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Development of a MEP HyperCel IgG purification process for a Commercial Polyclonal Antibody Product

Richard Francis
Process Development Director, Protherics

Mix Mode Chromatography, SCI conference London, June 27th 2007

Disclaimer

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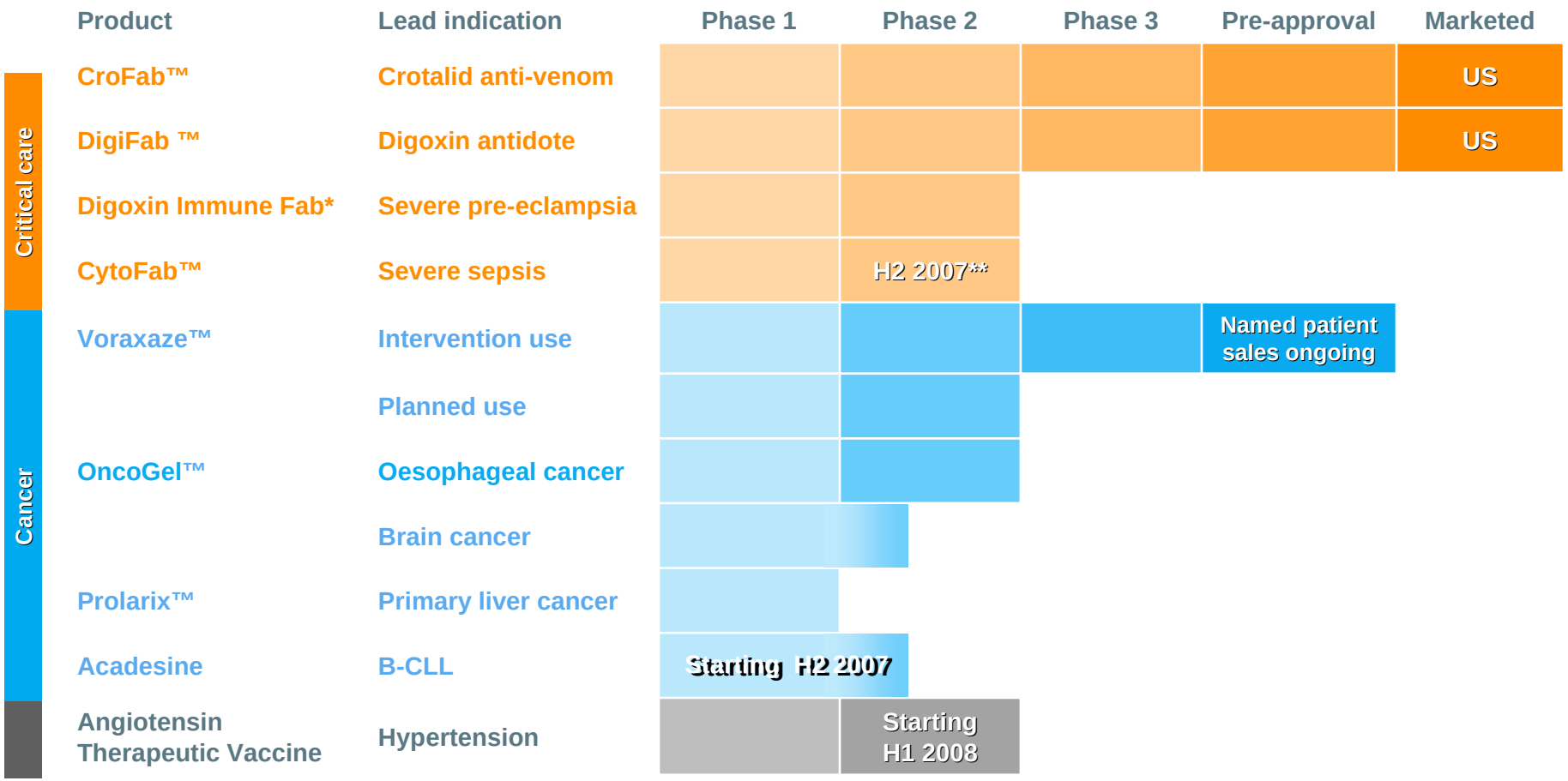
Overview

- Leading UK biopharmaceutical company in critical care and cancer
- Two approved biologics, seeking approval for third
- Broad, late stage pipeline with clinical studies in 9 indications in 2007/8
- Major £195m ($340m) licensing deal with AstraZeneca
- Strong cash position (£40.0m at end of FY 2007) & revenues (£30.1m in FY 2007) help fund pipeline

Expanded pipeline in 2007

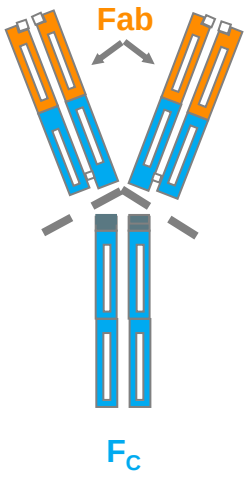


** Current study ongoing with Digibind® (GSK) ** AZ responsible for global development and commercialisation*

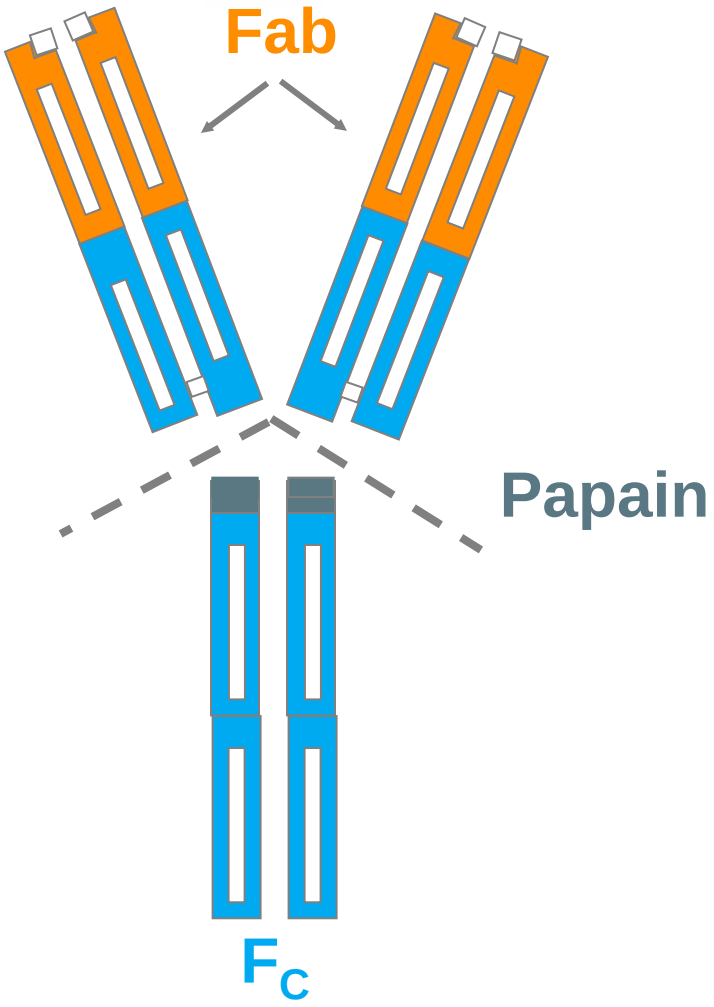
Critical care portfolio

Ovine polyclonal fragments

1. Immunize sheep against target antigen and collect serum
2. Purify and digest target antibodies
3. Purify Fab Fragments
4. Polyclonal antibody product is created for intravenous use e.g. CroFab™, DigiFab™, CytoFab™



