Recovery and purification of biosynthetic products: Downstream processing for the 21st century

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Cineworld: The O2, London, SE10 0DX UK
www.regonline.co.uk/down2013

The growing interest in biotechnology development and the high demand for biopharmaceuticals have contributed to the growth of the global biopharmaceutical market, which now comprises over 200 products. This market has reached $106 billion in 2009 and is forecasted to grow with a CAGR of 11.2% by 2016. The main challenges of the biopharmaceutical industry now are to increase the efficiency and reduce the costs of the manufacturing process, with respect to the protein recovery, purity and efficacy. Therefore, new strategies are being implemented for the upstream and downstream processing. In the upstream, genetic engineering and glycoengineering approaches, as well as single-use bioreactors are being developed and applied. However, as culture yields are continuously increasing, the bottleneck in the manufacturing process has shifted to the downstream processing, which accounts for ~ 80% of the total production cost. Many aspects are being considered for improving economics in downstream processing, such as simplification of the chromatographic purification processes, application of membrane technology, crystallization and precipitation. This meeting will focus on the advances, innovation and challenges in the area of downstream processing of biopharmaceuticals.

This event has **CPD accreditation** and is part of **The 2013 BioProcessing Summit** - www.BioprocessingSummit2013.com

Meeting chair: *Professor Colin R. Harwood*, Centre for Bacterial Cell Biology, Institute for Cell & Molecular Biosciences, Newcastle University, UK

Who Should Attend
Biotech and Pharma Industry: CEOs, Chief Scientists, Group Heads, Senior and Junior Scientists, Research Managers
Academic and Research Institutes: Group and Lab Heads, Postdoctoral Scientists and Research Students

Talk times include 5 – 10 minutes for questions

9:30 – 10:15 **Registration**

10:15 – 10:30 **Introduction by the Chair:** *Professor Colin R. Harwood*, Centre for Bacterial Cell Biology, Institute for Cell & Molecular Biosciences, Newcastle University, UK

10:30 – 11:15 **Fitting non-platform processes into platform timelines**
Dr Alison Tang, Associate Scientist II, MedImmune Ltd, Cambridge, UK
As therapeutic proteins become more complex, the purification challenge increases; in addition, the time allocated for process development reduces due to an ever expanding portfolio. To alleviate the problem, high throughput process development and automation was implemented. In the meeting the challenges and process development tools will be discussed.

11:15 – 12:00 **Semi-selective protein precipitation using salt-tolerant copolymers for industrial purification of therapeutic antibodies**
Mr Florian Capito, Institute for Organic Chemistry and Biochemistry, Technische Universität Darmstadt, Germany
Semi-selective precipitation employing customized salt-tolerant ionic copolymers was evaluated as antibody (mAb) purification step. Precipitated mAb was easily re-dissolved in small volume, enabling concentrating mAb up to hundred-fold, while residual polymer could be removed to > 98 %. mAb recovery of > 90 % and host cell protein clearance of > 80 % were achieved, not requiring any pre-dilution of the cell culture fluid. Precipitation showed no impact on mAb binding affinity. Compared to protein A based purification, yield and purity were lower; yet for high titer

processes already being implemented, precipitation is more cost-effective and easier to scale.

12:00 – 13:15 **Lunch, poster exhibition and trade show**

13:15 – 14:15 **Discussion session**

This discussion session is an informal question and answer session. This is an ideal opportunity to get advice and opinion from experts in this area. This session is not for questions about specific talks, which can be asked after the speakers session, but for discussing either general topics or specific issues. There are three ways you can ask questions:

1. *Before the session* you can submit your question to Euroscicon staff at the registration desk,
2. *Before and during the session* you can submit a question or comments, by email, which will be provided on the day of the event
3. *During the session* you can put your hand up and join in

14:15 – 14:45 **Afternoon Tea, last poster session and trade show**

14:45 - 15:30 **Liquid-liquid Extraction of Biomolecules; have they a role to play in current bioprocessing strategies?**

Dr Jonathan Huddleston, Consultant Biochemical Engineer, Advanced Bioprocessing Centre, Brunel University, UK

Liquid liquid extraction of biomolecules has periodically been promoted as an alternative to packed bed chromatography in downstream processing. The nature and properties of aqueous biphasic extraction systems will be discussed and their potential for application in the downstream recovery of bioproducts will be assessed. Potential applications in biomolecular analysis will also be briefly considered.

