

# Future Technologies in Downstream Processing

The Penridge Suite, London, N11 1NL, UK : Friday, 16 September 2011 09:00 - 17:00

The increased titres and productivity of the latest generation of cell lines are now well documented and understood. The rate limiting steps and capacity constraints within biopharmaceutical production have now moved to the downstream unit operations. This meeting aims to explain each of these unit operations and may offer some possible solutions.

This event has CPD accreditation and will have a discussion panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

Meeting Chair: John Moys, Sartorius Stedim, UK

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *John Moys*, Sartorius Stedim, UK

10:00 – 10:30 **Harvest & Recovery of High Value Biopharmaceuticals**

*Jennifer Edwards*, Eden Biodesign, UK

10:30 – 11:00 **Selection and Scale-up of Ultrafiltration Membranes**

*Martyn Paddick*, BPL, Elstree, UK

Considerations for the selection of ultrafiltration membranes and issues regarding the scale up of ultrafiltration processes from lab scale to full production scale.

11:00– 11:30 **Mid-morning break, Poster Viewing and Trade Show**

11:30 – 12:00 **Running a single use platform process - Advantages and Obstacles.**

*John Milne*, Technical Manager, BioUETIKON, Ireland

Within the biopharmaceutical industry single-use technologies are increasingly evolving and becoming commercially available for a wide variety of applications and processing steps. When compared with traditional reusable systems, single-use or disposable technologies can reduce costs and increase flexibility and speed which are important drivers within the industry. This presentation will look at the integration of a single use platform process in a contract manufacturing setting and in particular at the perceived advantages and obstacles that are presented using such an approach. As a contract manufacturing organisation (CMO) specialising in the manufacture of mammalian cell culture derived therapeutics, BioUetikon has seen at first hand the benefit to its development, pilot-production business of adopting a single-use strategy. The flexibility to incorporate a variety of projects by utilising existing facilities with reduced turnaround times has been deemed advantageous by clients and satisfies the obvious need for a competitive business approach to the Company. Some practical challenges to the efficient incorporation of disposable consumables will be considered using the example of the manufacture of a therapeutic monoclonal antibody for a recent clinical trial.

12:00 – 12:30 **Sequential Multi-column Chromatography.**

*Chris Davis*, Process Group Manager, **Mott MacDonald**, UK

Whilst there have, over the last 10 to 20 years, been significant developments in the area of upstream processing there have been fewer developments in the area of purification. The development of sequential multi-column chromatography has the potential to offer significant cost reductions over traditional column chromatography. The presentation will report on a desk-top study evaluating this new technology.

12:30 – 13:30 **Lunch, Poster Viewing and Trade Show**

13:30 - 14:30 **Question and Answer Session and Speakers photo**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:30 - 15:00 **Affinity Platform Approaches for the Purification of Non-Antibody Bio-Therapeutics**

*Sharon Williams*, Product Development and Downstream Processing Manager, ProMetic BioSciences Ltd. UK

Non-antibody proteins represent 65% of the bio-therapeutics market and include growth factors, hormones, cytokines, plasma proteins, therapeutic enzymes and protein vaccines. Whilst Protein A has provided a useful affinity capture platform for monoclonal antibody purification, equivalent platforms for non-antibody molecules have proved elusive.

Synthetic ligands, obtained through the use of computational chemistry and ligand library screening can be targeted to almost any protein. Such ligands provide a means of capturing and purifying non-antibody proteins and are also very robust and can be reused for many purification cycles. Ligands may be generated by screening general ligand libraries, modelling known binding compounds or identifying potential ligand binding sites on the protein of interest and modelling complementary binding ligands in-silico. Techniques such as molecular docking provide useful tools for predicting how potential ligands might interact with a particular region of a protein and virtual screening algorithms can be used to pre-select potential ligand candidates for synthesis and in-vitro screening.

15:00 – 15:30 **Afternoon Tea/Coffee, Last Poster Viewing and Trade show**

15:30 – 16:00 **Innovative Technologies for Downstream Biomanufacturing**

*Omar Wahab, Sartorius Stedim, UK*

The separation of different biomolecules from the functional drug product is fundamental when designing any biopharmaceutical manufacturing process. This separation should leave a high purity drug product of high efficacy, but still maintain a recovery that leaves the process economical at campaign scale. The downstream processing has seen many challenges over the past few years; from handling high product titres into the so called "purification bottle neck" to running single-use platforms. The recent surge in single-use technologies has helped reduce personnel, validation costs and risk to product safety, as well as reducing time to market. This presentation will discuss some of these cutting edge single-use technologies/hybrid systems in the downstream purification process, and give an insight to the process economics of such technologies.

