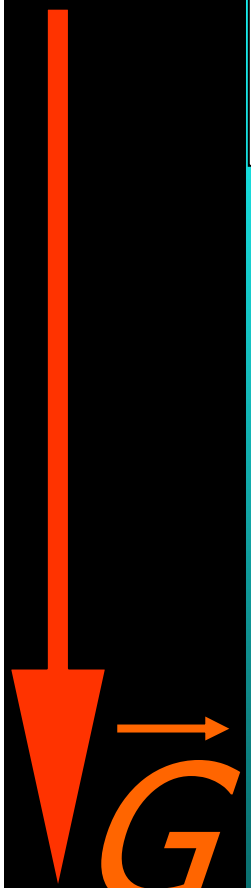
Ascending mode





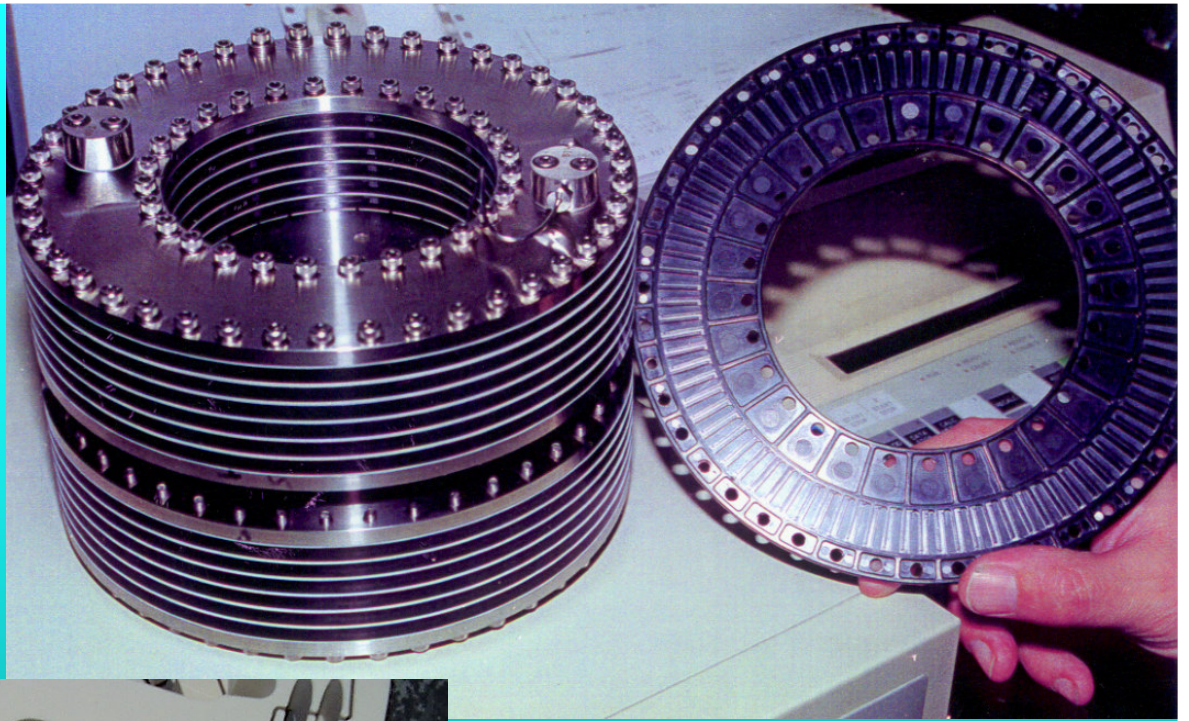
Descending mode

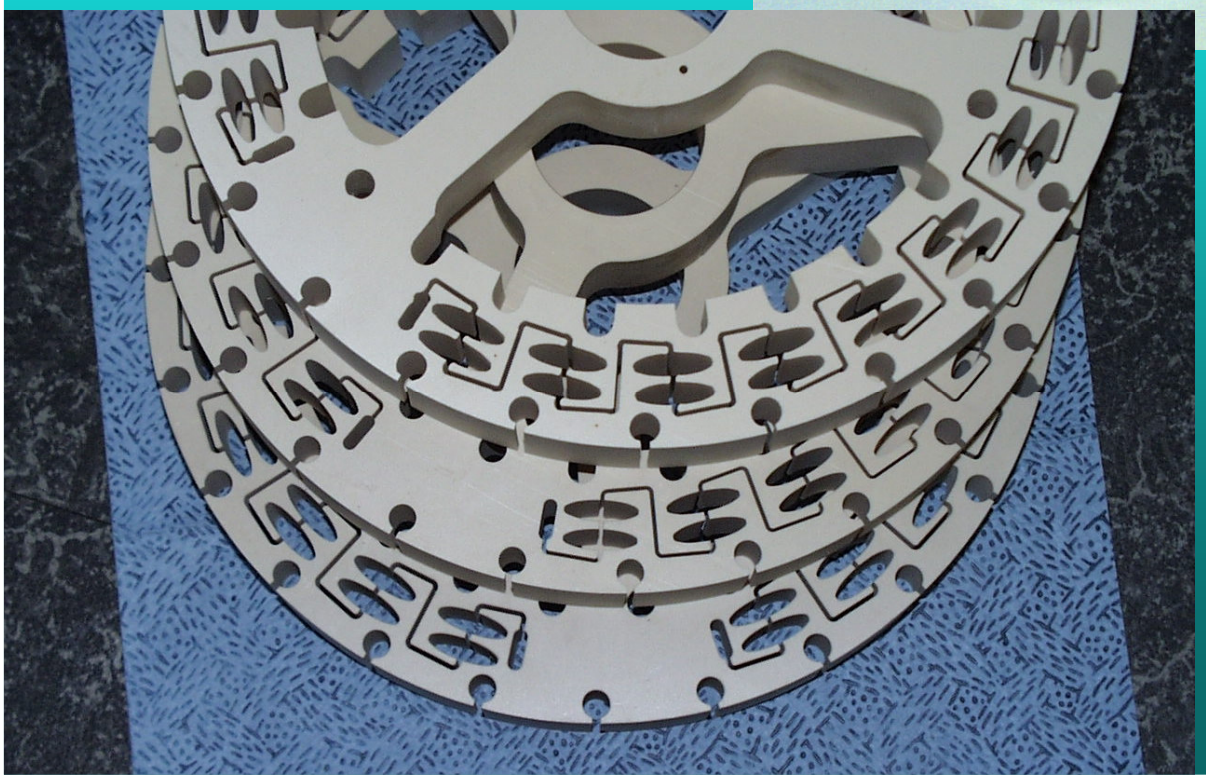




Sanki HPCPC, analytical scale

1 channel ≈ 90 µL



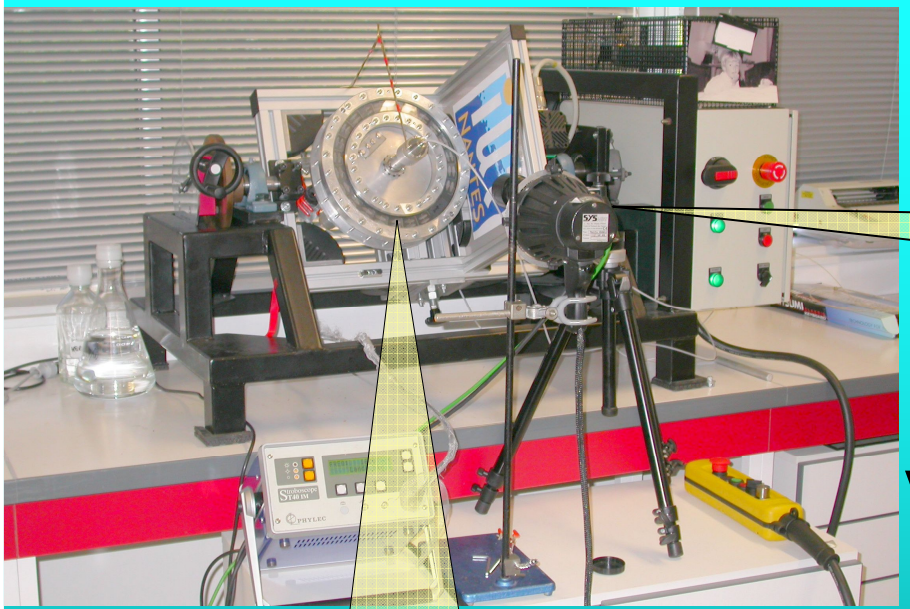


Armen Elite, industrial scale

Twin cells ≈ 12 mL

For the 12.5 liter instrument

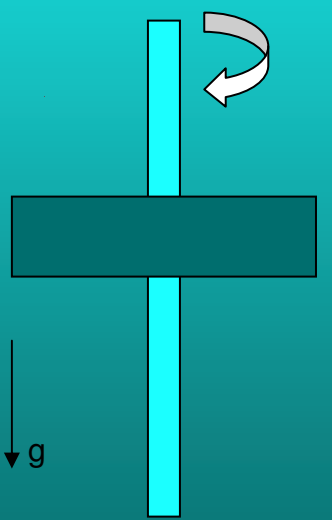
Visual CPC II



Stroboscopic flash and fast response camera

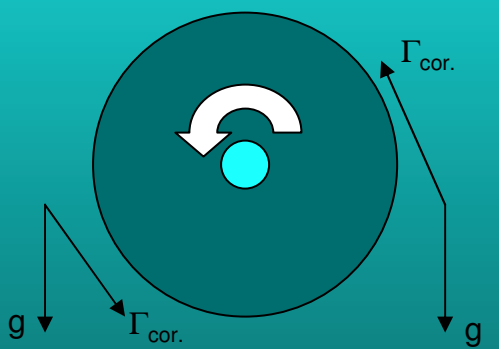
Stroboscopic looking at the same cell, observing the spray formation

Vertical pack



Earth gravity : possible sedimentation of the lower phase in deep channels

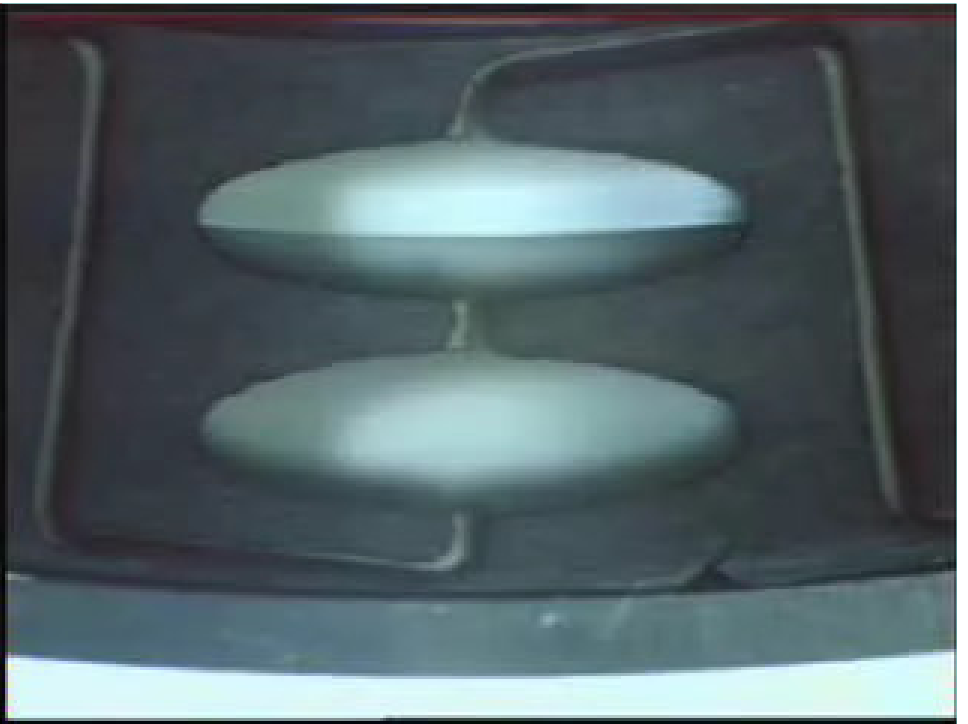