Production of polyclonal antibody therapeutics



Immunise Sheep

Collect Serum

Purify and digest Antibodies

Purify Fab Fragments

Formulate products for intravenous use

Application of modern biopharmaceutical development

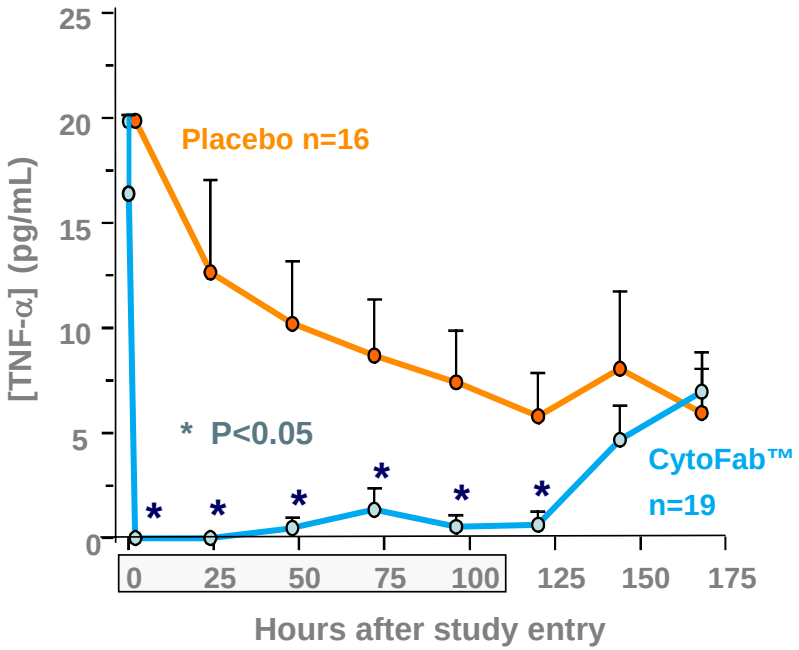
Images used with permission of The Wellcome Trust Medical Photographic Library, London UK.

CytoFab™ - anti-TNF-α polyclonal Fab

A new hope in the treatment of sepsis

- Sepsis is a life-threatening condition resulting from serious infection
  - Mortality is estimated at ~30%
  - Patients require intensive care
- "TNF-α Hypothesis" suggests that neutralisation of TNF- α in patients should improve outcomes in sepsis
- A large number of products have failed to neutralise TNF-α
- CytoFab has clearly been shown to effectively neutralise TNF-α in the blood of sepsis patients

Plasma TNF-α concentrations significantly lower in CytoFab™ group



- Box shows infusion period
- Values are mean + SEM
- P=0.001 for period 24-120 h

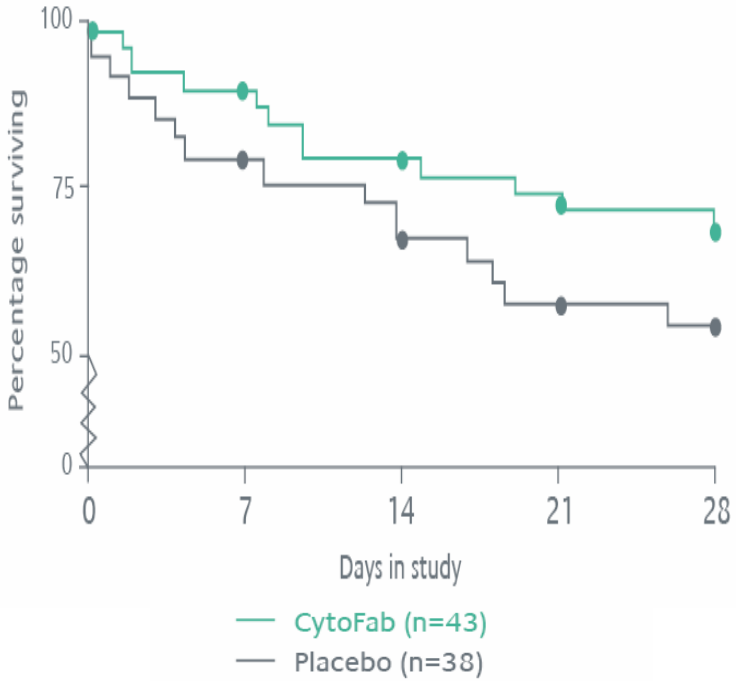
TNF-α= Tumour Necrosis Factor alpha

CytoFab™

Phase 2b trial summary*

- 81 patients, placebo-controlled, double-blind, randomised phase 2b study
- 5.2 more ventilator-free days than placebo (p = 0.04)
- 5.0 more days out of ICU than placebo (p = 0.03)
- Fewer deaths in CytoFab group (26% vs 37% mortality; p=0.24)
- Well tolerated during 10 infusions over 5 days

Kaplan-Meier survival by treatment group



Mortality Rate:
26% CytoFab
37% Placebo

Average time to death:
11.6 days CytoFab
10.3 days Placebo

** Crit Care Med 2006 Vol. 34, No. 9*
ICU= Intensive care unit

CytoFab™

A major market opportunity

- Major out-licensing deal with AstraZeneca (AZ) signed in Dec 2005
  - Upfront and milestone payments worth £195m ($340m)
  - Protherics receives 20% royalty on global net sales
  - AZ responsible for development and commercialisation
  - Protherics responsible for bulk manufacture and receives additional payments for supply
- Severe sepsis market is potentially worth up to $8bn pa globally*

- Xigris® is the only approved product for the treatment of severe sepsis
- Use is restricted due to risk of serious bleeding
- Relatively few products in late stage development

** assumes all severe sepsis patients receive a $7000 course of treatment with an anti-sepsis product*

CytoFab Manufacturing process summary

- The phase I and II manufacturing process operation was reviewed and determined not to be suitable for commercial operations
- Suitability was defined on several levels:
  - Product Quality, the process could be developed to deliver a safer product in terms of viral clearance capability and purity
  - Capacity and capability, the phase I and II process would not have the capability to supply clinical and commercial demands

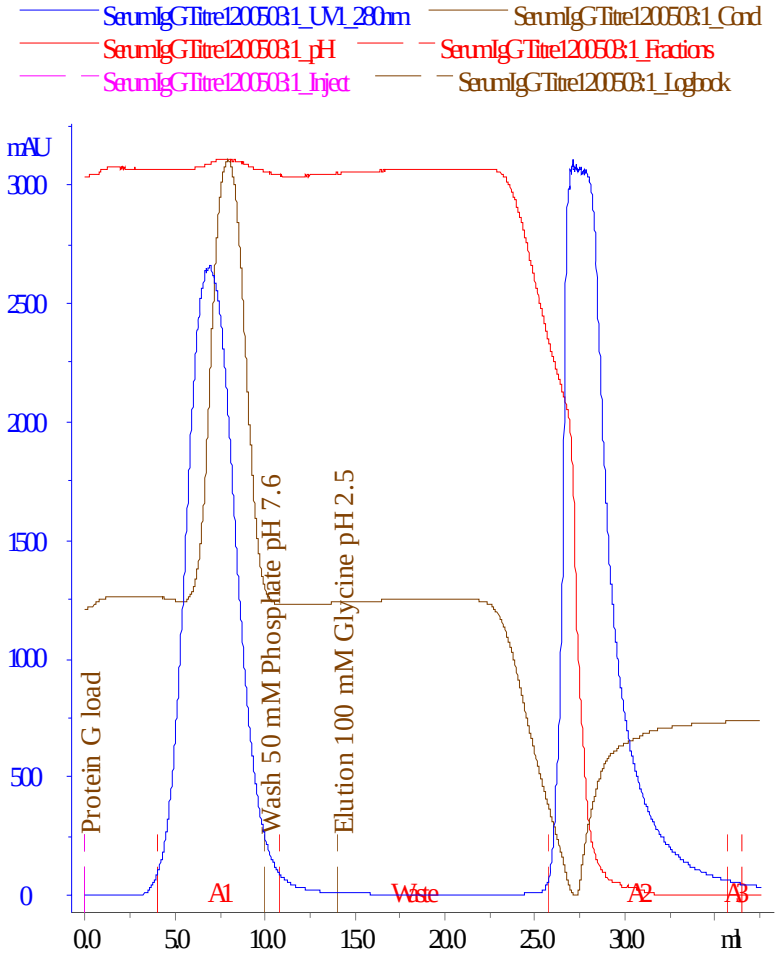
Strategy for development of a commercial process

