the Medicine Maker

SPECIAL SERIES:

Biopharmaceutical Drug Development

SUPPORTED BY

actylis

IN MY VIEW

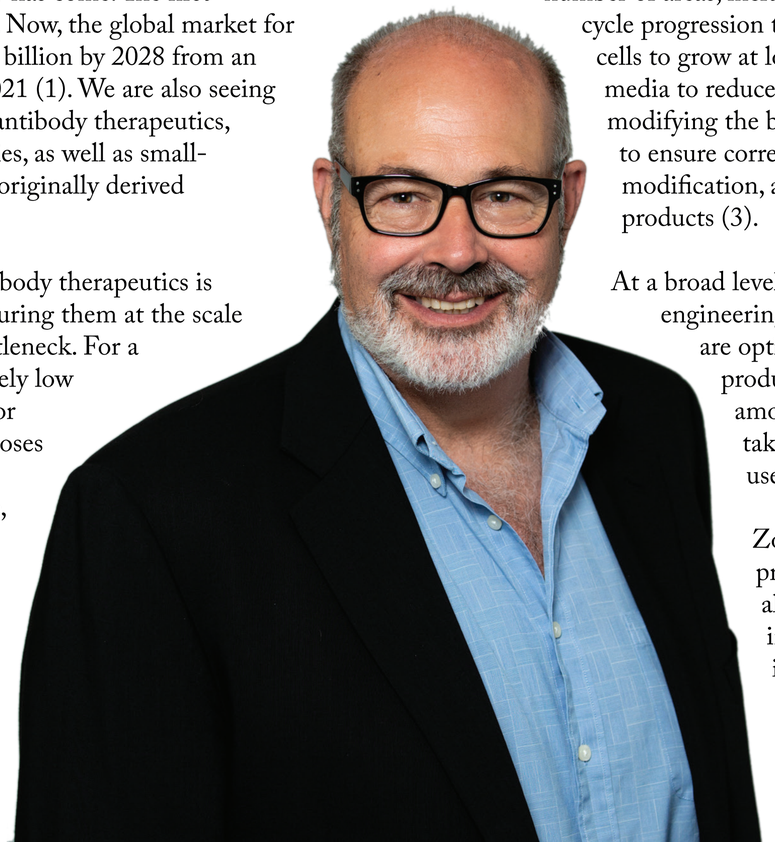
Welcome to the CRISPR Powerhouse

Why antibody manufacturers should embrace CRISPR gene editing

By Eric Rhodes, CEO at ERS Genomics, Ireland

It's incredible how far the mAb industry has come. The first therapeutic mAb was approved in 1986. Now, the global market for mAbs is anticipated to grow to $451.89 billion by 2028 from an already-impressive $178.50 billion in 2021 (1). We are also seeing the emergence of increasingly complex antibody therapeutics, including bi-and multi-specific antibodies, as well as small-format single domain VHH antibodies originally derived from camelids.

But while the pace of innovation in antibody therapeutics is accelerating, the challenges of manufacturing them at the scale required to meet demand remains a bottleneck. For a start, antibody bioproduction is a relatively low yield process, with each liter of bioreactor volume typically producing around 10 doses of a mAb compared with 1,400-2,000 doses of a viral vaccine (2). Furthermore, antibody production can affect cell growth and viability, even triggering apoptosis. There can also be issues with expression, post-translational modification, folding and purification, adding further layers of complexity to the manufacturing process.



Antibody manufacturers are continually looking for ways to improve and optimize production. So at this point I'd like to turn everyone's attention to CRISPR/Cas9 – the gene editing technology invented by Nobel prizewinners Emmanuelle Charpentier and Jennifer Doudna. As many of you may know, CRISPR/Cas9 (often just referred to as CRISPR) is a standalone genetic modification tool used to delete, add, or alter specific regions of the genome with high precision. It can be used in a wide range of cell types and species, including the commonly-used bioproduction workhorses of HEK293 (derived from human embryonic kidney) and CHO cells.