Horizontal pack



Earth gravity modulates the Coriolis acceleration and promotes spray formation

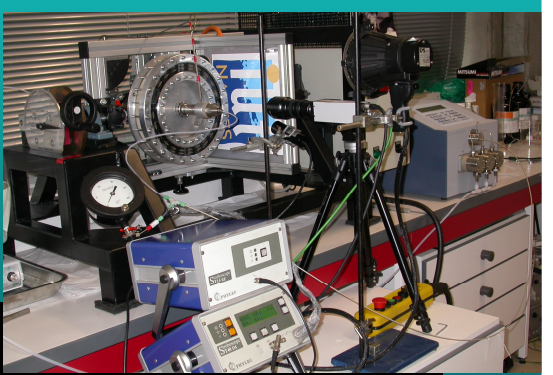
1) *Marchal L, Foucault AP, Patissier G, Rosant J, Legrand J (2002) Centrifugal partition chromatography: an engineering approach, In: Comprehensive Analytical Chemistry (A Berthod, Ed), Elsevier, 115-157.*

2) *Marchal, L., Legrand, J., Foucault, A. (2003) Centrifugal partition chromatography: a survey of its history and our recent*



506 rpm

1290 rpm



Flow rate (mL/min)

0

BuOH / Water

Ascending Mode

From 0 to 500 mL / min in 1 min then back to 0 mL / min in 1 min

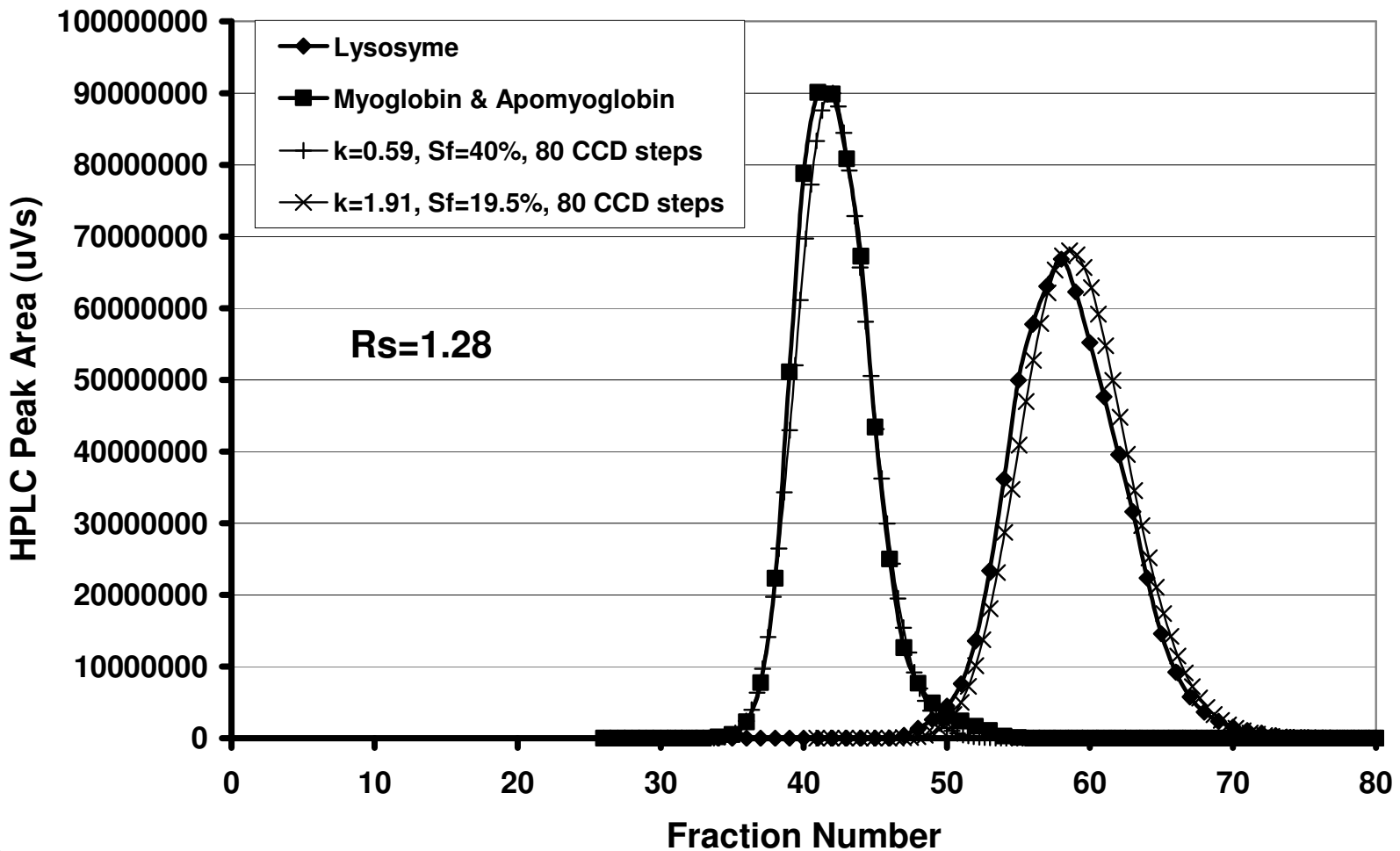
Advanced Bioprocessing Centre (ABC)