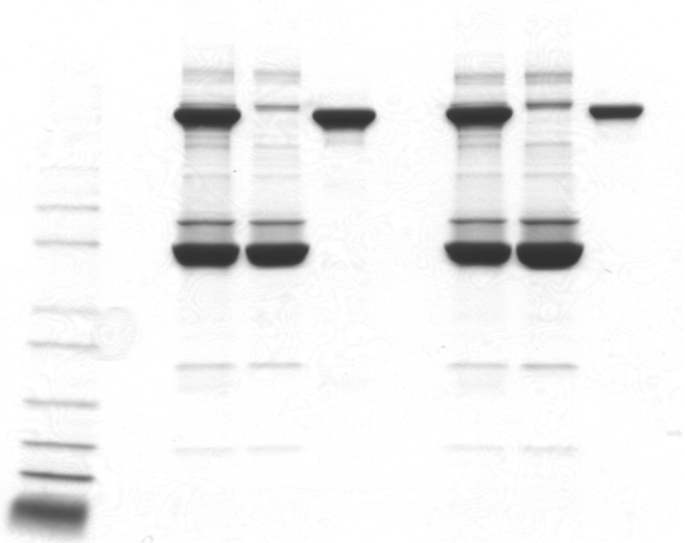
- Determine process stages which constrained capacity and or affected product quality
- Look to best practice in similar product types, i.e. Monoclonal antibodies and incorporate process stages
- Highlighted for development the following process stages:
  - IgG capture and purification – Replacement of sodium sulphate precipitation
  - Virus inactivation and removal – Inclusion of orthogonal methods into the process flow
  - Affinity chromatography – Replacement of immobilized TNFα affinity chromatography step

IgG capture options

- Ovine polyclonal product derived from ovine serum obtained from hyper immune sheep
- Standard in-house method sodium sulphate precipitation, limited scale up options
- The IgG has to be “captured” in a manner that allows for the other materials present to be removed from the process, while purifying and recovering the IgG at high yield
- These are other ovine proteins (albumin), DNA and potential adventitious agents
- Similar to the requirements for monoclonal antibody Protein A chromatography step
- BUT, the feedstock is significantly different in terms of protein concentrations, viscosity and much hasher cleaning methodologies would be required potentially significantly shortening the column life time
- Protein A / G used to prove the concept, but another resin needed to support commercial manufacture in a cost effective manner

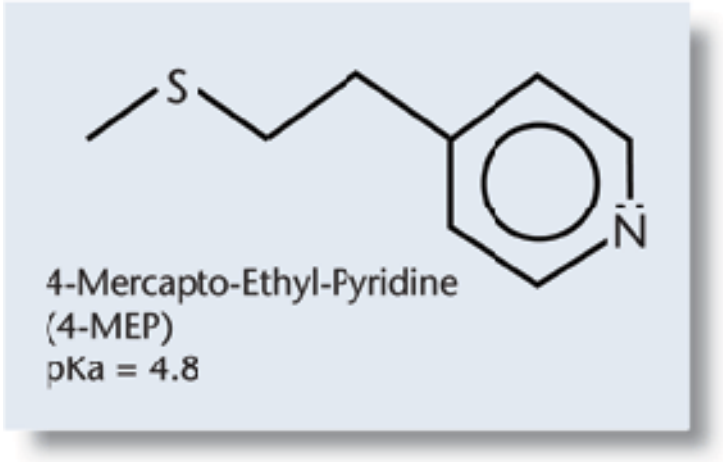
Purification of polyclonal IgG using Protein G Sepharose

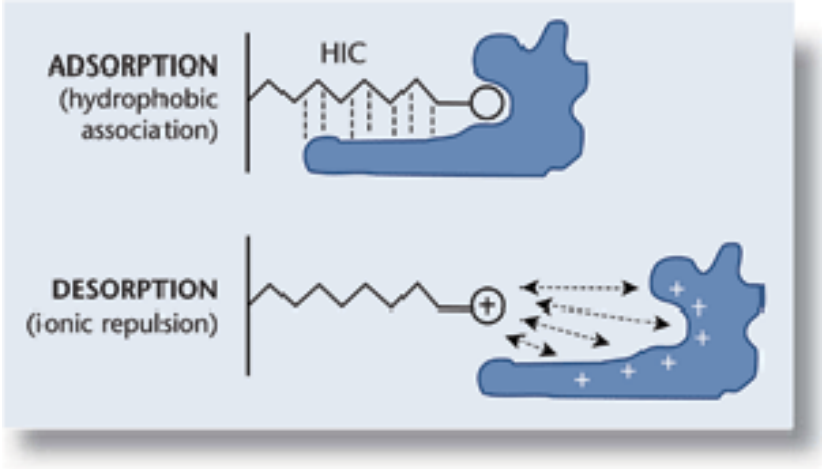




1. MW markers
2. Neat serum
3. Flow through (unbound), 1 in 2 dilution
4. Eluted protein
5. Neat serum
6. Flow through (unbound), 1 in 4 dilution
7. Eluted Protein

