FALL 2015 • Volume 14/Issue 3 • ISSN 1538-8786

BioProcessing JOURNAL

Trends & Developments in BioProcess Technology

A Production of BioProcess Technology Network

BioProcessing JOURNAL

TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

TECHNOLOGY REVIEW

Article Published Online: October 9, 2015

Selection of Clarification Methods for Improved Downstream Performance and Economics

By Sarah Le Merdy

Introduction

The production of biopharmaceutical drugs typically involves a biological expression within a bacterial, yeast, or mammalian cell expansion system. Getting to the final product requires multiple purification steps, from primary clarification to the final formulation and sterile filtration. The aim of the initial purification steps is not to purify the stream perfectly but rather, to prepare the stream for finer and more specific purification steps further downstream. Apart from efficiently removing contaminants, the clarification stages also need to maintain high product recovery whilst being consistent and robust.[1] The finer downstream purification stages represent the largest portion of production costs; and for monoclonal antibodies (MAb), they can represent up to 80% of the overall cost. This cost is, however, heavily impacted by the quality of the feed stream that has been produced.

Early clarification and purification steps, although seemingly crude, are critical in removing contaminants and maintaining a cost-effective process further downstream. Selecting them scientifically, based on chosen performance markers, could thus improve the performances of downstream operations. After reviewing the typical feed stream compositions, this article reviews the existing literature and shows the advantages of thoroughly investigating and then selecting a clarification method that provides the best downstream performance and economics.

Typical Characteristics and Expected Purity of a Feed Stream Product

Feed Composition and Characterization

Depending on the nature of the product being produced using live cells (*e.g.*, plant, microbial, or mammalian) to extract biopharmaceutical active ingredients, the composition of a feed stream to be clarified and purified can vary significantly. An example of the feed characteristics for a MAb process is shown in **Table 1**.

Based on the cell viability and the primary clarification (centrifugation, flocculation) or lysis (chemical, mechanical) methods selected, the impurity profile may differ considerably. For example, low cell viability can mean high levels of contaminants released in the feed stream due to cell lysis.[3]

Among the typical main contaminants, DNA, host cell proteins (HCPs), lipids, and bigger particles such as cell debris can be identified. The proportion of solids in the feed are usually a good indicator of the challenges ahead in purifying it (~6–8% in Chinese hamster ovary [CHO], 10–13% and greater, up to 40%, in yeast).

HCPs compose a very heterogeneous family of molecules, in terms of molecular weights and properties (pI, immunogenicity, *etc.*). They are released during both the cell culture itself and the lysis process. Although HCPs are rarely fully characterized, a better knowledge of their properties could help in their removal during the downstream steps.

As for HCPs, nucleic acid material content depends on the type of biological product and its manufacturing process. Different strategies can be put in place to reduce the average size (measured in base pairs [bp]) and con-

TABLE 1. Typical process feed characteristics for MAbs.[2]

At Various Stages	Mode	HCP (ng/mg)	DNA (pg/mg)	Aggregate (%)	Protein A (ng/mg)	Yield (%)
Clarified Harvest	—	250,000–1,000,000	10,000–1,500,000	5–15	—	—
Protein A (capture)	Bind and elute	200–3,000	100–1,000	5–15	3–35	>95
CEX (purification)	Bind and elute	25–150	<10	<0.5	<2	75–90
AEX (polishing)	Flowthrough	<5	<10	<0.5	<2	>95

NOTES: Chromatography processes – protein A removal, cation exchange (CEX), and anion exchange (AEX)

centration of DNA in the final product. Endonucleases (such as Benzonase®) can be used for their digestion, and techniques like chromatography or tangential flow filtration (TFF) have been proven to efficiently reduce DNA contaminant levels.[4, 5]

Although the average particle size of a CHO preparation is around 10 µm[6], the larger particles are easier to remove by either centrifugation or depth filtration. The smaller particles, below 5 µm, are more likely to impact capacity or penetrate the primary clarification process and present challenges to membrane filter protection or chromatography performance downstream.