1 litre Hydrostatic CPC Set-up at BIB

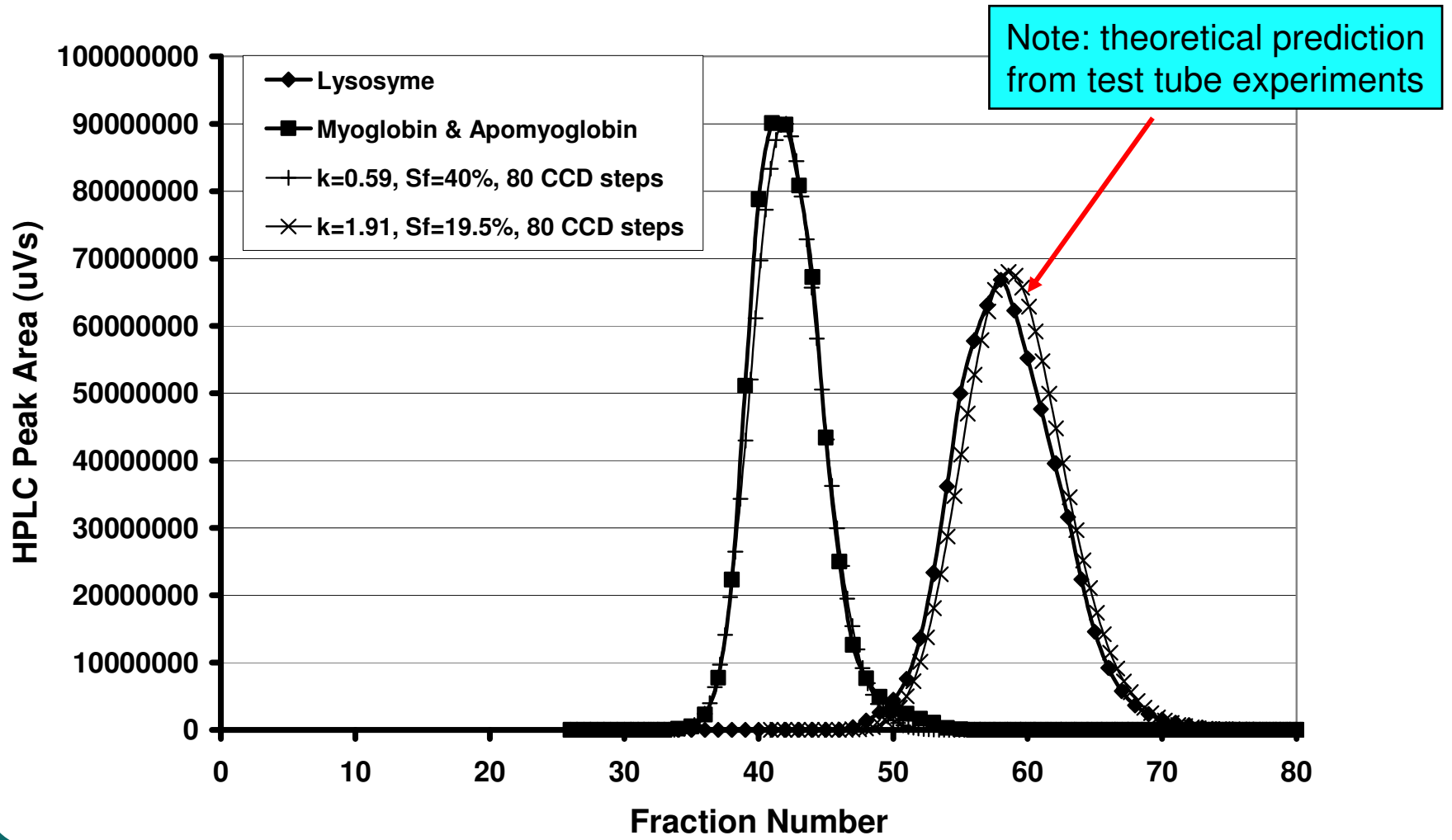
Run Conditions

- **Centrifuge:** Armen Elite 1 litre CPC
- **Capacity:** One rotor only - volume 500ml, cell volume 429ml
- **Phase System:** ATPS: 12.5% PEG1000-12.5% K_2HPO_4
- **Sample:** 90mg lysozyme, 90mg myoglobin in 40ml (~10% CV) 50:50 ATPS mix
- **Speed:** 2000 rpm (224g)
- **Flow:** 10 ml/min
- **Mobile Phase:** lower salt phase
- **Stationary phase retention (Sf) at breakthrough:** 52%
- **Stationary phase retention (Sf) at end:** 19%