15:30– 16:15 **Bacillus protein secretion, an aid to downstream processing**

Professor Colin R. Harwood, Centre for Bacterial Cell Biology, Institute for Cell & Molecular Biosciences, Newcastle University, UK

Bacillus species are prolific secretor of proteins that are directed into the culture medium. The main secretion pathway, the Sec translocase, requires targeted secretory proteins to be in a translocation competent, substantially unfolded, state. Consequently, secretory proteins must fold as they emerge on the trans side of the membrane. While this has the advantage of facilitating their folding into their native and therefore functional configuration, it adds an element of vulnerability to proteins that intrinsically fold slowly. Approaches to address this issue and improve the yield of proteins in the culture medium will be discussed.

16:15 – 16:30 **Chairman's Summing Up and Close of Meeting**

About the chair:

Colin Harwood is a graduate of London and Leeds Univerities and is currently Professor of Molecular Microbiology at the Centre for Bacterial Cell Biology, Newcastle University. He has studied Bacillus protein secretion for more than 25 years and Bacillus molecular biology for more than 40 years. He has collaborated widely with some of the major producers of industrial enzymes, and uses his knowledge of the fundamental processes involved in protein secretion to engineer strains with increased yields.

About the speakers:

Alison Tang is a process development scientist at MedImmune Ltd. She focuses her technical development in novel scale-down technology with automation, aiming to minimise time and material required for process design. She obtained a PhD in Biochemical Engineering at University College London in 2010, specialised in scale-down chromatography and modelling. She successfully transformed breakthrough curves obtained from micro-scale columns to a milli-litre scale column using mathematical models; making scale-down and scale-up using constant linear velocity possible.

Florian Capito's current scientific focus is on new purification strategies within production of biotherapeutic proteins, bioanalytics and bio- process monitoring. After obtaining his Bachelors degree in molecular Biology at Johannes- Gutenberg University Mainz, he did his Masters in Proteinscience at Lunds Universitet, Sweden, before starting his PhD thesis at Technical University of Darmstadt in conjunction with Merck KGaA, which he will finish beginning 2014. Besides his current research, his expertise is within protein-structure modeling, structure- function comparison, protein bioinformatics and protein chemistry.

Jonathan Huddleston spent 17 years in the Biochemical Recovery Group, Chemical Engineering, University of Birmingham Received his PhD for work on downstream processing using Aqueous Biphasic Systems. He then spent 6 years at the Centre for Green Manufacturing, University of Alabama working on Green Chemistry issues including the characterisation of ionic liquids and the processing of

ligno-cellulosics. He received the Presidential Challenge Award in Green Chemistry in 2005. Subsequently he spent several years with the Millipore Corporation developing and characterising adsorption bioprocesses. He is now an independent consultant working closely with the Advanced Bioprocessing Centre at Brunel University.

POSTER PRESENTATIONS

SEMI-SELECTIVE PROTEIN PRECIPITATION USING SALT-TOLERANT COPOLYMERS FOR INDUSTRIAL PURIFICATION OF THERAPEUTIC ANTIBODIES

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Semi-selective antibody precipitation employing customized salt-tolerant ionic copolymers was evaluated using pure monoclonal antibody (mAb) solutions, mAb- BSA binary protein model systems and different cell culture fluid. Precipitated mAb was easily re-dissolved in a small volume, enabling concentrating mAb up to hundred-fold, while residual polymer was removed to > 98 % using cationic polymer attached to silica flakes. Depending on copolymer chain length and composition, mAb recovery of > 90 % and host cell protein clearance of > 80 % can be achieved, not requiring any pre-dilution of the cell culture fluid. Copolymer- based precipitation showed no impact on mAb binding affinity. Compared to protein A based purification, yield and purity were lower; yet for high titer mAb purification processes being implemented in the future, precipitation is more cost-effective and easier to scale.
References:
Capito, F., Skudas, R., Stanislawski, B., & Kolmar, H. (2013). Polyelectrolyte–protein interaction at low ionic strength: required chain flexibility depending on protein average charge. *Colloid and Polymer Science*,
1-11. Capito, F., Bauer, J., Rapp, A., Schroeter, C.A., Kolmar, H., & Stanislawski, B. (2013) Feasibility study of semi-selective protein precipitation with salt-tolerant copolymers for industrial purification of therapeutic antibodies. *Biotechnology & Bioengineering*, in press. Capito, F., Skudas, R., Kolmar, H., & Stanislawski, B. (2013). Selectivity and yield optimization in polymer-driven protein purification depending on protein and copolymer charge density, hydrophobicity and copolymer molecular weight. *Biomacromolecules*, submitted.