Unsurprisingly for such a flexible and useful technology, CRISPR gene editing could improve antibody manufacturing processes in a number of areas, including regulating apoptosis and cell cycle progression to enhance growth, engineering cells to grow at lower temperatures or in cheaper media to reduce manufacturing costs, and modifying the biological pathways within cells to ensure correct expression, post-translational modification, and folding of the resulting products (3).

At a broad level, CRISPR can be used for genome engineering of host cells to create lines that are optimized for large-scale cell antibody production. Industry leader Lonza is among a group of companies that have taken a license from ERS Genomics to use CRISPR for just this purpose.

Zooming in on the antibody production process, CRISPR can also be used to precisely control the insertion of an antibody cassette into a specific location or multiple locations within the genome of the cell. This approach reduces the likelihood of epigenetic silencing effects and helps guarantee high levels of stable gene expression. It also facilitates the rapid development of new antibody producing lines by cutting down the time required to clonally isolate high-producing cells.

CRISPR can be used to engineer the molecular chaperones that are responsible for ensuring correct protein folding, which is particularly useful for increasing the yield of antibodies that are more difficult to express (4). Similarly, genome engineering can be used to modify the enzymes involved in post-translational modification, such as the addition of N-glycan sugars, which have an important role in antibody activity, efficacy, and safety.

Unwanted binding of endogenous proteins is another problem in bioproduction. These proteins can affect antibody secretion or co-purify with the antibody being produced, causing problems during purification or downstream processing, adding time and cost to manufacturing. CRISPR can remove or alter these problematic proteins.

Another potential application of CRISPR exists in the area of antibody-drug conjugate (ADC) development. With a global market expected to reach $13.8 billion by 2028 (5),ADCs offer a more precise way of treating cancer, improving efficacy and reducing side effects. However, the addition of therapeutic payloads can disrupt antibody stability and affinity, and it can also be difficult to control the number of drug molecules that are added to each antibody. Precision engineering of modification sites using CRISPR can result in more efficient and reliable drug conjugation – and far faster and more accurately than conventional antibody engineering techniques (6).

That's just a snapshot of the possibilities. The past decade has seen exceptional growth in the market for antibody therapeutics, and this trend is only set to continue. As the demand for these next-generation biotherapeutics continues to grow at pace, manufacturers should start embracing the great potential of CRISPR.

REFERENCES AVAILABLE ONLINE

ARTICLE

Format and Function: Optimizing Gene Therapy Manufacturing Workflows

Gene therapy is a rapidly evolving industry with the potential to transform patients' lives, but promising treatments for a wide range of diseases require optimal cell culture media solutions

By Elpidia Gamez, Senior Manager, Product Management, Thermo Fisher Scientific, USA

Format choice for cell culture media can have a significant logistical impact on gene therapy development. Understanding the suitability of a format for use at commercial scales and making the appropriate choices throughout process development can help streamline operations and reduce the risk of delays.

The use of media in a liquid format is well-established for small-scale gene therapy development because liquid media are ready to use and require few preparation steps. Saving time and reducing in-house workload, the liquid format is ideal for helping developers create a more convenient process.

However, as workflows scale up and liquid media volumes increase, so too do the logistical and financial challenges of shipping and storage. Careful planning and forecasting are required so that media are available when needed and used before expiration. However, liquid media are heavy, which means they can be expensive to ship and challenging to move around facilities. Moreover, large areas of manufacturing facilities may be needed for storage, reducing production space. Using third-party warehousing can lead to substantial additional costs, and storage is further complicated by the relatively short shelf life of liquid media.

Furthermore, while liquid media do not require reconstitution, the addition of supplements can increase complexity by adding to the number of preparation steps.

Because of the challenges associated with liquid media, many companies choose to use dry powder media (DPM). DPM is more compact, saving on storage and shipping costs, and leaves more facility capacity for operations, providing the opportunity for greater productivity. DPM also has a longer shelf life, which allows for the stockpiling of supplies and results in less pressure on accurate forecasting – as well as the reliance on supply chains.