Analytical Runs



Analytical Runs



Transfer to Pilot Scale Unit in France

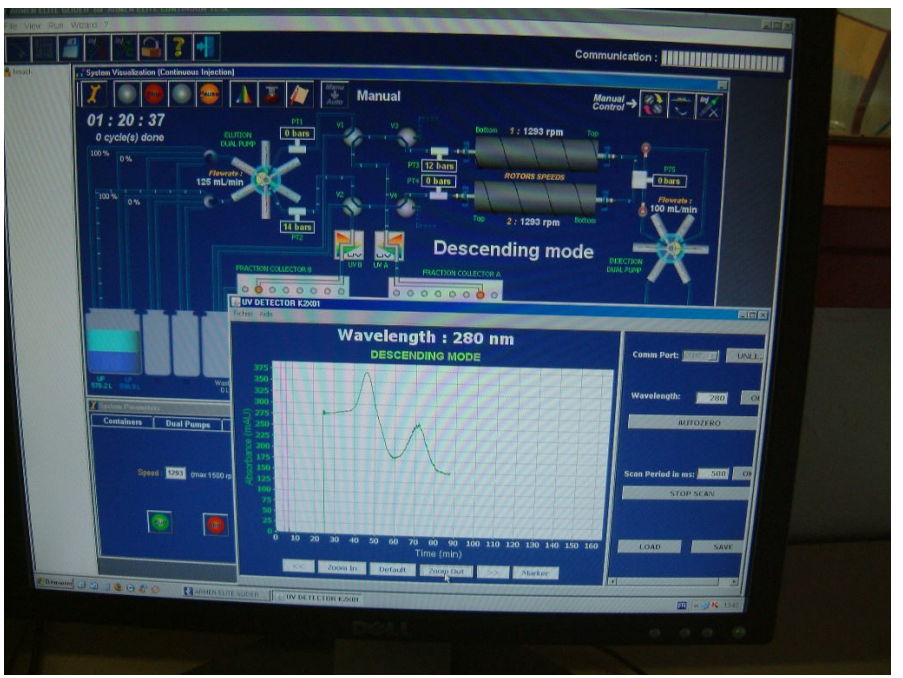
Armen 12.5 litre CPC at Archimex

Run Conditions

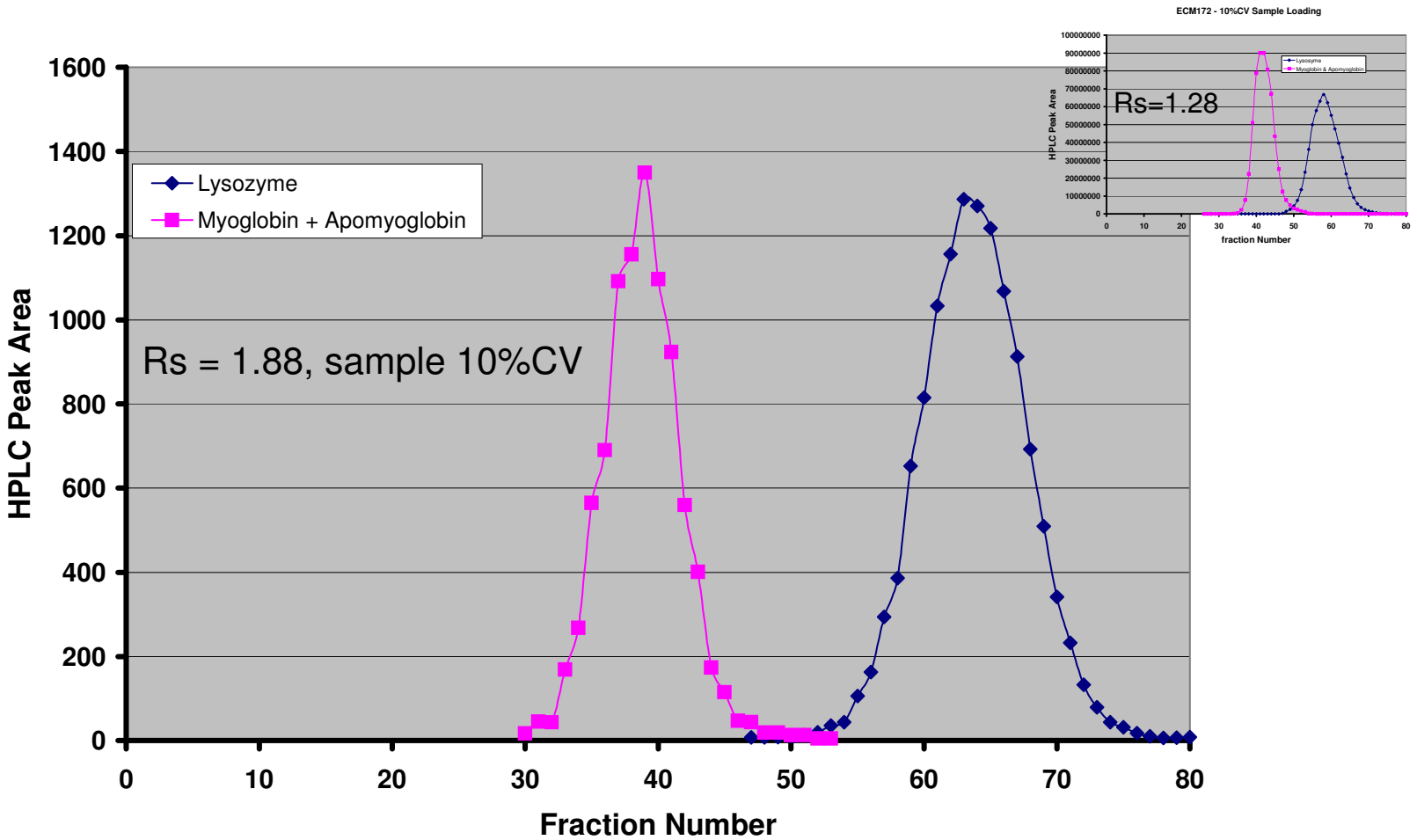
- **Centrifuge:** Armen Continuum 12.5 litre CPC
- **Capacity:** One rotor only - volume 6.25 litre
- **Phase System:** ATPS: 12.5% PEG1000-12.5% K_2HPO_4
- **Sample:** 1.1g lysozyme, 1.1g myoglobin in 500ml (~10% CV) 50:50 ATPS mix
- **Speed:** 1293 rpm (224g)
- **Flow:** 125 ml/min
- **Mobile Phase:** lower salt phase
- **Stationary phase retention (Sf) at breakthrough:** 63%
- **Stationary phase retention (Sf) at end:** 22%

Some of the team at Archimex

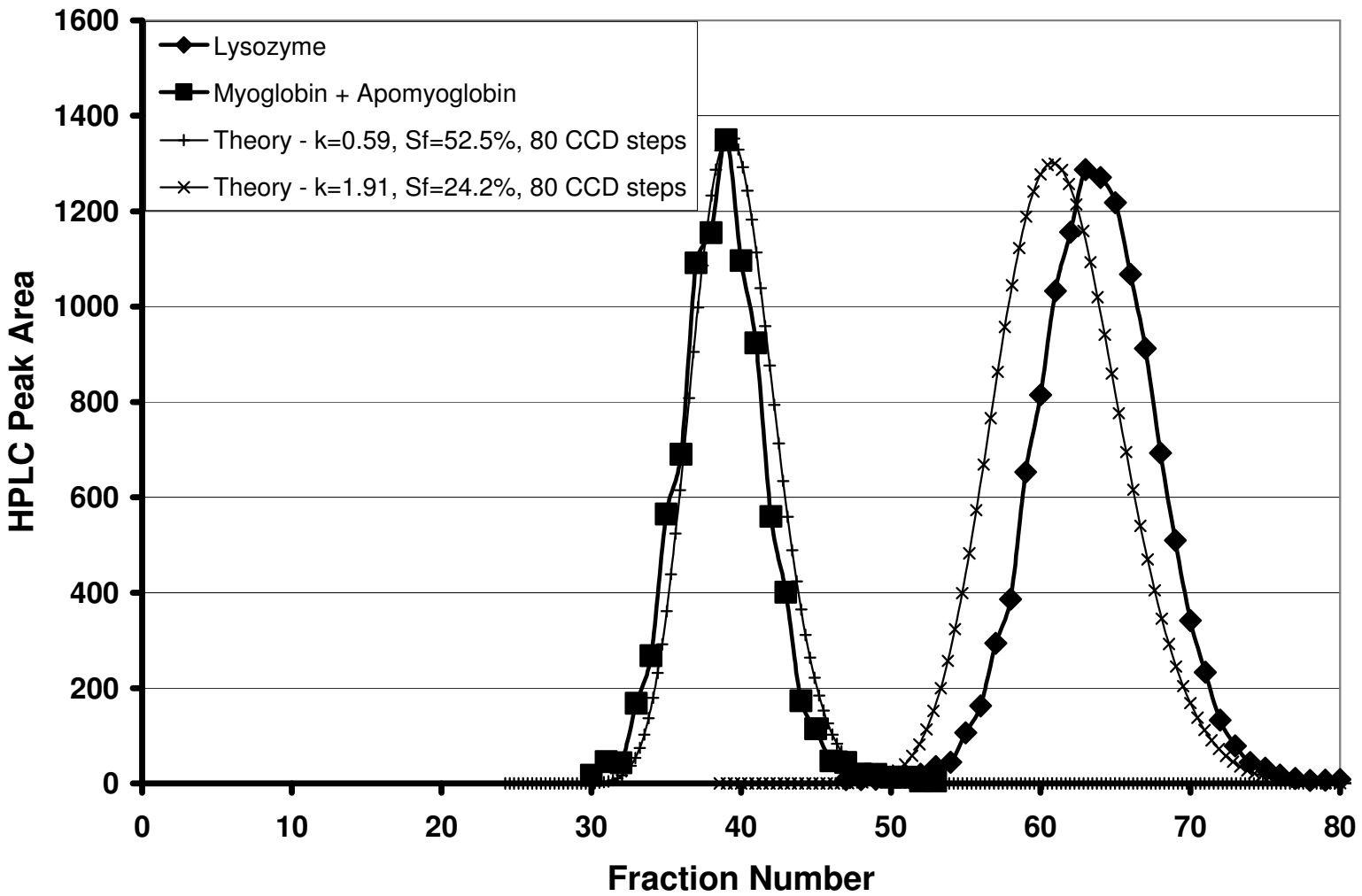
Fraction collection & on-line monitoring