Examples of technologies used in the manufacture of recombinant proteins, monoclonal antibodies and vaccines will be shown together with detailed case studies for the separation of impurities such as host cell proteins, DNA, viruses, endotoxins, detergents and aggregates from the target protein. Membrane chromatography is fast becoming a preferred choice for the polishing chromatography step. This technology will be discussed in detail, alongside the new trends in more longstanding technologies such as depth and crossflow filtration. Finally novel technologies for virus inactivation will be discussed, such as Ultraviolet – C Irradiation and automated systems for the low pH holding step will be presented.

16:00 – 16:30 **A novel chromatography technique for purifying large molecules**

*Dr Tomas Nyhammar, GE Healthcare, Kent, UK*

By designing layered beads with different ligands in inner and outer layer new properties and features of the chromatography resins can be obtained. Layered beads has potential to give unique functionalities and solve difficult separation problems as well as improve process economics. Examples with novel resin designs and their properties are shown, and with examples on how layered beads can improve process economics and throughput in vaccine processing. Many downstream vaccine processes in current production either use centrifugation with sucrose gradients or size exclusion chromatography as unit operations in the purification strategy, with limited throughput. The presentation will show how impurities such as host cell proteins and DNA fragments are eliminated in one single chromatographic step with layered beads using multimodal ligands in combination with size exclusion. Process examples of this generic process will be shown with purification of human influenza virus, as well as adeno virus.

16:30 – 17:00 **Implementing biologics filling with implementation of single use systems**

*Brendan Fish, GlaxoSmithKline, UK*

17:00 **Chairman's summing up**

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**Keywords:** Purification, include downstream processing, primary separation, primary capture, chromatography, filtration, polishing, fill & finish, Single use Technologies, chromatography, purification, biopharmaceutical

### About the Chair

*John Moys* is the head of applications support, North Europe for Sartorius Stedim Biotech. He has over 15 years experience with Sartorius in both technical and commercial roles and has carried out projects in the UK, Europe and Asia. Prior to joining Sartorius he had over 8 years working in industry in manufacturing, quality control and R&D roles in the in-vitro diagnostics and antibody manufacturing sectors.

### About the Speakers

*Omar Wahab* started his career working at Abbott Laboratories, where he worked in downstream process optimisation and technical transfers. In 2007 Omar moved to Sartorius Stedim to take over the role as downstream process development specialist covering North Europe. He has been working on developing client's downstream manufacturing platforms and incorporation of new technologies.

*Martyn Paddick* is a Project Scientist at Bio Products Laboratory Ltd working on the development of intravenous immunoglobulin products.

*John Milne* is a Science graduate from University College Dublin and was awarded a PhD in Biochemistry in 1996. Following a series of postdoctoral research positions in the areas of molecular enzymology and protein chemistry, he joined BioUetikon Ltd (formally Archport Ltd.) in 2000 employed as Downstream Processing Manager specialising in the development, optimisation and validation of protein purification protocols for molecules proceeding to clinical trial and commercial manufacture. Following an investment 2007 by CPH Chemie + Papier Holding AG he has been employed as Technical Manager responsible for technical operations and supporting new project assessment. BioUetikon provides high quality process development, optimisation and Bioproduction services for mammalian cell culture derived therapeutics in a multi-suite GMP certified facility.

*Chris Davis*, Process Group Manager for the Nuclear and Process Division of Mott MacDonald, and is a chartered chemical engineer with 21 years of experience in process engineering, consultancy, and design management on projects of every scale in the biopharmaceutical industry. He has been involved in all stages of process development, production operations, design, commissioning, and validation. His responsibilities have included consultancy and concept studies, feasibility, preliminary engineering, detailed design, installation, and validation.

*Tomas Nyhammar*, organic chemist, graduated from chemistry department, Swedish University of Agriculture, Uppsala, Sweden in 1986. He started the same year to work for LKB Produkter AB as section manager within R&D and has had positions as project manager or section manager through all mergers with Pharmacia, Amersham and finally General Electric. The last years he has worked as senior project manager for different development projects mainly resulting in novel chromatography media for biomolecule purification.