However, standard DPM requires a multistep rehydration process before it can be used, which can add to the in-house workload. The need for manual handling steps during the rehydration process, such as pH and osmolality adjustments, can increase the risk of inconsistency. When using DPM, it is important for developers to have strict quality protocols in place to reduce any process variability.

There is also an alternative option that can bridge the gap between production-ready liquid media and DPM: granulated media formats. Granulated options provide a simpler reconstitution process and lower dust generation without the need for pH or osmolality adjustments, increasing efficiency and reducing the risk of inconsistency. Moreover, supplements can be integrated into a granulated format, effectively resulting in a convenient single-component product.

By providing similar storage and shipping benefits as DPM, alongside helping support more efficient preparation and improved consistency, granulated media can help many developers reduce operating costs and increase productivity.

"Saving time and reducing in-house workload, the liquid format is ideal for helping developers create a more convenient process."

Transitioning from liquid to dry powder or granulated media during scale-up can be challenging. Carefully considering the optimal format early during process development is essential to help avoid costly delays, such as reformulation or requalification of a medium.

Choosing an off-the-shelf medium that is available in multiple formats could prepare a workflow for future changes. Similarly, validating that a proprietary formulation is suitable for conversion can help streamline the transition. This validation step can still be beneficial for developers who plan on using liquid media at all scales. Knowing that a formulation can be used in multiple formats means developers have a backup option should they face complications.

That said, using the same format at all stages can support the more rapid progression of the therapy. Liquid, dry powder, and granulated media formats offer a variety of benefits and potential drawbacks, depending on the specific process requirements. The challenge is finding a medium that provides consistent quality and performance in a format that can also help optimize logistics.

Ultimately, developers need to consider scalability, cost-effectiveness, and convenience during process development to find the format that will be most suitable for their current and future needs. By choosing the right format, developers can optimize their development and manufacturing processes to help them accelerate the speed to market for their gene therapy product and confidently meet future demand.

MANUFACTURING

Top Tips to Optimize Downstream Processes

From resins to buffers to single-use technologies – there are many opportunities to improve downstream processes

By Nandu Deorkar, Senior Vice President, Research & Development, Jungmin Oh, Manager, New Product Development, Pranav Vengsarkar, Manager, Process Development, and Jonathan Fura, Manager, R&D, all at Avantor

Emerging treatments, including cell and gene therapies, are exciting and are certainly starting to expand pipelines, however, traditional biologics; monoclonal antibodies, still dominate the world of biopharma. Research has shown that the clinical pipeline of antibody therapeutics grew by 30 percent over the past year (1) – excluding COVID-19 antibody therapies – highlighting the importance of these treatments and the need for their efficient production.

Given that 60–80 percent of mAb production costs can be attributed to downstream processing (2), removing downstream bottlenecks, and improving yields will continue to be an important priority for mAbs manufacturers – especially amidst rising demand. Below, we offer a few suggestions.

Considering resins and buffers

In the capture step, protein A is the most widely used resin. Protein A is simple to implement as a standard purification process and holds a strong regulatory track record (3); however, the costs of the resin are substantial, making it important to optimize the process to maximize cost and efficiency. A key consideration in process optimization is understanding the role dynamic binding capacities (DBC) plays in overall protein A performance. Use of a resin with higher DBC can improve capture step productivity while maintaining column sizes and minimizing facility modification – especially when it comes to high titer cell culture processes.

To prove the point, we performed a simulation using BioSolve software, calculating the number of bind/elute cycles, process time, and volumes of buffer required for a 2000 L bioreactor batch. We looked at three model resins with DBCs ranging from 30 g/L–65 g/L. Assumptions made for the calculations are summarized in Table 1. We maintained column size at 68.6 L for 2000 L cell culture reactor with 5 g/L titer value. We evaluated process productivity based on the number of cycles required per batch as well as process time.