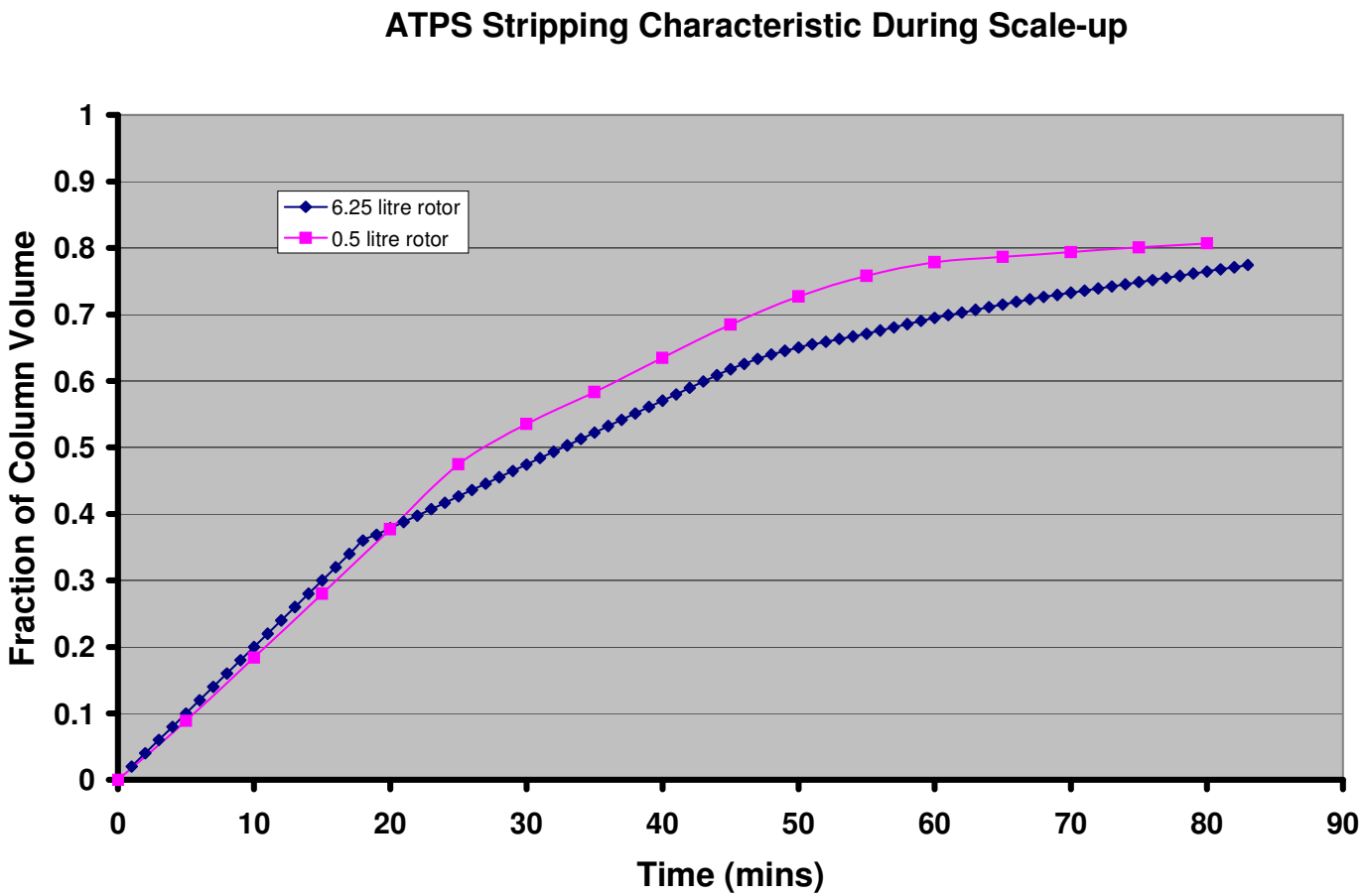
Armen 12.5 litre CPC at Archimex



Theoretical Predictions – Process Scale



ATPS Stripping Characteristic – Scale-up

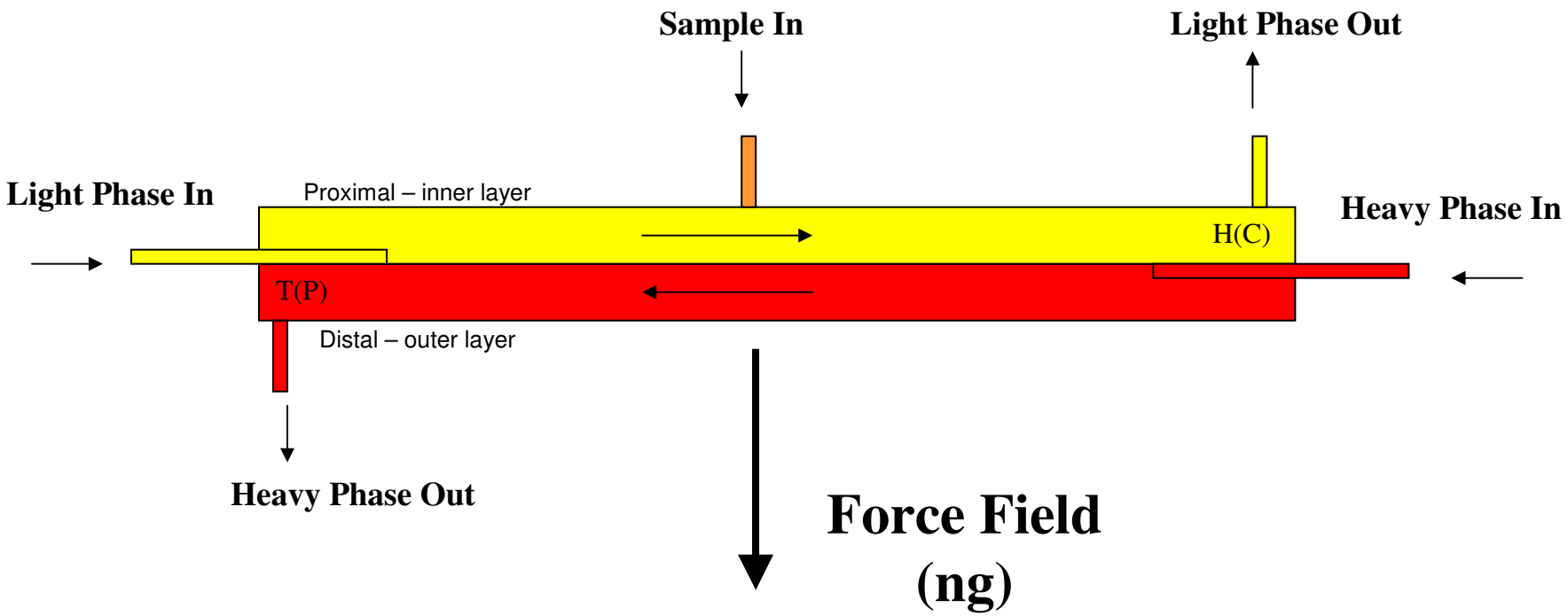


Conclusions – Batch Isocratic

- Linear scale-up feasible
- Robust technology – easily transferred from the test tube to a production run
- Note that with Hubbuch's robotic high throughput screening with ATPS systems, combined with this rapid scale-up approach – small footprint, rapid new approaches to manufacture are becoming feasible

Intermittent – True Moving Bed (i-TMB)

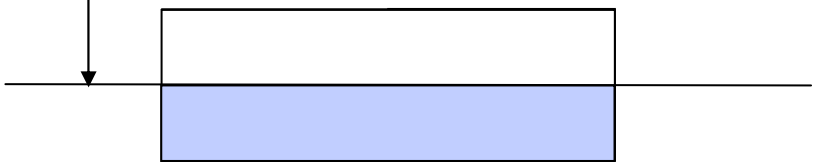
True Moving Bed

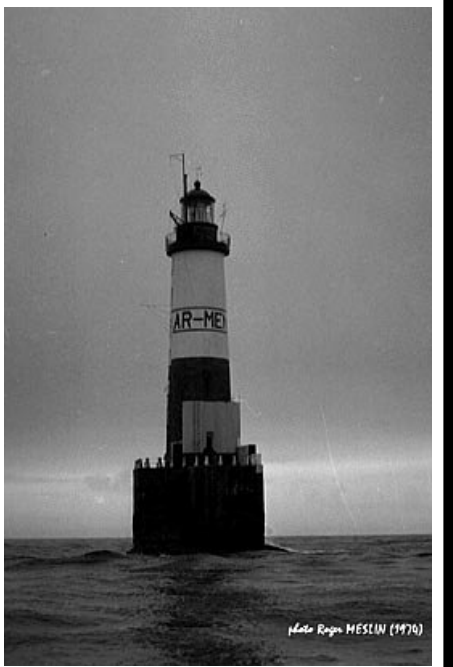


Sutherland, Scientific Update, San Francisco March 21-22, 2005

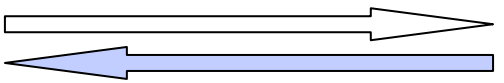
Novel Liquid-liquid Extraction Technology