*Brendan Fish* is Director of New Product Introduction and Process Technology for GSK at Barnard Castle. With a career spanning over 20 years, he was Director of Bioprocess Sciences at MedImmune Cambridge where he was responsible for all aspects of the development of purification methodologies, product characterisation, QC, formulation and delivery for MedImmune products in relation to their use in commercial pharmaceutical processes. This included initial design and optimisation, scale-up, process cost modelling, process integration and technology transfer to GMP Production for clinical trial supply. Brendan was also at Delta Biotechnology Limited as a Consulting Scientist. He played a key role in the development of their biotechnology-based products providing expert opinion and strategies for QA, QC, Production, Marketing, Operations, Regulatory Affairs and Engineering on all aspects of Process Development. Early in his career, he was a Post-doctoral fellowship at University of Toronto in Canada working in the School of Nutritional Sciences, studying the anti-nutritional effects of lectins in the diet

*Sharon Williams* is the Product Development and Downstream Processing Manager for ProMetic BioSciences Ltd. This is a biotechnology company that produces and supplies materials for the production and purification of biopharmaceuticals and for the capture and removal of bio-contaminants. Her role is to manage the development of new products from conception to product launch and subsequent use in the field. She also oversees the development downstream processes using these materials. She has been working in downstream processing since she started her PhD in the Biochemical Recovery Group at University of Birmingham, where she investigated adsorbent design for nanoparticle recovery. She continued this work as a Post Doctoral Research Associate at the University of Cambridge where she investigated the affinity purification of viruses.

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## **POSTERS**

### **The Development of a Low pH Virus Inactivation Step During Cation Exchange Chromatography**

David Gruber (Scientist II Downstream Processing) - [gruberd@medimmune.com](mailto:gruberd@medimmune.com)

Additional Author: Richard Turner (Associate Director Downstream Processing)  
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The purification of monoclonal antibodies (Mabs) typically consists of three chromatography steps and two dedicated orthogonal viral reduction steps. Low pH virus inactivation is performed by adding acidic titrants to bring the pH of the product containing solution down a point where adventitious enveloped virus, theoretically present, will be inactivated.

The traditional low pH step is typically a manual process which involves pumping titrants at set flow rates or bolus additions, mixing and measuring pH, often with very narrow pH specifications. Due to the number of steps in this process there may be complications such as pH overshoots at either low pH or after subsequent pH neutralisation. The consequences of deviation in this step may be trivial and only require minor readjustments in pH however in more extreme deviations product quality could be adversely effected.

This study investigates the feasibility of performing low pH virus inactivation whilst the product is bound to a cation exchange chromatography matrix. Potential benefits, including increased process control and improvements to product stability during the inactivation step will be discussed.

### **RAMAN SPECTROSCOPY IN BIOPROCESSING: SIGNAL ENHANCEMENT AND HPLC DETECTION**

D. P. Cowcher, S. Webster, A. Smith and R. Goodacre.

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Raman spectroscopy provides the potential for real-time, online bioprocess monitoring, as well as providing complementary information to existing established techniques in offline analyses, such as high-performance liquid chromatography (HPLC).

Raman is a vibrational spectroscopy that measures the wavelength changes of laser light when inelastically scattered by a molecule, providing a vibrational fingerprint much like its complementary technique, infrared (IR) spectroscopy. However, Raman has significant advantages over IR spectroscopy for biological analysis in that bands tend to be sharper, leading to high information content and also water is only weakly Raman active, meaning background solvent interference is insignificant. Additionally, Raman has benefits over other analytical techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR), in that spectra can be collected in as little as one second, it is non-destructive and instrumentation is small, making it adaptable to online, real time process analysis. However, the one major drawback of Raman spectroscopy is its inherent low sensitivity, due to the small probability of a Raman scattering event compared to Rayleigh (elastic) scattering. Our research explores and demonstrates enhancement techniques that improve the sensitivity, such as surface-enhanced Raman scattering (SERS) and liquid-core waveguide technology. Analytes that were previously barely detectable even in saturated solutions, can now be quantified in tens of parts-per-billion by locating them near to a nanoscale metal surface, as we have developed and will illustrate for dipicolinic acid (DPA; pyridine-2,6-dicarboxylic acid), a biomarker for bacterial endospores.