Before primary clarification begins, it can be found that specific pretreatment of less common feed streams, such as those with high cell densities or high levels of specific contaminants, may well generate feedstocks with properties compatible with effective and efficient downstream purification steps.

Feed Pretreatment

Feed pretreatment is a well-known technique widely applied to wastewater treatment to build up sludge for the biomass removal, or in the food industry for cheese production.[7] In biopharmaceutical production, specifically with mammalian cell-based feed, pretreatment may lead to a shift in average particulate size from below 10 µm to between 20 and 60 µm after pretreatment (depending on the strategy used). Remaining particulates can be removed by depth filtration and improve the efficiency of a centrifuge step. A secondary effect can be the selective precipitation or flocculation of contaminant species.[8]

Pretreatment agents are diverse. For example, acids (acetic or citric), salts ($[NH_4]_2SO_4$, K_2SO_4, KH_2PO_4), cationic polymers (chitosan, pDADMAC, PEI) or other artificial polymers (PEG) are frequently used for such purposes.[9-11]

In order to assess the efficiency of the pretreatment agent, several parameters can be monitored, including supernatant turbidity and particle size distribution, for which a combination of absorbance at 600 nm and dynamic light scattering can be used. Product yield and potency is, of course, a key indicator of the impact pretreatment will have on product quality, and this must be monitored by in-house assays developed specifically for the feedstock.

Acidic treatment is one of the simplest methods to implement and is widely accepted by the regulatory authorities: pH is lowered down to ~5.0 (to be optimized during trials) by adding acid, thus modifying the charges of the solutes and leading to the aggregation or precipitation of medium-sized particles (20–30 µm).[12] From a regulatory point of view, it is not necessary to demonstrate the absence of any added agent in the final product as only the pH has been modified.[13,14] It is generally readjusted after filtration.

Using salts for protein precipitation is a broadly used method in biochemistry. Salts interact at the surface of contaminants, decreasing their solubility, which leads to their precipitation.

However, these two methods can denature the product of interest and decrease its stability, and/or have a negative impact on product recovery during the filtration step. Losing product via precipitation is also a possibility, and some level of yield loss has to be expected.

Flocculation agents such as cationic polymers act as a binder to aggregate contaminants in the solution, resulting in the formation of a loosely bound floc. Polymers have a positive or negative charge and bind cell debris, host cell proteins, nucleic acids, and other contaminants. Flocs are generally larger than particulates obtained via acidification, ranging from 30–60 µm. The success of a flocculation step depends mainly on polymer dosage. Its removal has to be validated, per regulatory standards, as polymer remnants are considered to be contaminants.[15]

Final Product Purity Levels

Regulatory agencies provide recommendations and requirements regarding acceptable residual amounts of contaminants in the final product. Host cell DNA in the final product, for patient safety and tolerance reasons, must be reduced to appropriate levels. In 1998, the World Health Organization specified the maximum residual DNA content in a vaccine to be below 10 ng/dose. More recently, the European Medicines Agency (EMA) proposed more stringent conditions based on the type of cell line (tumorigenic origin) used in the vaccine manufacturing, whereas the U.S. Food and Drug Administration (FDA) follows a case-by-case evaluation approach and recommends that manufacturers reduce both the size (~200 bp) and amount of DNA per dose. To date, a final DNA content of <10 ng/dose is commonly accepted for most biologics.[16–18] Similarly, a recombinant MAb product must reach clearance of impurities down to <100 ppm of HCP, ≤10 ng/dose of DNA, and <5% of immunogenic aggregates.[19] A summary is shown in **Table 2**.

TABLE 2. Acceptable remaining impurities in vaccine and MAb products.

Requirements	Specification
Reduction of DNA amount	≤10 ng/dose
Reduction of DNA strand size	~200 bp
Reduction of HCP	<100 ppm
Immunogenic aggregates	<5% (for MAb)

Feed Quality Evaluation Criteria

Several parameters can be used in order to assess the clarity or quality of a product, either during process development work or after each manufacturing process step. Some of them will be described in this article. Turbidity is an easy parameter to monitor and provides an immediate assessment of the feed quality. It allows for the detection of depth filter breakthrough, for example.