Injection



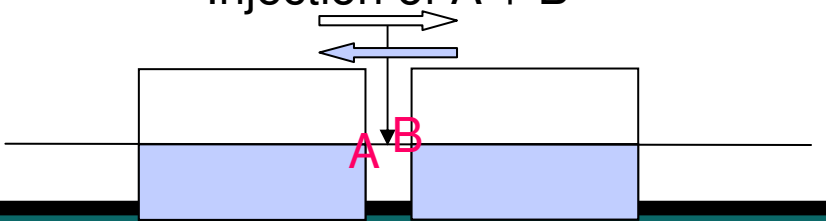


Ascending mode period



Descending mode period

Injection of A + B

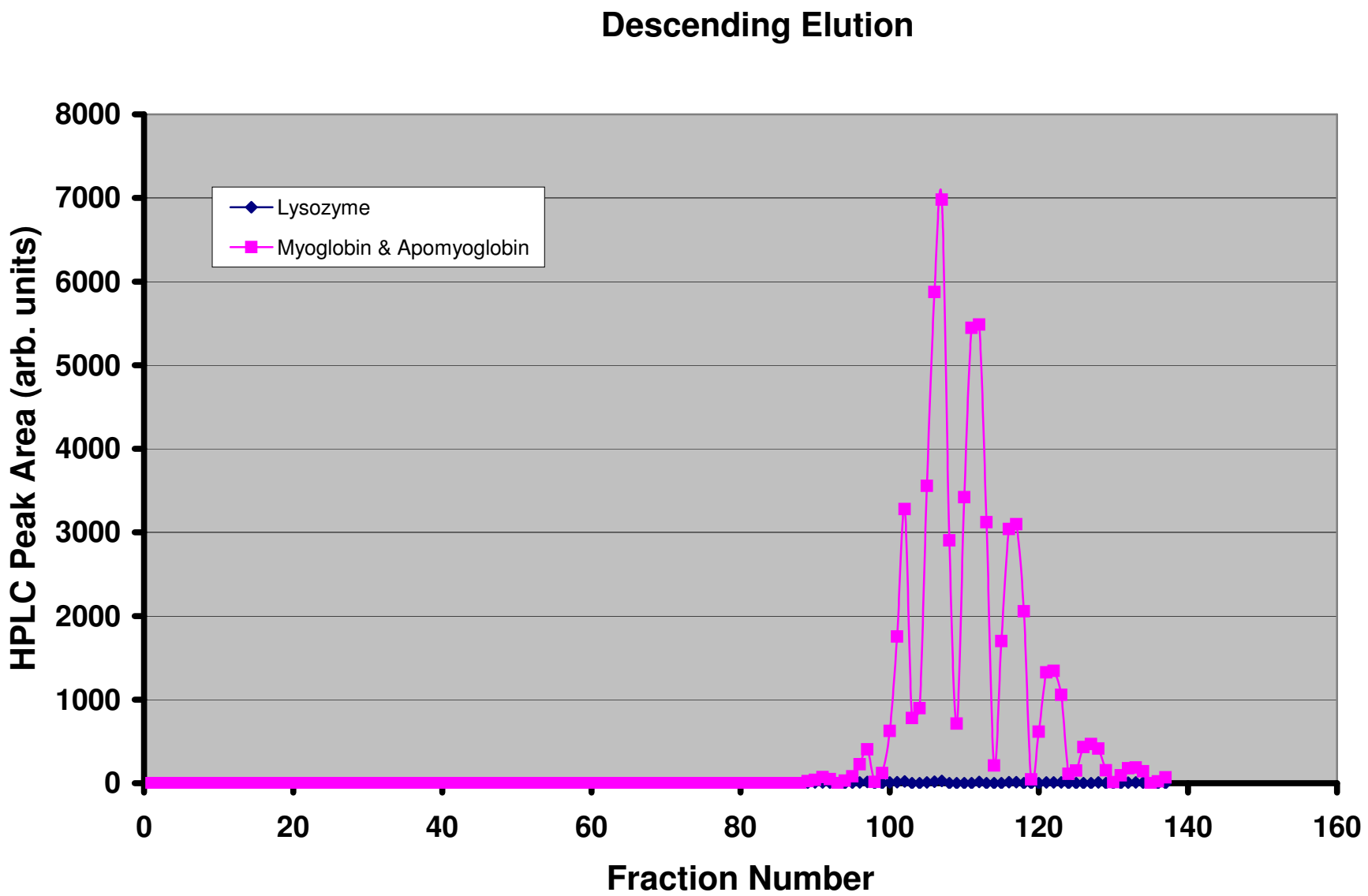


Run Conditions

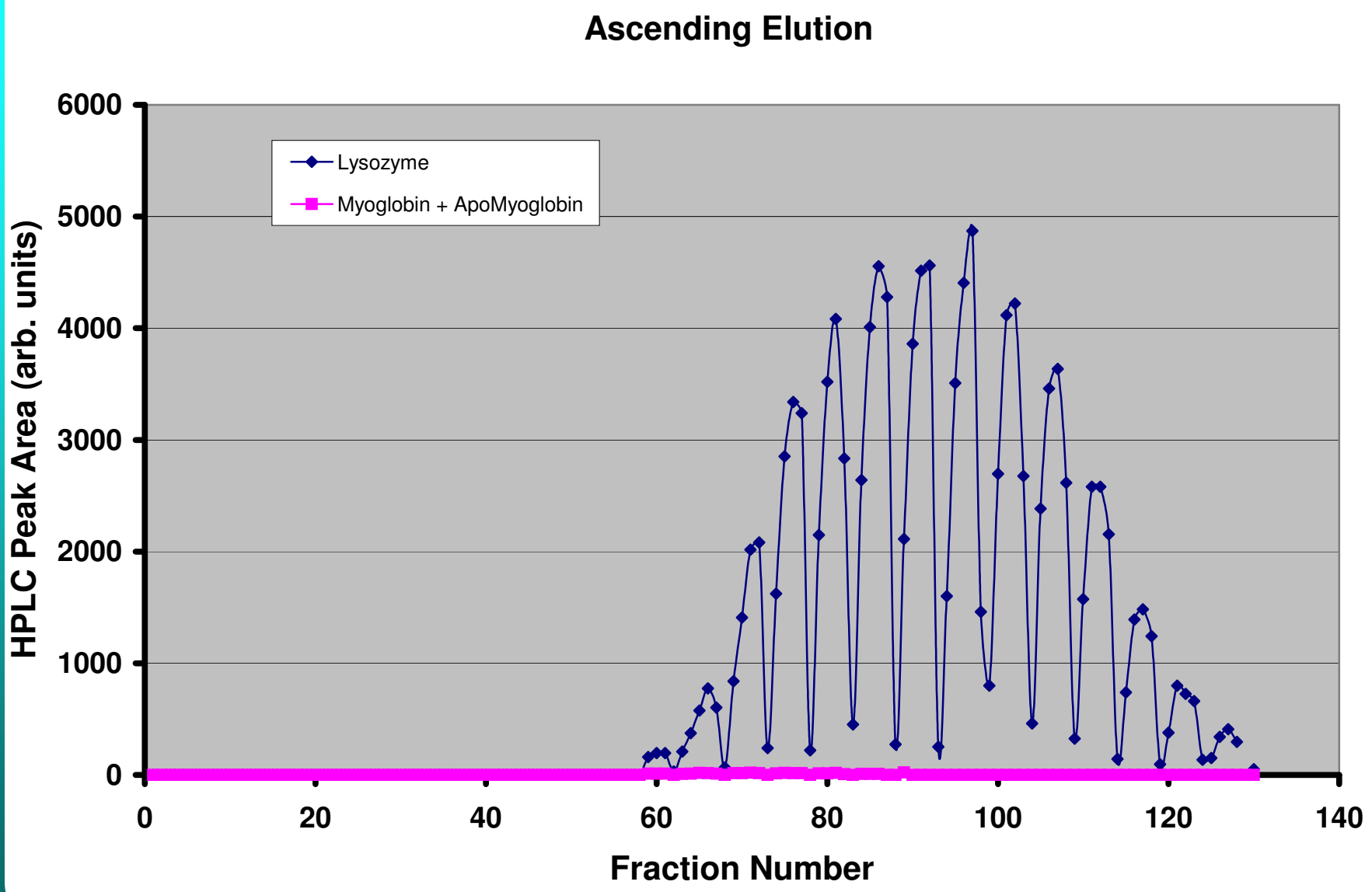
- **Centrifuge:** Armen Elite 1 litre CPC
- **Capacity:** Two rotors - volume 1000ml, cell volume 858ml
- **Phase System:** ATPS: 12.5% PEG1000-12.5% K_2HPO_4
- **Sample:** Continuous flow of 2.2mg/ml lysozyme, 2.2mg/ml myoglobin at 3ml/min in UP (ascending) and LP (descending) – 0.79g/hr – total 1.2g in 1.5 hours
- **Speed:** 2000 rpm (224g)
- **Flow:** 10 ml/min (Ascending) 5 minutes; 10ml/min (Descending) 5 minutes and so on …..
- **Mobile Phase:** alternating between between UP & LP