MEP Hypercel®

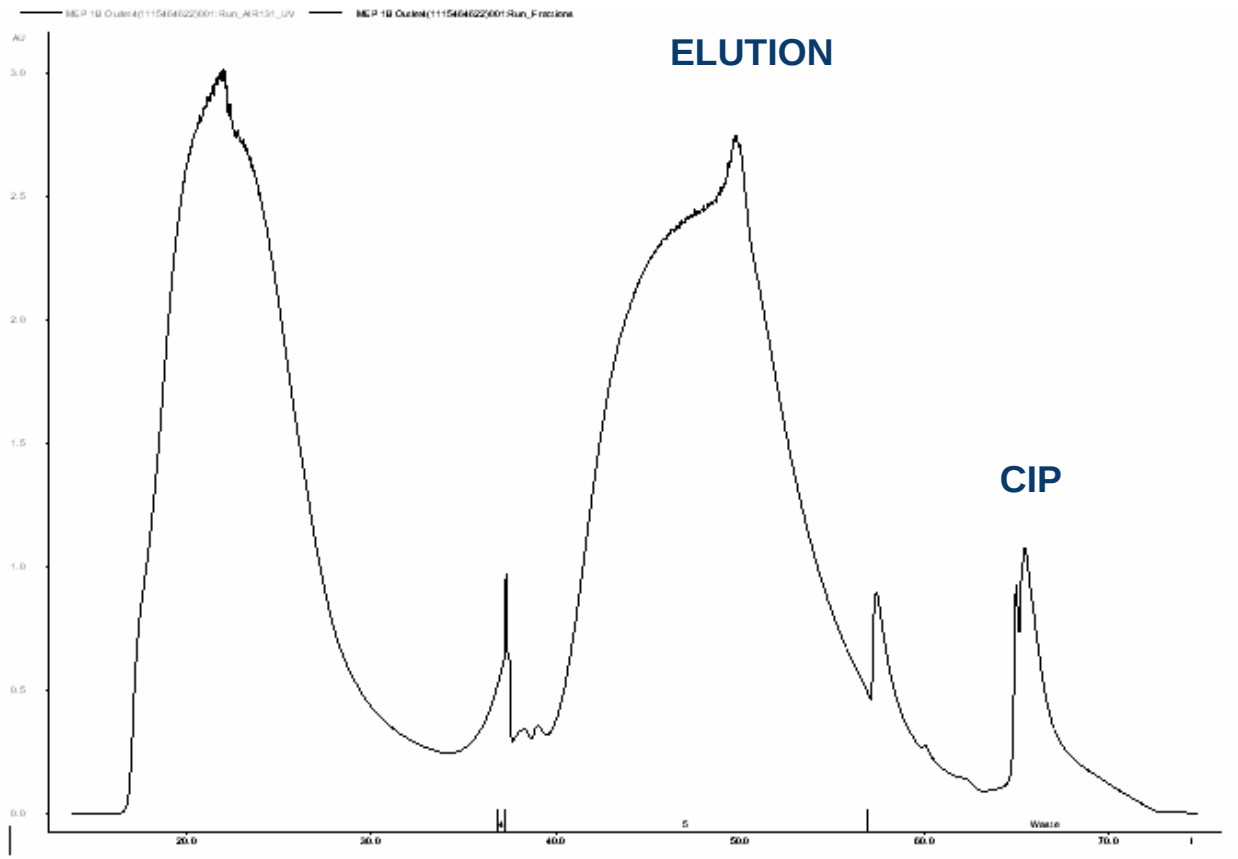




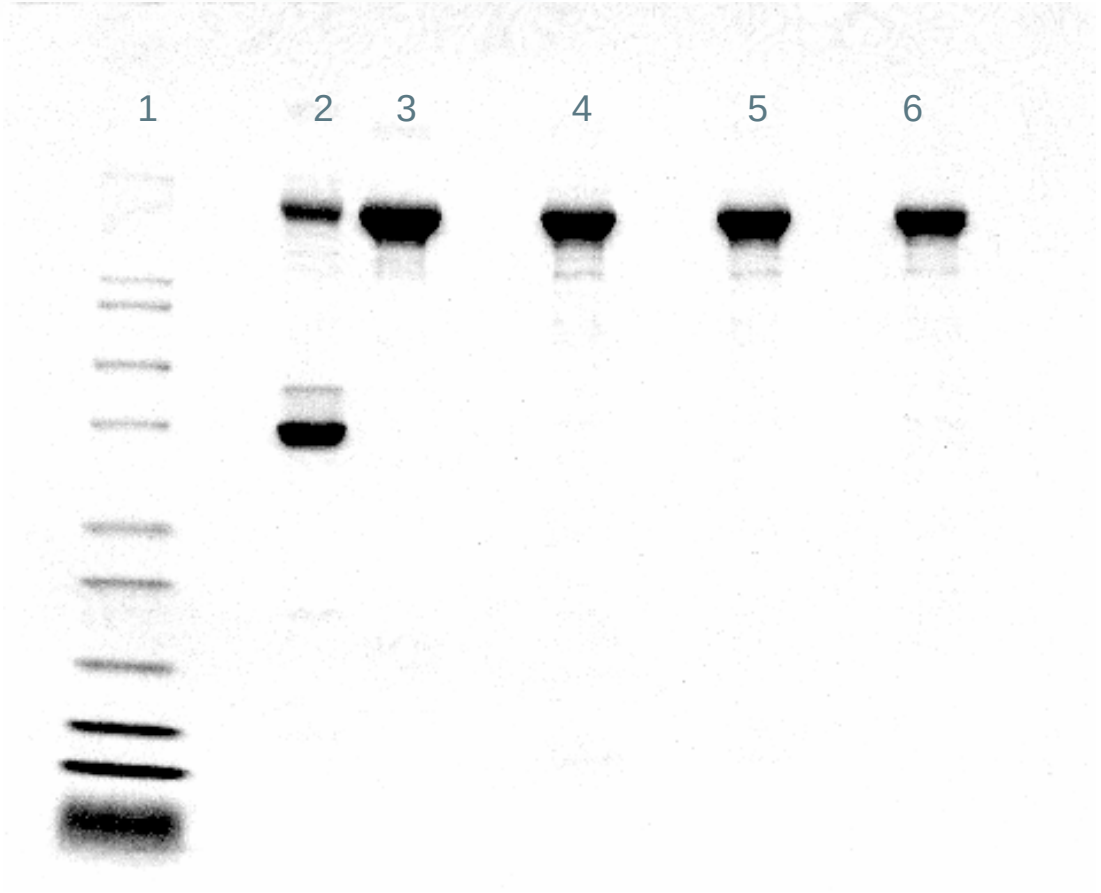
- Commercially available, (Pall BioSepra / Ciphergen)
- Regulatory support file available

Figures from Pall Life Sciences, http://www.pall.com/datasheet_biopharm_35677.asp

MEP Hypercel® – IgG purification

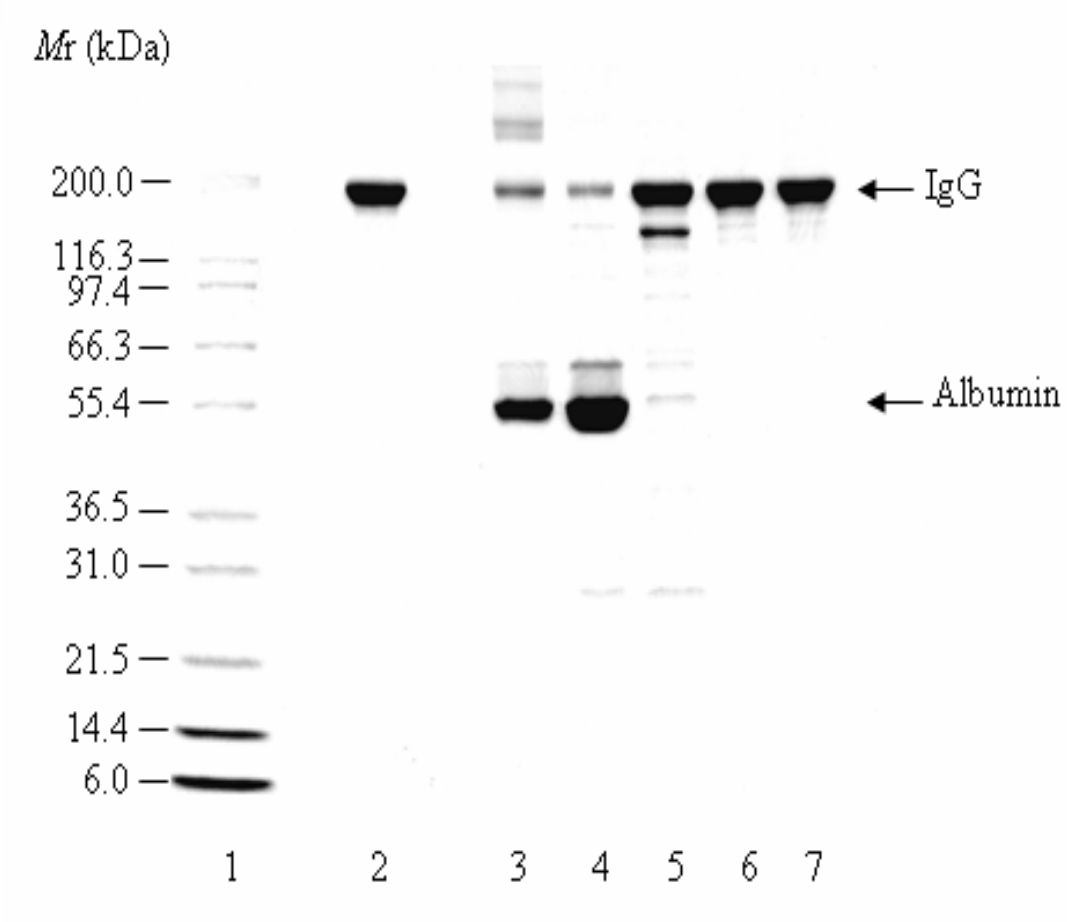


Purification of polyclonal IgG using MEP HyperCel



Lane	
1	Molecular weight markers
2	Ovine Serum
3 – 6	Eluted product, 5.0µg loading repeated purification cycles

MEP Hypercel® – Polyclonal IgG Purification (cycle 110)



1. Markers
2. IgG reference
3. Flow through
4. Wash
5. Pre-collection
6. Collected IgG
7. Sanitisation

MEP HyperCel

- MEP HyperCel selected as the resin utilised for the IgG capture step
- IgG purified to acceptable level, equivalent to that seen with Protein G
- Impurities cleared to levels were the remaining process stages ensured removal
- Cleaning was robust and sufficient to support many hundreds of purification cycles of thawed, filtered serum application
- Facilitated the introduction of a low pH hold step to allow for virus inactivation
- Purification method could be developed using DoE factorial experimental design approach