What did we find? Higher DBC resins significantly reduce the number of cycles and total downstream processing time (see Table 2 and Figure 1). Notably, by reducing the number of cycles, one can also reduce operational risks and per-cycle costs for labor and consumables. Similarly, a lower volume of buffer consumption not only reduces raw material cost, but also buffer preparation time, buffer tank size, and method of preparation. In our model, Resin C reduced total buffer consumption by approximately 40 and 30 percent when compared to Resin A and B, respectively.

Creating buffers in-house is a well-established method suitable for manufacturing large volumes, however, preparation of buffers often involves utilities and resources, such as Water for Injection (WFI), which may be constrained due to demand in other systems such as clean-in-place or other process lines. Further, the sheer number and volume of buffer solutions required for the entire downstream purification process may cause scheduling issues for the buffer prep team trying to meet the demands of the production schedule. Reduced buffer solution requirements offer additional flexibility as these operations require significant infrastructure, including warehouse space

"New developments in single-use technology have added flexibility in buffer preparation methods, allowing small- and medium-scale facilities to move to single-use tanks for buffer preparation."

for holding raw materials prior to their use, a weighing and dispensing area for raw materials, and space to store the prepared solutions which are often stored in corridors due to lack of space. In addition, the stainless-steel tanks themselves can require a considerable footprint in the facility and frequently experience corrosion issues due to the caustic nature and high chloride content of commonly used buffers.

New developments in single-use technology have added flexibility in buffer preparation methods, allowing small- and medium-scale facilities to move to single-use tanks for buffer preparation. This has enabled faster changeovers in buffer preparation, saving both time and cost in manufacturing processes (4). Single-use fluid handling systems can help reduce bottlenecks, particularly in cell therapy manufacturing where downstream processing is often slowed by lack of the suitable closed manufacturing systems. The closed, automated systems that are available are often unsuitable for large volumes of allogeneic cell therapies. If a biomanufacturer uses single-use equipment, a reputable supplier with a multiple-source supply chain is key to avoid disruptions.

A hybrid approach

Combining both in-house systems and outsourced buffers in a hybrid approach can help streamline downstream purification unit operations. Moreover, in-line dilution (ILD) systems can improve the efficiency of critical buffer component production.

- Clean-in-place solutions: Usually a fixed normality of NaOH, it can be prepared in-house using concentrate or purchased as a 1X concentration thanks to the smaller volumes needed, lowering safety concerns.
- Storage buffer: Due to low consistent volumes typically required (irrespective of resin DBC), storage buffers, such as 20% ethanol, can be managed in-house in the same way as the cleaning buffer.
- Equilibration and wash buffers: Volumes of these buffers (for example, 1X PBS or 50 mM Tris, pH 7) significantly decrease with an increase in resin DBC, as shown in Figure 1. Whether these buffers are prepared using in-house or single-use systems, high volumes can cause several operation challenges. When preparing these buffers, inline dilution (ILD) systems using multicomponent concentrates (for example, 10X PBS) can provide operational advantages. For example, ILD can help minimize facility footprint, reduce raw material management, and increase availability of buffer on demand.
- Elution buffers: Use of these buffers (for example, 0.1M acetate buffer, pH 3.4) can also be streamlined through the use of an in-line dilution system.

Workflow improvements in buffer preparation

Broadly, there are three options for buffer prep system/process in downstream purification:

Cell culture volume	2000L
Titer	5g/L
Protein A column bed height	20cm
Protein A column volume	68.6L
Step yield	90%
Flow rate	150cm/hr
Protein A process phase	Duration (Column Volume)
Flush (WFI)	3CV
Equilibrium	5CV
Load	N/A
Wash	5CV
Elution	5CV
CIP (0.5M NaOH)	2CV
Storage	5CV

Table 1. Simulation process parameters

	Resin A	*Resin B*	*Resin C***
DBC	30g/L	40g/L	65g/L
# of Protein A cycle/batch	4	3	2
Protein A column size	68.6L	68.6L	68.6L
Process time	18.8 hours	15.8 hours	12.8 hours
Total buffer consumption per batch	4,365L	3,429L	2,496L

Table 2.