Armen CPC i-TMB System at Archimex

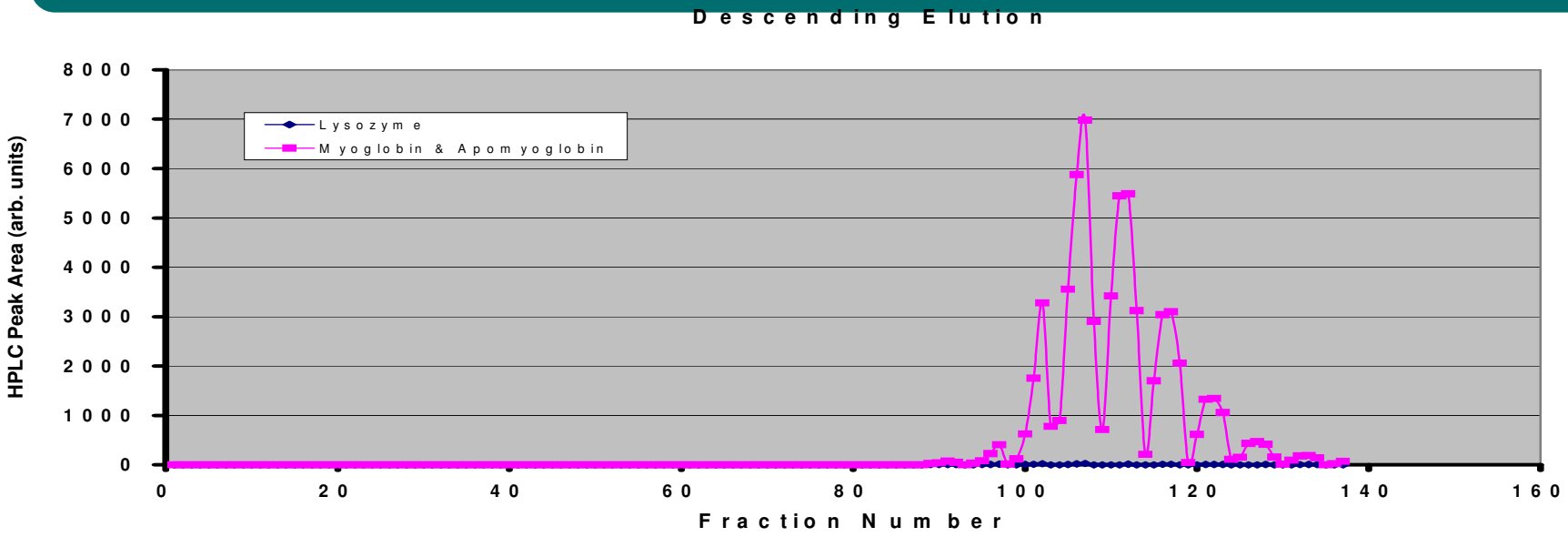
Novel Liquid-liquid Extraction Technology