There are two primary methods of ascertaining the

effectiveness of the clarification step, be it centrifugation or depth filtration. Turbidity can be monitored simply by absorbance/scatter in the visible range, providing an immediate assessment of the particle load within the filtrate or centrate. This also relates to filter capacity, which is the volume of feed a depth filter can process before the pressure drop breaches specifications. Capacity relating to both pressure drop and turbidity breakthrough are linked and specifications for both should be set during process development. For some processes, particularly those with a smaller average particle size in the feed, the turbidity breakthrough will be the limiting factor in sizing the filtration train. More commonly, the capacity limit is due to pressure drop, but as high pressures or flow rates can cause premature turbidity breakthrough, the two mechanisms are related.

In most cases, yield is an off-line measurement done at the end of given process steps. Depending on the success criteria for each step (sometimes a compromise between clearance of a contaminant and product yield), yield may be the main parameter to consider for selecting one option over another for a given step. But some additional parameters can be used as well, like dosage of the main contaminants, for example. Numerous methods for their quantification have been described in detail in published literature.[20, 21]

Factors Impacting Chromatography Performance and Processes

Common parameters generally considered in evaluating chromatographic performance are dynamic binding capacity, recovery, product purity, and resolution. What affects them can be different depending on the type of chromatography applied. For example, packed bed chromatography is especially sensitive to changes in feed stream characteristics, particularly to fouling species like lipids, DNA, and solids. We recommend early removal of HCPs and DNA, prior to selected chromatography processes.[20, 22, 23] Among others, depth filtration can be an efficient method for downstream protection.

Chromatography Equipment and Components

The average particle size in unclarified lysates such as *Escherichia coli* (*E. coli*) and CHO is around 10 µm. Typical packed bed chromatography columns present a frit (also called a bed support), which is a mesh available in different sizes (typically 10, 20, and 30 µm).[24] Column frits/bed supports and resin bed heads can therefore retain particles as small as 1–2 µm in size, leading to fouling.[6] This is typically the case with small solid particles (below 5 µm), which are not efficiently removed by centrifugation.[1] They challenge the downstream process by compromising membrane filter protection as well as the downstream chromatography.

Protein A Chromatography

Protein A chromatography is one of the most effective technologies to capture and efficiently purify antibodies. However, the cost of the media used in this step is significant and users attempt as many reuses/regeneration cycles as possible whilst still retaining acceptable binding capacities and contaminant clearance profiles, often up to several hundred cycles.[2] Predictably, product yields decrease while contaminants—such as DNA and HCP—rise. Low pH conditions are mandatory for elution of product and may lead to the formation of proteins aggregates or precipitates (antibodies or HCPs). This can lead to fouling and may reduce the resin lifespan.

Precipitates are composed of the product and HCPs, highlighting an interest in their early removal or prevention of formation in the first place.[25] In parallel, some studies show that HCPs in the feed can interact with the product of interest on the column and lead to their increased retention.[3] Therefore, an efficient reduction of contaminants prior to protein A is key to maintaining the longevity and efficiency of the resin.[3]

AEX Chromatography

It has been shown that after the protein A step, fouling agents impacting the AEX polishing step are mainly composed of HCPs. Excessive contaminant binding can seriously impair cleaning effectiveness and reduce the lifetime of the resin, which is why such contaminants should be removed prior to chromatography.[23]

CEX Chromatography

Feed quality has been shown to dramatically impact CEX performance as the complexity of the product loaded on the column increases with the concentration of contaminants. Competitive binding is indeed more pronounced in less clarified feeds with cell debris components acting as adsorbents. This also reduces the window of operation for good performance.[6]

Hydrophobic Interaction Chromatography (HIC)

Lipids are a specific challenge for HIC when used in yeast applications, for example. They tend to accumulate run after run and coat the outer surface of the beads.[26, 27] They can impact the HIC capacity by competing or even shielding the binding surface and changing the binding properties of the matrix. Harsh clean-in-place conditions do not represent a robust method to recover column capacity. Therefore, another method for lipid removal prior to column loading must be considered.