DEVELOPMENT OF A DOWNSTREAM PROCESS FOR THE PURIFICATION OF LIPID A FROM ENGINEERED *Escherichia coli* K4: A POTENTIAL SCAFFOLD TO OBTAIN SEMI-SYNTHETIC VACCINE ADJUVANTS

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The Lipid A from *Salmonella minnesota R595* is widely used as a raw material for the construction of effective adjuvants, some of which are now at an advanced stage for human applications. It is a powerful immunostimulatory which acts as molecular signal of microbial invasion (PAMP - Pathogen Associated Molecular Pattern) and it is able to activate vigorously innate and adaptive immunity (Roberts et al., 1996). The Lipid A, despite its great potential to stimulate the immune system, is not directly usable as an adjuvant in vaccines for humans for its high pyrogenicity and often lethal toxicity (Chung JY et al. 2001). It can be obtained from the processing of the lipopolysaccharide (LPS), an essential component of the outer cell membrane of Gram-negative bacteria. Its primary structure is composed of three chemically and genetically distinct domains: the Lipid A, the and the O-chain. For this reason we focused on the development of a fermentation and downstream process to obtain Lipid A from an engineered *Escherichia coli K4* that overproduces LPS. In particular, the purification of Lipid A is based on the use of proteolytic systems for the removal of protein contaminants, on the treatment with solvents, on membrane processes to remove other contaminants and hydrolysis for the recovery of the product.

References: Chung J.Y., Boyce J.D., Townsend K.M., Frost A.J., Ghoddusi M and Adler B., 2001. Role of capsule in the pathogenesis of fowl cholera caused by Pasteurella multocida Serogroup A. Infect Immun. 69: . Roberts I.S., 1996. The biochemistry and genetics of capsulated polysaccharide production in bacteria. Annu Rev Microbiol. 50: 285-315.

OBTAINING NATURAL dsRNAs AND THE STUDY OF THEIR PHARMACOLOGICAL AND TOXICOLOGICAL PROPERTIES

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At present, a steady increase in the prevalence of infectious diseases is observed in the world. The high degree of antigenic variation of some virus families can result in new outbreaks of epidemics in the population and, therefore, prophylactic is not always effective. A huge range of infectious agents and the prevalence of immunodeficiency states in the population urge researchers to develop not only new antivirals, but also drugs affecting the status of the nonspecific resistance system and having an immunomodulatory effect. A preparation of this type, Ridostine, was developed at the Institute of Medical Biotechnology FBRI SRC VB "Vector" (Berdsk, Novosibirsk Region, Russia). The preparation is a product of biological synthesis. The active ingredient of Ridostine is double-stranded ribonucleic acid (dsRNA) from virus-like particles of a killer strain of yeast Saccharomyces cerevisiae. The process of dsRNA isolation comprises the stages of dsRNA extraction with saline solutions and the deposition of extracted material; dsRNA fractionation with lithium chloride solution; the solution deproteinization; obtaining of alcohol precipitate and dsRNA aqueous solution; lyophilization. Ridostine in injections and ointment is currently used as an interferon inducer and immunomodulator for therapy of viral infections (influenza, ARVI, herpes) (Masycheva V.I. et al, 2004). Given the evidence of generalized nature of dsRNA effect on a macroorganism, it seems appropriate to develop a drug for oral administration. The aim of this work was to compare the toxicity levels and some indicators of activity of the nonspecific resistance system at intramuscular and intragastric administration of Ridostine substance to mice. An experimental study was performed on ICR mice weighing 19-21g. Ridostine substance containing 18.2% dsRNA was used in the work. The evaluation of pharmacological and toxic properties of the preparation included the study of the effect of maximum tolerated and effective doses at intramuscular and intragastric administration on physiological (body temperature and weight) and immunological parameters (total amount of leukocytes, the number and redox activity level of peritoneal exudate macrophages in NBT-test , the weight of lymphoid organs). It was shown that none of the animals died after intragastric administration of Ridostine at the dosage range between 100 and 1250 mg/kg, indicating its low toxicity for this route of administration. The most pronounced changes in the state of mice were revealed at the dose of 1250 mg/kg. The clinical presentation of toxicity was characterized by retardation, reduced body weight of some animals one day after administration. For intramuscular administration, deaths were recorded in mice given the doses between 130 mg/kg and 310 mg/kg. The maximum tolerated dose was 100 mg/kg; the clinical picture was characterized by body weight loss (10%) and temperature decrease (to 36°C in some animals) one day after administration. The evaluation of immunological indices against the background of Ridostine administration showed that the preparation administered intramuscularly and intragastrically at maximum tolerated doses (100 and 1250 mg/kg, respectively) reduced the number of peritoneal macrophages, splenic weight indices and amount of leukocytes. The activation of immune system was observed one day after the drug administration at the doses of 10 and 20 mg/kg, which manifested as increased metabolic activity of peritoneal macrophages (up to 232%) for intramuscular route and up to 143% for intragastric route. Thus, it was found that the toxicity level of dsRNA Ridostine differed depending on the route of administration. The drug administration both in injections and intragastrically activates the macrophage component of protection reaction, influences the state of immunocompetent organs, the intensity of reactions being dose-dependent.