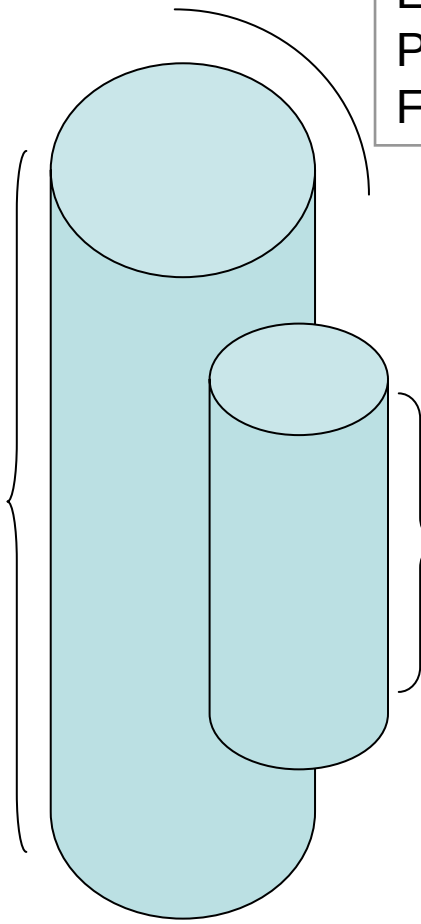
Biopharmaceutical scale up may be challenging

Image source: Dr. Uwe Gottshalk, Sartorius AG

A robust development process

- Combine the utilisation of scale down process models with state of the art analytical methods
- Labs on chips, micro-array and Biacore type systems for high through put screening of process samples and process parameters
- Map out process performance and develop process models
- Traditional approach is to defer these studies until phase III
- Rational approach is to perform this work early following proof of principle and aim to define both process and product pre phase III

Process Parameter operating Ranges

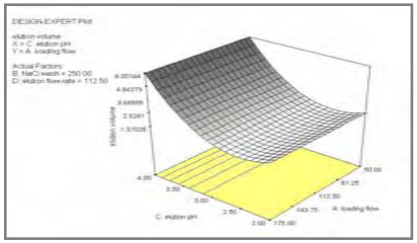


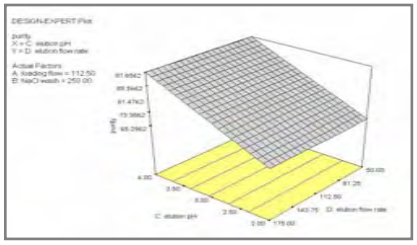
Edges of Process Failure

Proven Acceptable Range

Routine Operational Parameters

Factorial Experimental Design Scale down process models

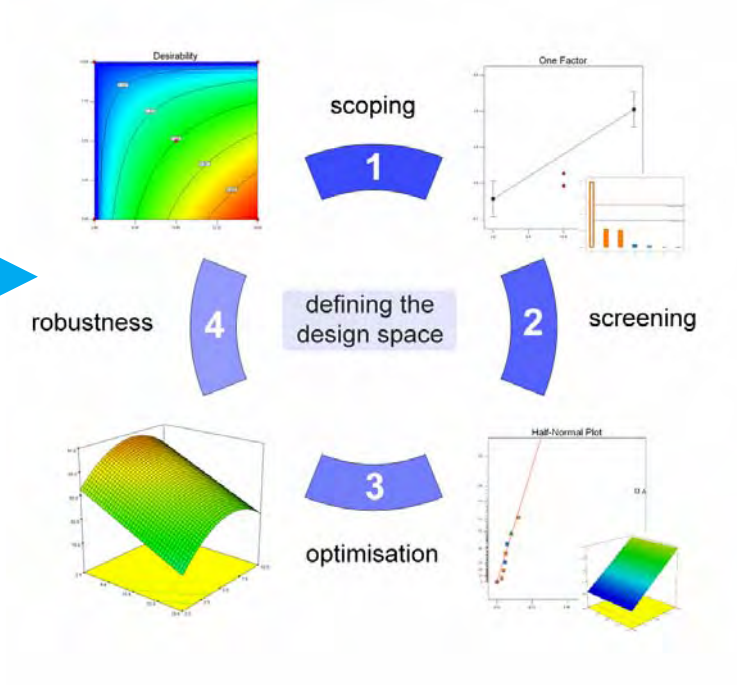




Application of Scale down process models

Process Development

Scaled down process models / High through put testing



Critical parameters / optimised processing & process monitoring

Analytical development

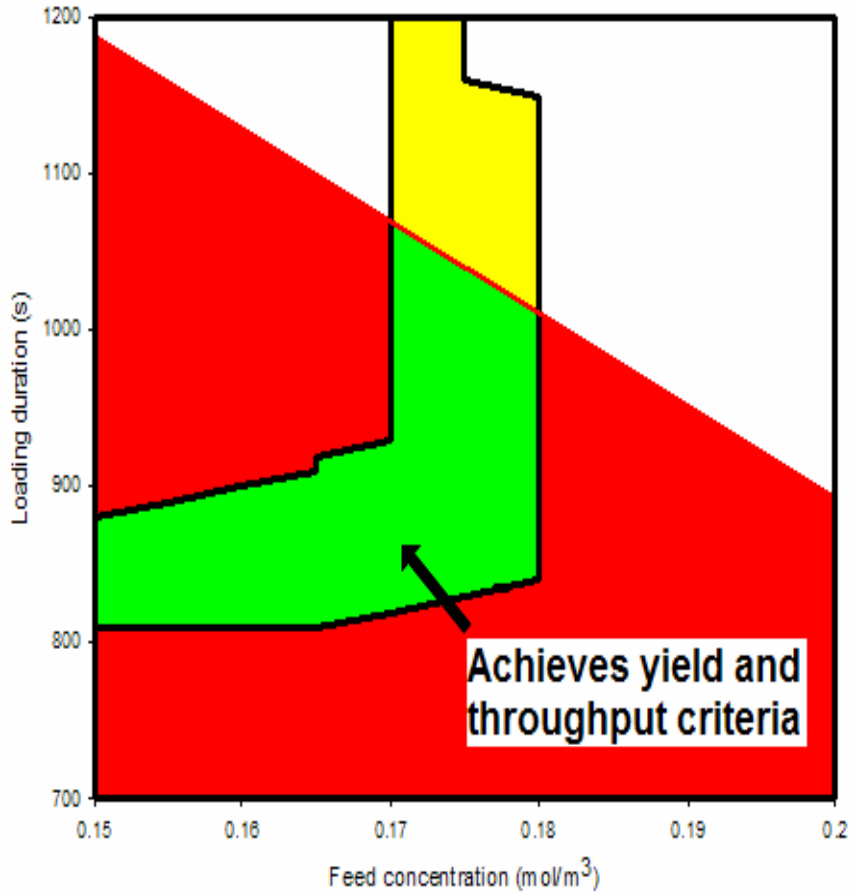
Commercial Production

Build the house on rock not sand

- **Traditional approach to process development**
- **involves experiment at full scale**
  - Costly, Time consuming and high risk
  - Limits data collection and process understanding
  - Poorly defines process response variables and control parameters
  - Limits optimisation (yields, COG and cycle times)
  - Not suitable for commercial routing manufacture
  - House on sand!

- **Small scale process model process development**
  - Eliminates the need for full scale process development, driven by process understanding
  - Rapid, cheap and cost effective
  - Facilitates total process characterisation studies at phase I/II CTM manufacture
  - Builds a robust and detailed database from process inception
  - Defines outer limits and edges of failure and process control parameters
  - House on rock!