Depth Filter Clarification Effects on Feedstream Characteristics

Structure and Composition of Depth Filters

Included in the Millistak+® family of primary and secondary clarification filters are traditionally depth filters

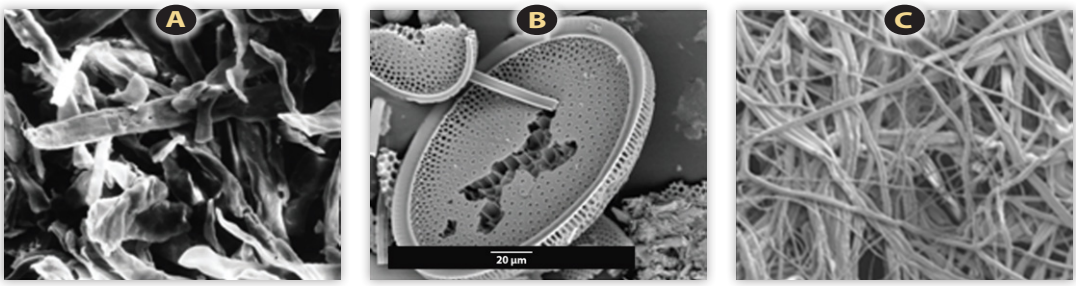

FIGURE 1. Main components of depth filters: **(A)** cellulose fibers; **(B)** diatomaceous earth; and **(C)** polypropylene fibers.

composed of cellulose fiber (with a high permeability matrix and good mechanical properties), an inorganic filter aid (such as diatomaceous earth) presenting an inherent binding capability for biological material, and a positively-charged polymeric binding agent (with adsorptive charge-based properties) (**Figure 1**).

Other materials are used for prefiltration like glass, cellulose ester, or activated carbon. Their structures are made of a single layer, or specific combinations of media layers, for different feed qualities and origins. Furthermore, specific media aimed at a higher filtrate purity are constantly being developed. For example, Merck Millipore recently launched a new media (Clarisolve®) for handling pretreated feed that is designed to more fully optimize the overall media depth, and therefore, enhance retention and capacity (**Figure 2**).[28]

Mechanisms of Retention

Depth filters ensure a normal flow separation of particles and solutes via two main mechanisms: sieving and adsorption. Their high capacity is due to the fact that contaminants are trapped and retained within the whole depth of the media rather than being held only on the surface of the filter.

Sieving is based on size exclusion, where rigid particles are retained at the surface and within the depth of the filter. Adsorption works independently from size. It is based on the interactions between filtration media and particles through electrostatic attraction, hydrophobic interaction, or Van de Waals forces. Adsorption is often effective for the removal of smaller particles (below 1 µm) but is limited by available adsorption sites within the media.