STUDY ON CULTURE MEDIUM COMPOSITION FOR BIOSURFACTANT PRODUCTION FROM *BACILLUS SUBTILIS* AND ITS INFLUENCE ON DOWNSTREAM PROCESS

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Currently, industries focus environment friendly processes. An alternative is replacement of chemicals by bioproducts with similar properties – obtained from biotechnological processes - (eg. surfactants to biosurfactants). The main bottlenecks implementing of this technology are: culture medium and its downstream process. The first represents ≈ 25 - 35% of production cost whereas the latter ≈ 60 - 85%. Cassava wastewater (CWW) was well-described as culture medium to biosurfactant (BS) production, on the other hand, a few papers evaluated the activated carbon (AC) and whey. BSs (amphiphilic compounds of microbial origin) are the backbone of biological membranes and except for a few, their physiological role is completely uncharacterized. The industrial interest on BSs is at the bulk product markets such as laundry detergents and domestic cleaning products. Nevertheless, they have potential applications within wide sorts of industries, since compared with their chemical counterparts (surfactants), show antimicrobial and functional activities, bioremediation, lower toxicity, biodegradability and activity at extreme conditions (temperature, pH and salinity). The aim

of this work was evaluated different concentrations of CWW, whey and AC – as a culture medium – to surfactin production (a sort of BS) from *Bacillus subtilis* LB5a. Whey and CWW were dried by spray dry and lyophilizer, respectively. Then, they were rehydrated at different concentration. Central composite design (CCD), erlenmeyer experiments, identified the best concentration of CWW, AC and whey; in which the effects were surface tensions (ST). Then, it was scale-up to bioreactor (7.5L). Foam (BS) was collected, centrifuged, recipitated (pH 2), neutralized, rehydrated (200mg.L^{-1}), analyzed by HLPC and dynamic light scattering (DLS). AC may support the strain to growth as biofilm and improve the transfer coefficient of oxygen, in its turn, induce BS production. Also, since surfactin can be recovery by foam overflow the AC adsorb BS, which increase the BS concentration in the foam, obviously, the foam volume is lower. It is can be advantageous for the next steps of downstream. CCD indicated the ideal culture medium composition (g.L^{-1}): ≈ 74 (CWW), 27.699 (whey) and 25 (CA). The foam volume collected ≈ 230mL, which after acid precipitation reached ≈ 55% of purity. DLS indicated a micelle size ≈ 100nm. Therefore, the production of surfactin using that culture medium resulted in a good BS production with may make easier the downstream process.

Keywords: Purification, downstream processing, primary separation, primary capture, chromatography, filtration, polishing,
Single use Technologies, purification, biopharmaceutical, nanofibres; productivity, Membrane Adsorbers, Scale Up, Process Development, Biosimilars, Process Development, protein secretion, Bacillus, proteases, translocase, chaperone, biopharmaceuticals, membrane technology, precipitation, antibody purification, customization, copolymer, bioseparations, liquid-liquid extraction, Mab, ATPS, Downstream Processing, Biomolecular Analysis

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