Finally, enhanced Raman spectroscopy has the potential to interface with HPLC and provide complementary vibrational structure information to the more established, routinely used detection techniques such as UV and MS. This is an area we are currently developing.

# VIBRATIONAL SPECTROSCOPY AND CHEMOMETRIC MODELING: APPLICATIONS IN DOWNSTREAM BIOPROCESS MONITORING.

V L. Brewster, L. Ashton, E S. Correa and R. Goodacre

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At-line, real-time measurement of biopharmaceutical production has long been a goal for biopharmaceutical companies. The development of robust process analytical technologies (PAT) for monitoring bioprocesses is essential for both quality control and product yield optimisation.

There are two popular vibrational spectroscopic methods that have scope for use for the analysis of bioprocesses; Fourier transform infrared (FT-IR) spectroscopy and Raman spectroscopy. Both techniques utilise the way that light interacts with matter to yield information on the functional groups or molecules present in the sample. FT-IR spectroscopy is concerned with the absorption of light from the mid IR region, whereas in Raman spectroscopy, it is the inelastic scattering of light from a laser usually in the visible region of the spectrum which is measured. In both cases, the resulting spectrum provides a unique spectral fingerprint which contains both qualitative and quantitative information. Due to the large number of variables, vibrational spectroscopic data are often combined with multivariate analysis (chemometric) strategies, which aim to simplify the spectral matrix and turn the data into useful knowledge about the samples under study.

Vibrational spectroscopy has a rich history in protein analysis and is an ideal technique to be investigated for the non-invasive, on-line monitoring of bioprocesses as it is non-destructive, relatively inexpensive, rapid and quantitative. In the case of Raman spectroscopy the confocal nature of the technique also makes it possible to focus through transparent vessels.

A major area of interest in biopharmaceutical characterisation is post translational modifications (PTMs). Raman spectroscopy and principal components analysis (PCA) have been used to distinguish successfully between a non-glycosylated protein (RNase A) and its glycosylated equivalent (RNase B) and also deglycosylated RNase B (Brewster *et al.*, 2011). We have also shown that Raman spectroscopy can be used in conjunction with partial least squares regression (PLSR) to determine the concentration of glycoprotein (RNase B) in a mixture of protein and glycoprotein (RNase A and B). Further to this we present data that conclusively shows that the glycosidic based vibrations are the ones that are discriminatory, this evidence is two-fold: (a) the PLSR chemometric model indicates specific bands which can be assigned to the glycan, and (b) when the glycosylated protein RNase B has the sugar residues removed (either enzymatically or chemically) its whole spectrum reverts to the 'native' non-glycosylated RNase A protein.

We also present data demonstrating the suitability of Raman spectroscopy for discrimination between the glycan component of a glycoprotein. We have successfully differentiated between monosaccharides, various glycan fragments (di/trisaccharides), and finally, different whole glycans. Again, PCA has been used in conjunction with Raman spectroscopy to distinguish between glycans with the same sugars residues in different spatial arrangements, inspection of the PCA loadings here has highlighted the importance of the glycosidic bond vibrations.

Moreover, FT-IR spectroscopy has been applied to biopharmaceutical analysis, in the detection of protein contaminants in biopharmaceutical products. We present for the first time a vibrational spectroscopic method which when combined with appropriate chemometric modelling is able to classify pure proteins and proteins 'spiked' with a protein based contaminant, in some cases, in concentrations as low as 1%.

Finally, we have also demonstrated the utility of vibrational spectroscopy in monitoring the stability of biopharmaceutical products; RNase A and B were been subjected to unfolding using guanidine hydrochloride in order to assess the potential of Raman spectroscopy to monitor such changes. Fluorescence spectroscopy was also used as a corroborative technique. Unfolding curves for both fluorescence and Raman data correlate well, both showing that the  $[d]_{50}$  (concentration of denaturant needed to unfold half of the protein molecules) is in good agreement for both techniques.

Brewster, V.L., Ashton, L. & Goodacre, R. (2011) Monitoring the glycosylation status of proteins using Raman spectroscopy. *Analytical Chemistry* **83**, 6074-6081.

Registration Web Site: [www.regonline.co.uk/downstream2011](http://www.regonline.co.uk/downstream2011)