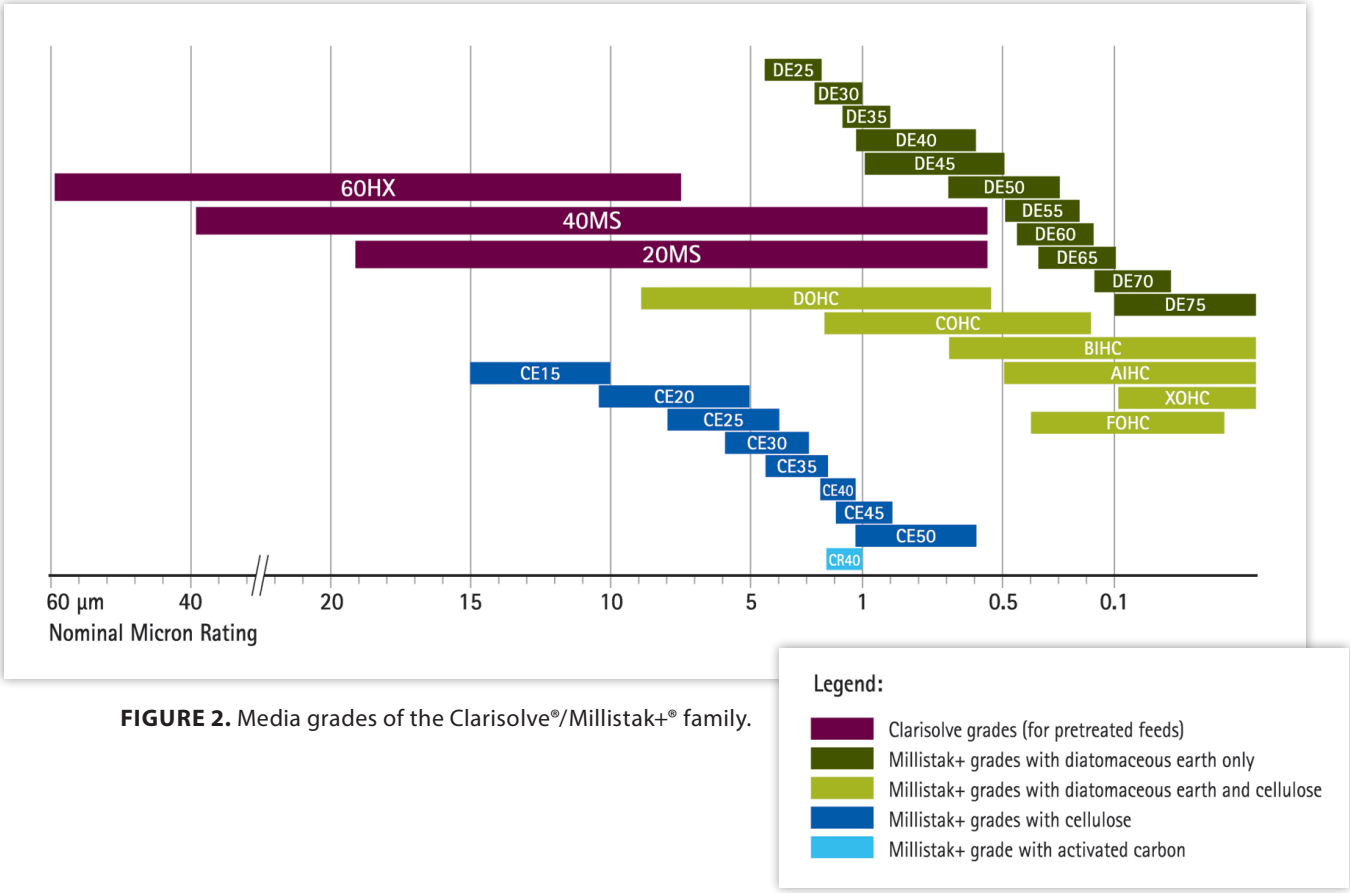

FIGURE 2. Media grades of the Clarisolve®/Millistak+® family.

Particles

The largest particles present in feed stocks can be retained via sieving at the surface of depth filters. The smaller ones penetrate the depth of the filter and are retained by entrapment. When any pretreatment is applied to the feed, the average size of particles shifts significantly (up to 60 µm), leading to an increase in sieving/entrapment and therefore may require the use of specific media.

DNA

DNA removal by depth filtration occurs mostly via the mechanism of adsorption. DNA adsorption is usually due to both hydrophobic interactions and electrostatic effects. Electrostatic interactions take place along the polyphosphate backbone of the DNA, whereas hydrophobic interactions happen within the grooves of the DNA helix.[22] Therefore, adsorption depends on feedstock solution composition, with pH and conductivity (lower conductivity suggests higher retention) playing a main role.