Window of operation model for IgG Capture
(Sunil Chhatre, IMRC Bioprocess program – UCL UK)

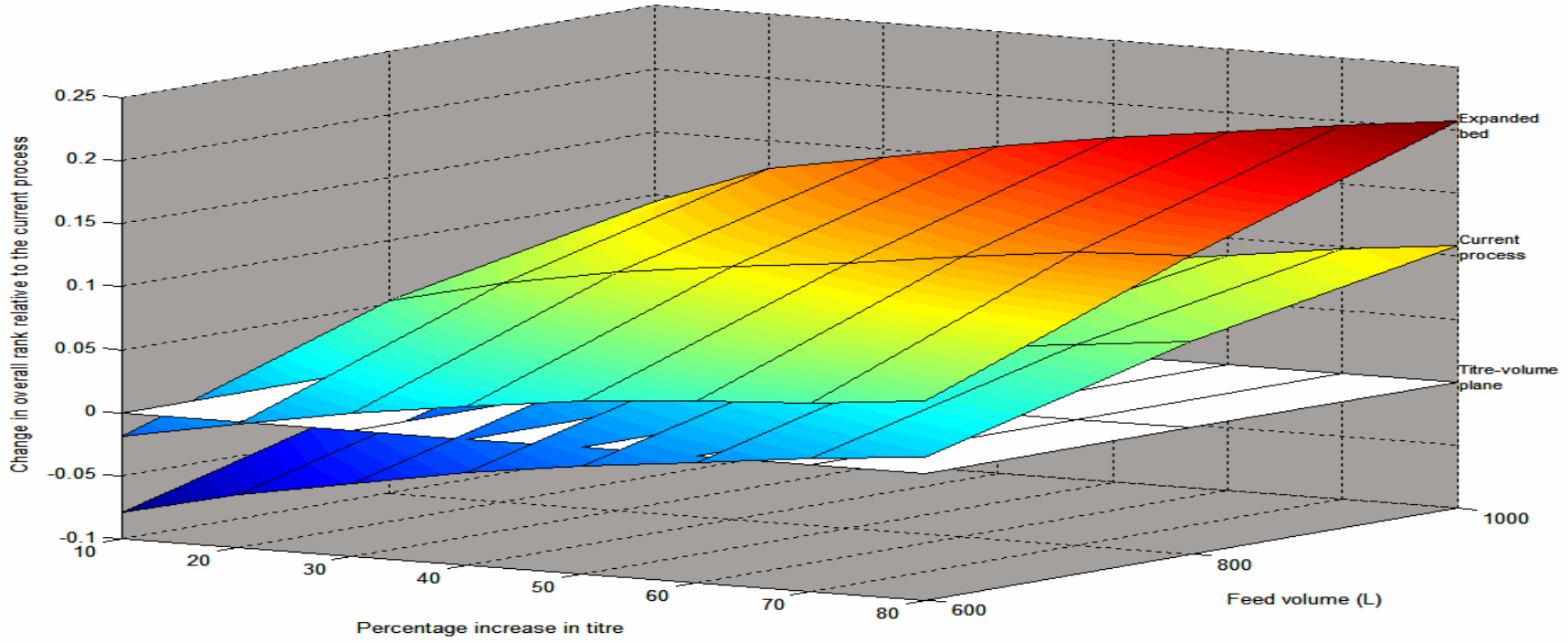


- Target Yield>85%
- Target through put
- 6000 – 6500 g IgG/h
- Window of operation model determines desired operational parameters (Green) to achieve desired yield and through put

Evaluation of process changes using a global sensitivity model

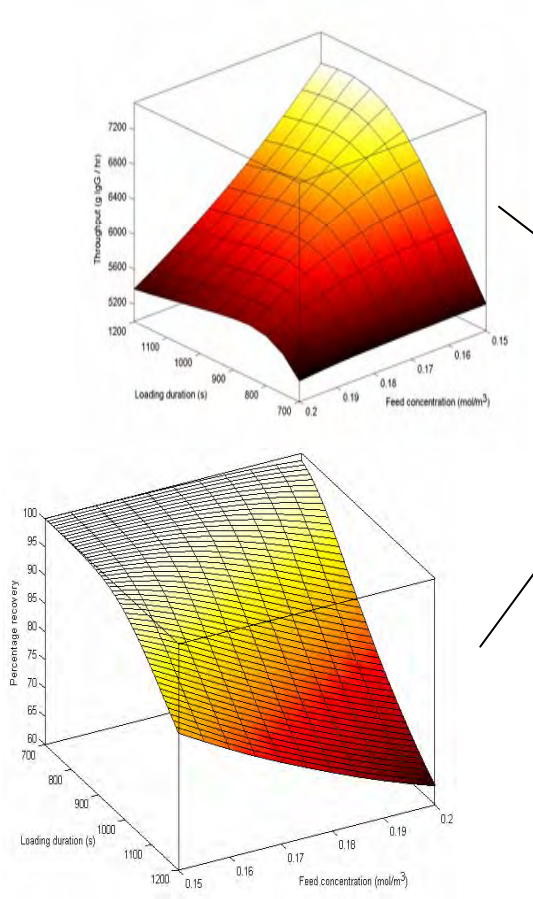
Facilitated comparison of packed bed, expanded bed and several column loading conditions to allow for selection of more optimal process step

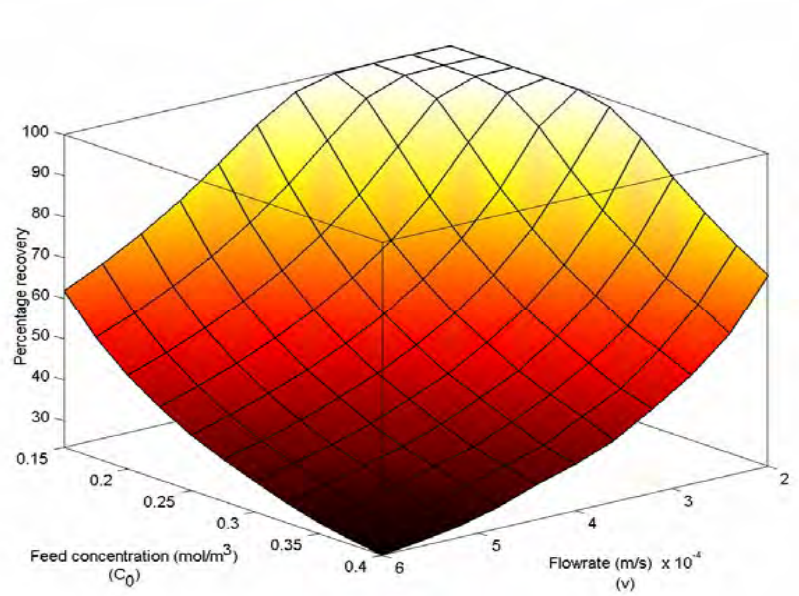
(Sunil Chhatre, IMRC Bioprocess program – UCL UK)



Global Sensitivity Analysis: IgG capture chromatography

Evaluation of Feed concentration (C_0); Duration of feed application (t); Matrix capacity (q_m) and Flow rate (v)
(Sunil Chhatre, IMRC Bioprocess program – UCL UK)