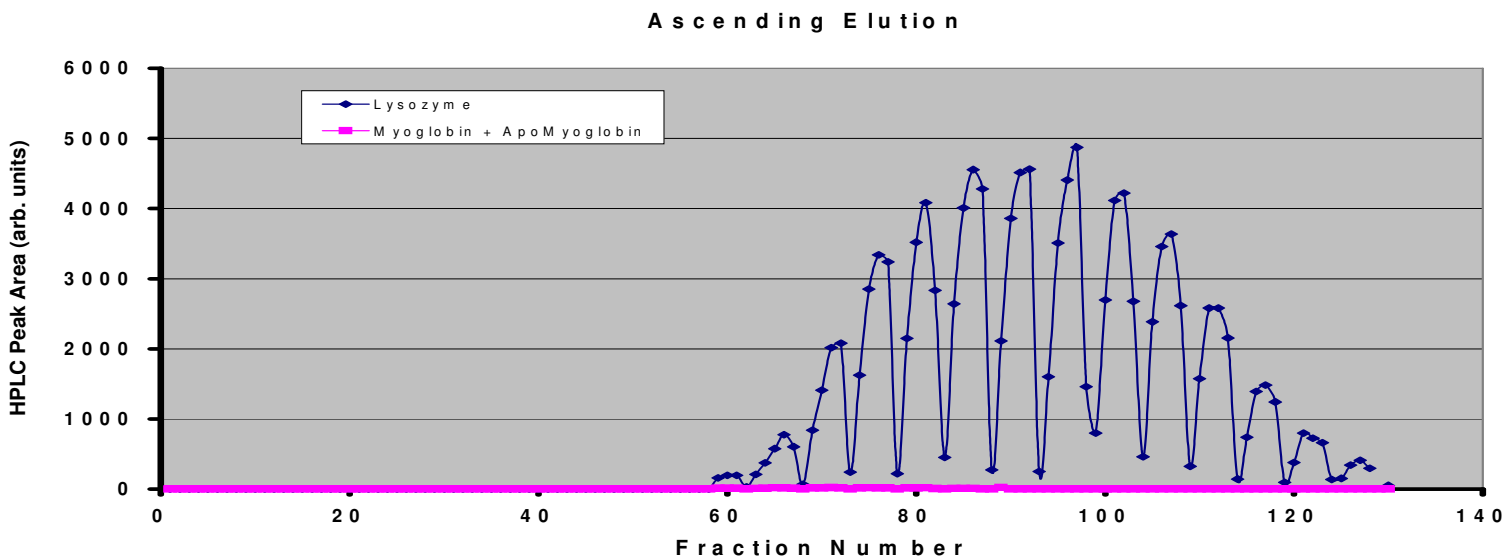


Novel Liquid-liquid Extraction Technology

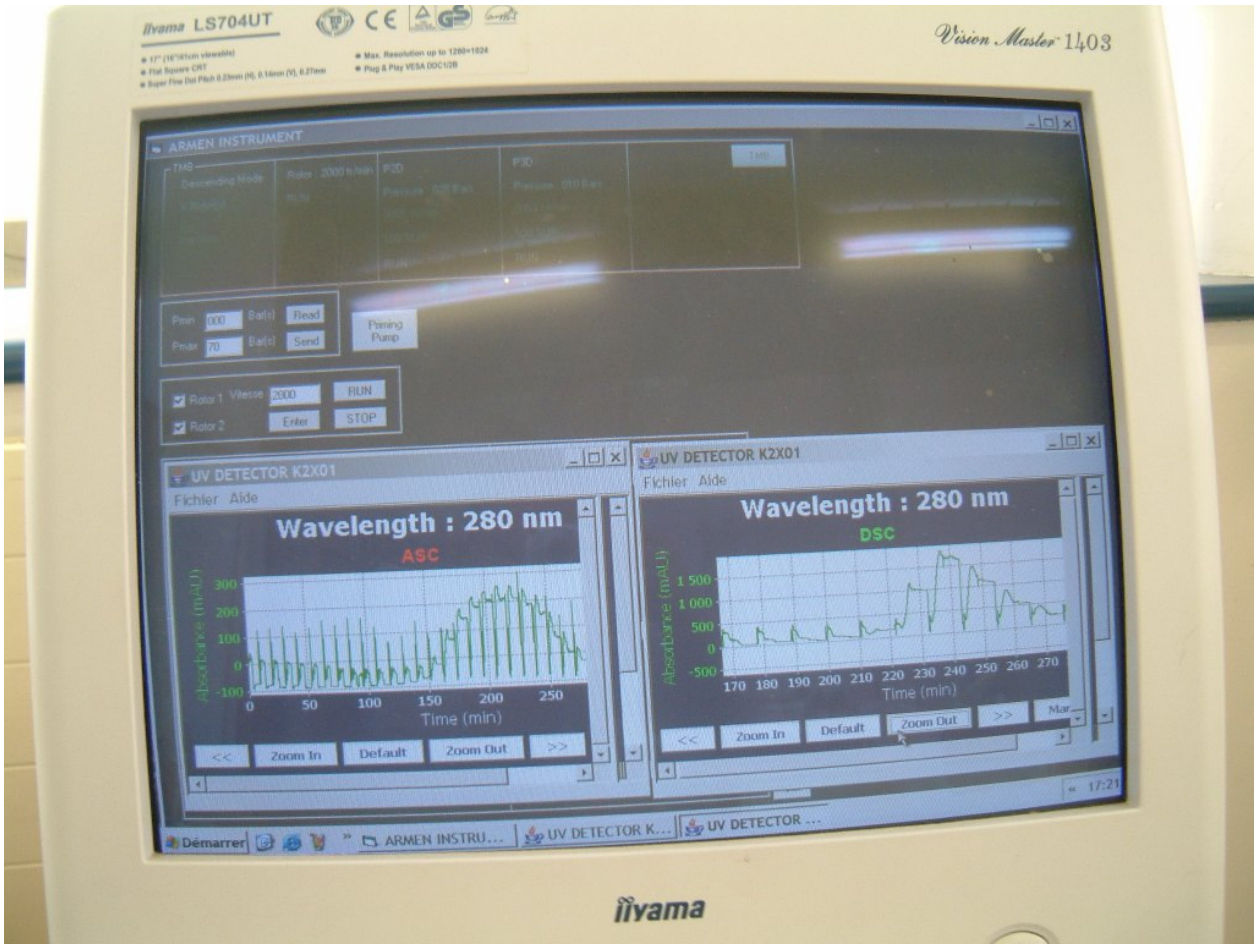


Novel Liquid-liquid Extraction Technology

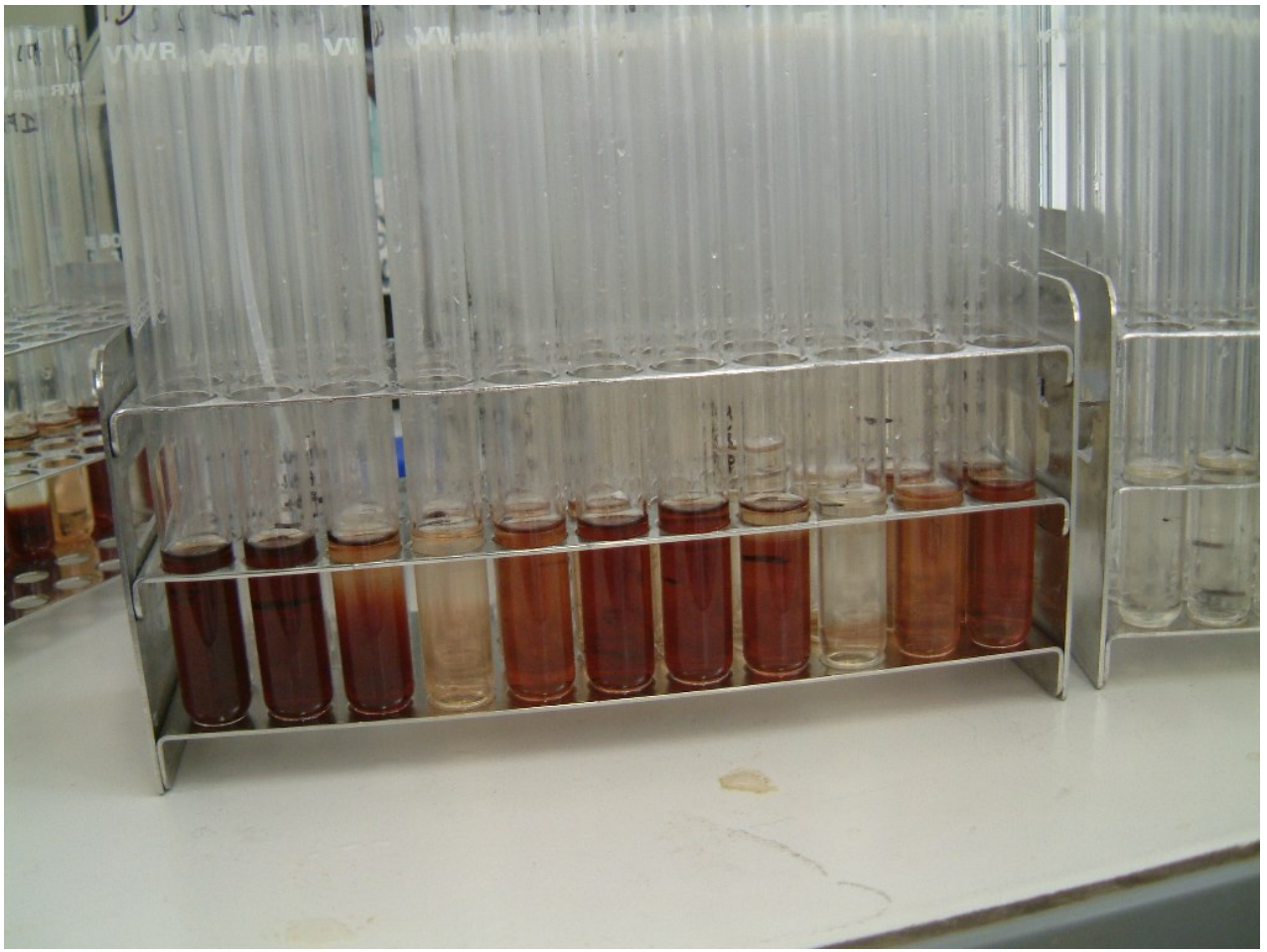




Elution control monitoring during run



Descending Elution - Myoglobin



Summary – Batch v. i-TMB

Centrifuge*		g/hour	g/day
1 litre CPC	Batch	0.14	3.24
	i-TMB	0.79	19.01
12.5 litre CPC	Batch	1.69	40.50
	i-TMB	9.90	237.60

** Non-Optimised*

Conclusions

- **Rapid scale up from the test tube (Hubbuch) to pilot scale (kg/day) is feasible for model systems in batch and continuous process mode**
- **Now needs evaluating with real systems**
  - Paula Rosa, Lisbon, Portugal (BPP2007)
  - Emma Bourton, Brunel, UK (PhD – Syngenta)
  - Other volunteers welcome

Challenges

- **New liquid-liquid phase systems**
  - Ionic liquids (QUILLS)
  - ATPS with higher solubility (Hans-Olef Johansson, Sao Paulo, Brazil)
- **Increased sample concentrations in ATPS**
  - Non-equilibrium sample loading & extraction (NESLE)
  - New continuous tube hydrostatic approach
- **Development of small footprint, versatile, multi-product facilities for "niche markets" highlighted by Dr Dana Andersen of Genentech**
  - Initially for high value added products
- **UK Government – future emphasis on basic and "user-driven" research**