HCP

Similarly to DNA, the removal of HCPs by depth filtration occurs by adsorption. Absorbance can be a good indicator of their removal as studies show that absorbance at 410 nm is mainly due to HCP (increased spiking with DNA does not affect its value).[25] Also, in a CHO process, most HCPs are negatively charged due to their pI <7.0.[20] Therefore, slightly basic pH and lower conductivity is preferable for efficient HCP removal by depth filtration. The efficiency of depth filters has been shown at different stages (prior to or after protein A)[3, 29] and highlights the necessity for a thorough selection of the media used for optimum clearance. As absorbance is a good tool to monitor the presence/absence of HCPs, it can be utilized effectively at scale and is one of the major parameters typically monitored during filter sizing trials.

Discussion

Column Protection with Clarification Depth Filters

Different chromatography modes have been shown to be sensitive to the nature and/or content of contaminants loaded onto them. Logic says that removing them earlier, and in a more effective way, would improve the protection and performance of the chromatography steps. However, available literature that clearly illustrates the cause/effect link is scarce.

Among existing data, the choice of the secondary clarification by depth filtration indeed impacts the HCP profile post-protein A column, showing that the retention properties of the depth filters could be used to reduce and influence this profile.[3] HCP profiles could also be used early in process development to select the most suitable clarification step for a specific feed. As well, the positively charged depth filtration of feed streams was shown to result in less turbid protein A eluates, a result of its contaminant removal qualities.[25] In another study, performed on AEX chromatography, data show that applying clarification by depth filtration on the feed stream beforehand improves the AEX capacity, robustness, and media longevity.[23] Also, a multi-stage depth filtration is more efficient in removing contaminants[30] and the use of pretreatment shows promise for contaminant removal and downstream protection.[28]

These published examples prove that there is a real interest in investigating further how to best apply clarification by depth filtration for early purification of feeds. It should still be kept in mind that to ensure consistent and robust clarification, variability of the feed stream and the clarifying media should be taken into account and built into the safety factors for designing and sizing this protective step. For comparable performances, the selection of a clarification train may depend on scale and/or preferred technologies. For example, at scales from 2,000–5,000 L of bioreactor capacity, using depth filtration (single or multi-stages) versus centrifugation for primary clarification is cost-equivalent. For smaller scales (<1,000 L), it offers real financial advantages.[31]

However, in all of the clarification scenarios involving depth filtration (clarification in one or two steps following pretreatment), further work should be performed to assess the overall impact on the downstream process. A number of questions can be raised:

- Does it affect the design of the downstream process itself or only its lifetime/performance?
- Does this increased early removal of contaminants impact only the first stage of the downstream cascade or the complete downstream train (to the point of modifying it by reducing its stages)?
- Could the performance of the clarification steps be pushed so far as to lead to a redesign (and ideally simplification) of the downstream cascade?

Until results from further clarification process efforts become available, and given the variability of feeds, case-by-case testing, supplier experience, and in-house process development remain the best ways to assess the actual impact of depth filtration for contaminant removal and to design an economical downstream process.

Conclusion

In this paper, the referenced literature illustrating depth filtration properties for the removal of contaminants such as HCP and DNA has been reviewed. Even though the impact on the overall downstream purification process, and notably the chromatography steps involved, is rarely made clear, published data show that resin lifetime, robustness, and/or capacity can be improved by using cleaner clarified feeds on different chromatography types. This opens distinct possibilities for further investigations on the advantages of improved clarification for downstream economics or redesign.

References

[1] Yavorsky D, Blanck R, Lambalot C, Brunkow R. The clarification of bioreactor cell cultures for biopharmaceuticals. *Pharmaceut Technol*, 2003: 62–76.

[2] Fahrner RL, Knudsen HL, Basey CD, Galan W, Feuerhelm D, Vanderlaan M, Blank GS. Industrial purification of pharmaceutical antibodies: development, operation, and validation of chromatography processes. *Biotechnol Genet Eng Rev*, 2001; 18(1): 301–27. http://dx.doi.org/10.1080/02648725.2001.10648017.

[3] Hogwood CEM, Tait AS, Koloteva-Levine N, Bracewell DG, Smales CM. The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnol Bioeng*, 2013; 110(1): 240–51. http://dx.doi.org/10.1002/bit.24607.