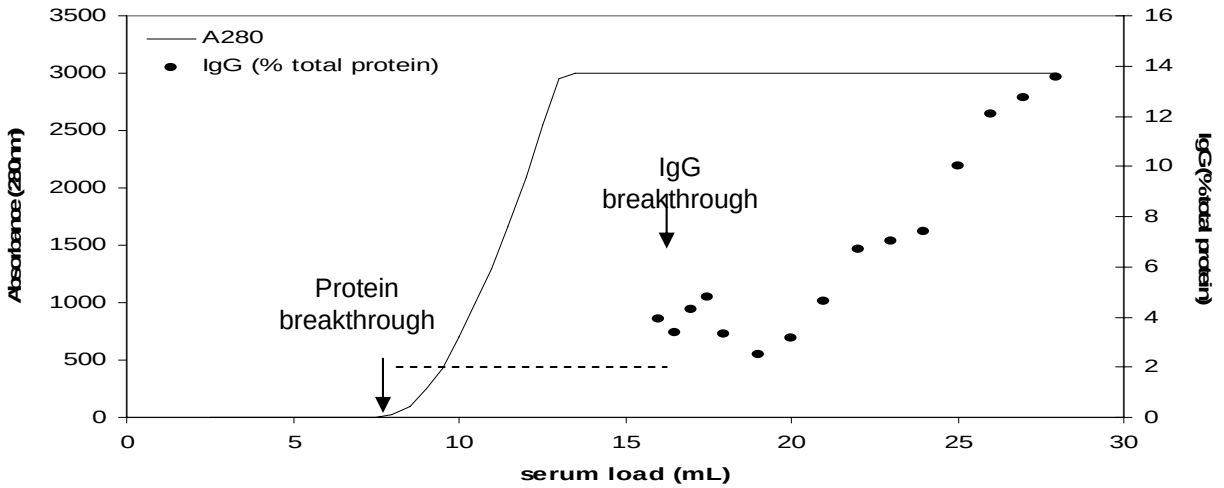


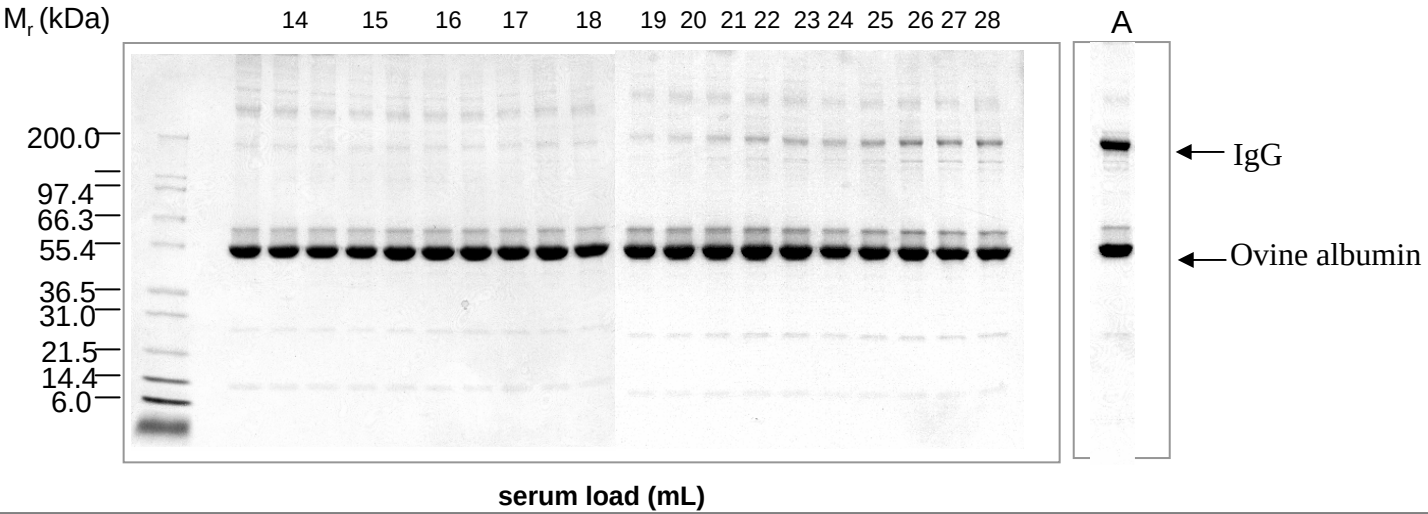


Final analysis relationship of feedstock concentration, flow rate and load duration

Test bed for process operation and
Determination of process optimisation benefit

MEP binding capacity studies



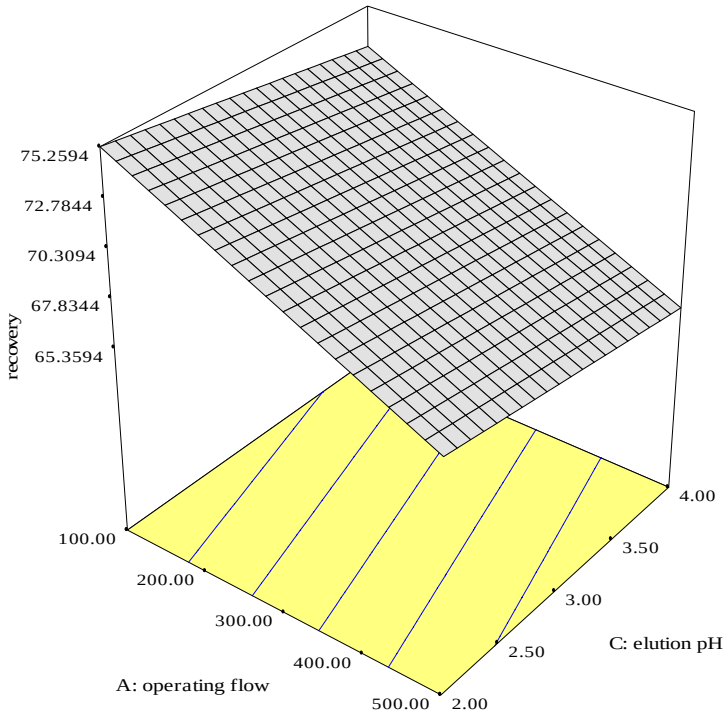


Elution pH relationship between recovery, flow and pH

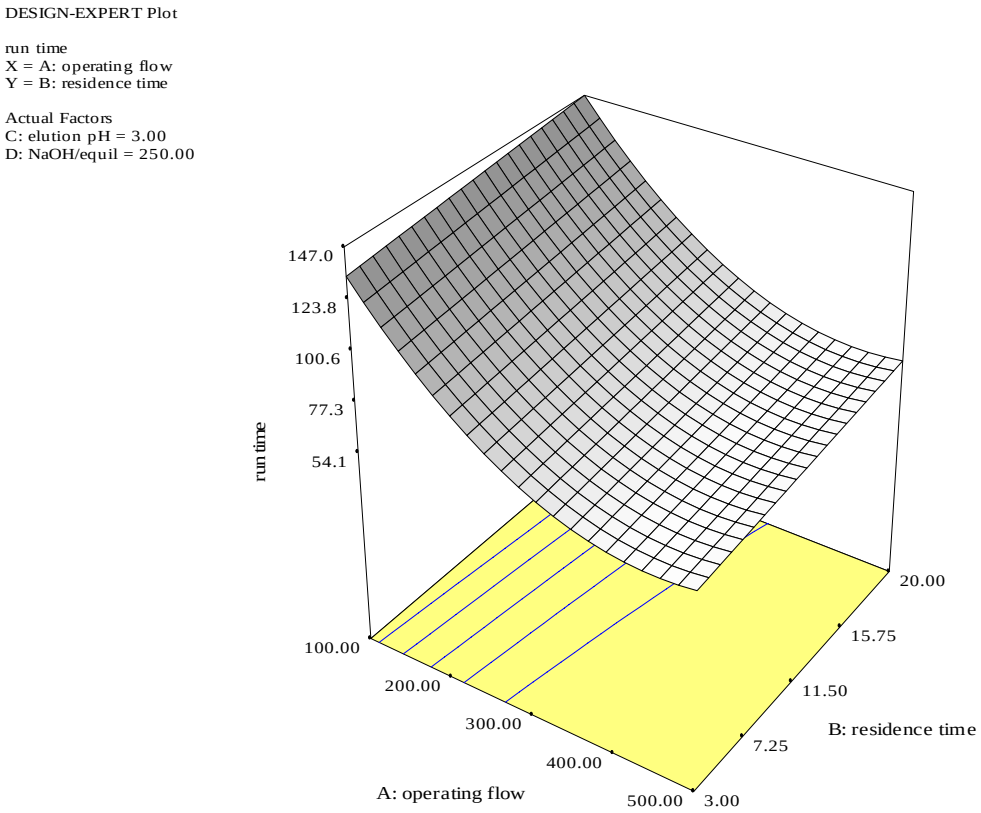
DESIGN-EXPERT Plot

recovery
X = A: operating flow
Y = C: elution pH

Actual Factors
B: residence time = 11.50
D: NaOH/equil = 254.05



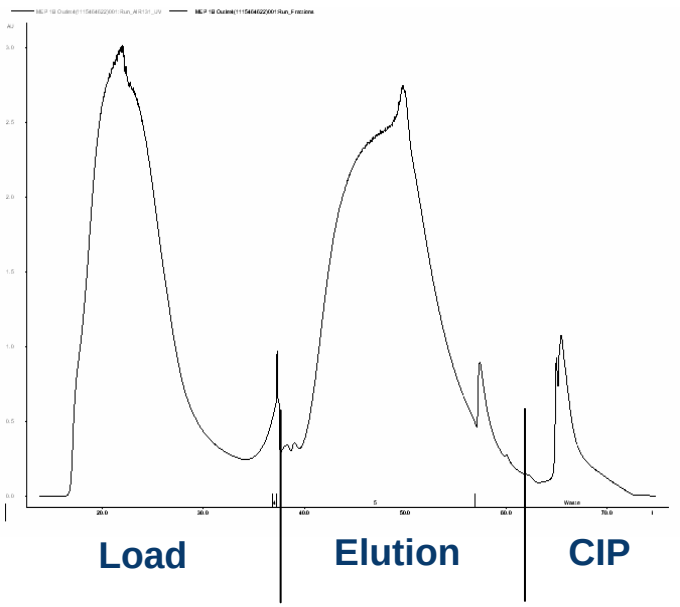
Effect of operating flow (cm / hr) and residence time (minutes) on total purification run time (minutes).



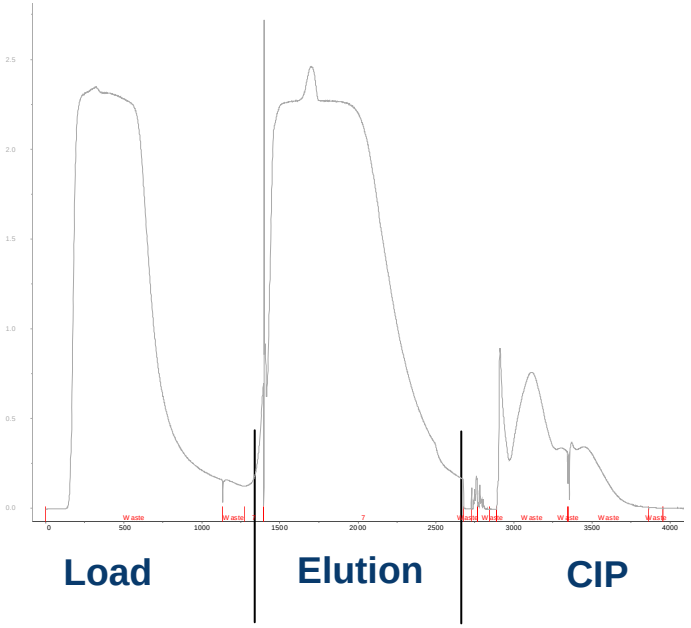
Commercial scale bio production

MEP HyperCel scale up elution profiles

15 mL packed bed

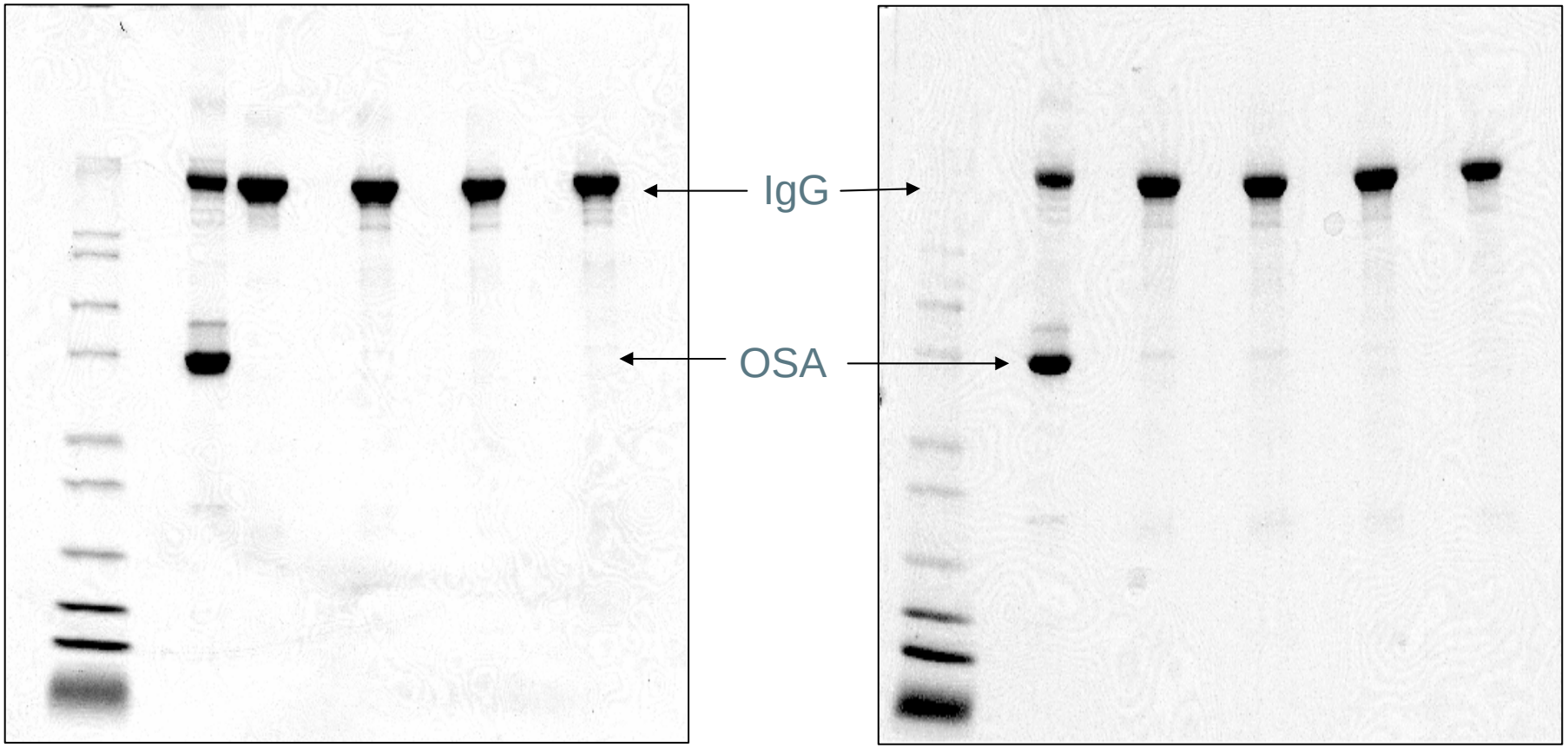


500L packed bed



The minor differences in peak shape are attributable to equipment differences at the 15 mL and 500L scales, the composition of each of the factions is comparable

Validation of the small scale model



16 mL IgG Capture Chromatography

500 L IgG Capture Chromatography

Commercial agreement with AstraZeneca for development of CytoFab™

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Last Updated: Thursday, 8 December 2005, 12:16 GMT

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Giant deal for small drugs firm

Shares in a small UK biotech firm Protherics soared more than 40% on Thursday after a lucrative deal with drugs giant AstraZeneca was announced.

Drug shares depend on new medicines to pep them up

Europe's third-biggest drugs maker has signed up for global rights to an experimental septic shock drug from Protherics worth up to £195m ($338m).

One industry analyst said the deal for the company's Cytofab drug was "stunningly good" for Protherics.

Protherics already markets an antidote to rattlesnake bites.

Septic shock - or sepsis - is a life threatening condition affecting three million people a year worldwide. If the drug is successful the rewards are likely to be substantial.

Image used for non-commercial purposes

CytoFab™

Manufacturing process scale-up completed

- Manufacturing process scaled to 3000 litre batch size
- Received a £10 million milestone payment from AZ
- AZ expanded phase 2 programme to start in Q3 2007, prior to global phase 3 study

Concluding remarks

- The application of small scale process models, DoE and high through put analytical methods facilitated the rapid development of a large scale high through put manufacturing process
- The predicted manufacturing process has been successfully operated at a scale approximately 30,000 fold from the original scale models
- The performance of the large scale process maps back to that demonstrated at the small scale
- This development program has successfully scaled an MEP HyperCel process to deliver a manufacturing process with a capability for processing up to 100 kg antibody per batch