[4] Method for purifying the rabies virus. Patent-US2010/0260798A1, Date: Oct 14, 2010 (Sanofi Pasteur).

[5] Sheng-Fowler L, Lewis AM Jr, Peden K. Issues associated with residual cell-substrate DNA in viral vaccines. *Biologicals*, 2009; 37(3): 190–5. http://dx.doi.org/10.1016/j.biologicals.2009.02.015. Epub 2009 Mar 14.

[6] Boushaba R, Baldascini H, Gerontas S, Titchener-Hooker NJ, Bracewell DG. Demonstration of the use of windows of operation to visualize the effects of fouling on the performance of a chromatographic step. *Biotechnol Progress*, 2011; 27(4): 1009–17. http://dx.doi.org/10.1002/btpr.617. Epub 12 May 2011.

[7] Hwang D-C, Damodaran S. Selective precipitation and removal of lipids from cheese whey using chitosan. *J Agric Food Chem*, 1995; 43(1): 33–7. http://dx.doi.org/10.1021/jf00049a008.

[8] Ko KY, Ahn DU. Preparation of immunoglobulin Y from egg yolk using ammonium sulfate precipitation and ion exchange chromatography, *Poult Sci*, 2007; 86(2): 400–7. http://dx.doi.org/10.1093/ps/86.2.400.

[9] Riske F, Schroeder J, Belliveau J, Kang X, Kutzko J, Menon MK. The use of chitosan as a flocculant in mammalian cell culture dramatically improves clarification throughput without adversely impacting monoclonal antibody recovery. *J Biotechnol*, 2007; 128(4): 813–23. http://dx.doi.org/10.1016/j.jbiotec.2006.12.023. PMid:17291617.

[10] Kang YK, Hamzik J, Felo M, Qi B, Lee J, Ng S, Liebisch G, Shanehsaz B, Singh N, Persaud K, Ludwig DL, Balderes P. Development of a novel and efficient cell culture flocculation process using a stimulus responsive polymer to streamline antibody purification processes. *Biotechnol Bioeng*, 2013; 110(11): 2928–37. http://dx.doi.org/10.1002/bit.24969. PMid:23740533, PMCid:PMC3812681.

[11] McNerney T, Thomas A, Senczuk A, Petty K, Zhao X, Piper R, Carvalho J, Hammond M, Sawant S, Bussiere J. PDADMAC flocculation of Chinese hamster ovary cells: enabling a centrifuge-less harvest process for monoclonal antibodies. mAbs, 2015; 7(2): 413–27. http://dx.doi.org/10.1080/19420862.2015.1007824. PMid:25706650.

[12] Westoby M, Chrostowski J, de Vilmorin P, Smelko JP, Romero JK. Effects of solution environment on mammalian cell fermentation broth properties: enhanced impurity removal and clarification performance. *Biotechnol Bioeng*, 2011; 108(1): 50–8. http://dx.doi.org/10.1002/bit.22923. PMid:20812295.

[13] ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). *Impurities in new drug substances Q3A (R2)*. Geneva, Switzerland; 2006. Available from: http://www.ich.org/products/guidelines/quality/quality-single/article/impurities-in-new-drug-substances.html.

[14] ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). *Impurities in new drug products Q3B (R2)*. Geneva, Switzerland; 2006. Available from: http://www.ich.org/products/guidelines/quality/quality-single/article/impurities-in-new-drug-products.html.

[15] ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). *Specifications: test procedures and acceptance criteria for biotechnological/biological products (Q6B)*. Available from: http://www.ich.org/products/guidelines/quality/quality-single/article/specifications-test-procedures-and-acceptance-criteria-for-biotechnological-biological-products.html.

[16] *Acceptability of cell substrates for the production of biologicals: report of a WHO Study Group*. In WHO Technical Report Series; World Health Organization: Geneva, 1987. 29-page book.

[17] Grachev V, Magrath D, Griffiths E. WHO requirements for the use of animal cells as *in vitro* substrates for the production of biologicals (requirements for biological substances no. 50). *Biologicals*, 1998; 26(3): 175–93. http://dx.doi.org/10.1006/biol.1998.0153. PMid:10208720.

[18] WHO Expert Committee on Biological Standardization in Biologics, 1997. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_889.pdf.

[19] Chon JH, Zarbis-Papastoitsis G. Advances in the production and downstream processing of antibodies. *New Biotechnol*, 2011; 28(5): 458–63. http://dx.doi.org/10.1016/j.nbt.2011.03.015. PMid:21515428.

[20] Jin M, Szapiel N, Zhang J, Hickey J, Ghose S. Profiling of host cell proteins by two-dimensional difference gel electrophoresis (2D-DIGE): implications for downstream process development. *Biotechnol Bioeng*, 2010; 105(2): 306–16. http://dx.doi.org/10.1002/bit.22532. PMid:19739084.

[21] Ahn SJ, Costa J, Emanuel JR. PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. *Nucleic Acids Res*, 1996, 24(13): 2623–5. http://dx.doi.org/10.1093/nar/24.13.2623. PMid:8692708, PMCid:PMC145983.

[22] Charlton HR, Relton JM, Slater NKH. Characterisation of a generic monoclonal antibody harvesting system for adsorption of DNA by depth filters and various membranes. *Bioseparation*, 1999; 8(6): 281–91. http://dx.doi.org/10.1023/A:1008142709419. PMid:10786277.

[23] Iskra T, Bolton GR, Coffman JL, Godavarti R. The effect of protein A cycle number on the performance and lifetime of an anion exchange polishing step. *Biotechnol Bioeng*, 2013; 110(4): 1142–52. http://dx.doi.org/10.1002/bit.24781.

[24] QuikScale® biochromatography columns: innovative chromatography columns for scalable, rapid purification. Merck Millipore Lit. No. DS12240000 Rev. C, 12/08.

[25] Yigzaw Y, Piper R, Tran M, Shukla AA. Exploitation of the adsorptive properties of depth filters for host cell protein removal during monoclonal antibody purification. *Biotechnol Prog*, 2006; 22(1): 288–96. http://dx.doi.org/10.1021/bp050274w. PMid:16454522.

[26] Wang A, Lewus R, Rathore AS. Comparison of different options for harvest of a therapeutic protein product from high cell density yeast fermentation broth. *Biotechnol Bioeng*, 2006; 94(1): 91–104. http://dx.doi.org/10.1002/bit.20816.

[27] Jin J, Chhatre S, Titchener-Hooker NJ, Bracewell DG. Evaluation of the impact of lipid fouling during the chromatographic purification of virus-like particles from *Saccharomyces cerevisiae*. *J Chem Technol Biotechnol*, 2010; 85(2): 209–15. http://dx.doi.org/10.1002/jctb.2290.

[28] Singh N, Pizzelli K, Romero JK, Chrostowski J, Evangelist G, Hamzik J, Soice N, Cheng KS. Clarification of recombinant proteins from high cell density mammalian cell culture systems using new improved depth filters. *Biotechnol Bioeng*, 2013; 110(7): 1964–72. http://dx.doi.org/10.1002/bit.24848. PMid:23334838.

[29] Haverstock R, Westoby M, Lam Y. *CHOP removal by depth filtration post-protein A capture*. Paper presented at: 239th American Chemical Society National Meeting. San Francisco, California; March 2010.

[30] Echeverry S, Steele A, Kim E, EMD Millipore. *MAb impurity clearance by operating depth filtration in series*. Poster, BPI October 2014.

[31] Felo M, Christensen B, Higgins J. Process cost and facility considerations in the selection of primary cell culture clarification technology. *Biotechn Prog*, 2013; 29(5); 1239–45. http://dx.doi.org/10.1002/btpr.1776.

About the Author

Sarah Le Merdy is Technology Manager, Downstream Processing, Millipore S.A.S. France, a subsidiary of Merck KGaA, Darmstadt, Germany

Email: sarah.lemerdy@emdgroup.com; Website: www.emdmillipore.com