* 2000L Bioreactor providing 5g/L titer

** DBC value of Resin C was taken from experimental value [3]

"Each facility and downstream process has unique requirements and bottlenecks, so having flexible process optimization options is important."

1. Single-use buffer prep reactors or chemical hydration in fixed stainless-steel tanks
2. Multicomponent buffer concentrates with in-line dilution or single component stocks with buffer stock blending
3. Ready-to-use cGMP 1X buffers

BioPhorum Operations Group (BPOG) and other industry organizations have offered insight into how buffer stock blending and in-line dilution generate overall improvements across unit operations (4, 5, 6). Choosing the right option will usually depend on an economic analysis of several factors, including scale, batches of drug produced per year, raw materials required, and other site attributes.

Table 3 offers workflow improvements for each of the three options.

The flexibility and productivity of the mAb capture process step can be improved by using high DBC resins along with optimal buffer management. High-capacity resin decreases process time by allowing less numbers of cycles required per batch — saving cost, mitigating risk, and reducing labor costs.

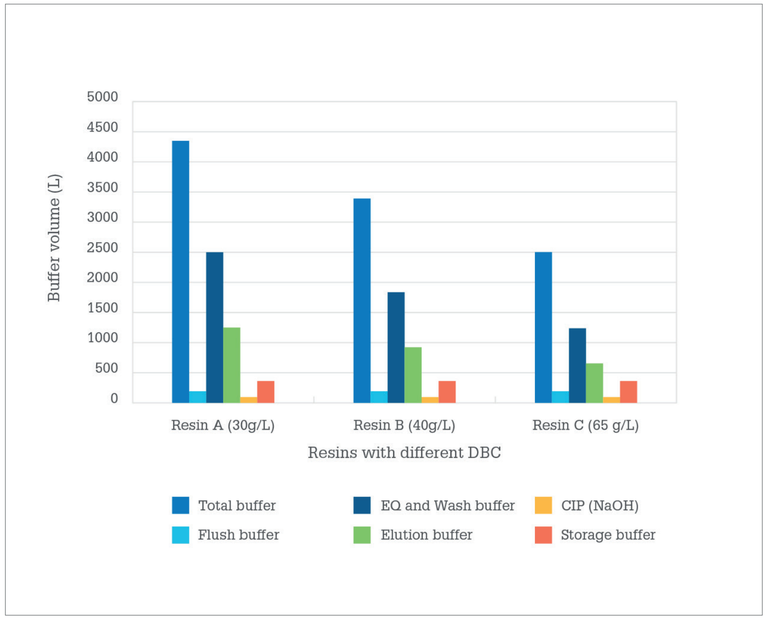


Figure 1. Buffer consumption of three protein A resins with different dynamic binding capacity (DBC) for processing of one 2000L bioreactor batch

In addition, implementing a high DBC resin decreases the volume of process buffers significantly, which allows the flexibility to adopt different buffer preparation processes based on facility requirements. Each facility and downstream process has unique requirements and bottlenecks, so having flexible process optimization options is important.

As innovative biologic treatments continue to emerge, manufacturers will almost certainly face even more hurdles – but, in every situation, the development of highly efficient, high yield manufacturing processes will be a key factor for success.

REFERENCES AVAILABLE ONLINE

Buffer Preparation Method	*Power Hydration in Stainless- Steel or Single-Use Tanks*	*Multicomponent Buffer Concentrates With In-Line Dilution (or Single Component Stocks With Buffer Stock Blending*	*Ready-To-Use Cgmp 1x Buffers*
Workflow improvements	— Supply of pre-weighed cGMP powdered raw materials in pails and drums, or in single-use powder delivery systems, to eliminate solid subdivision steps and streamline pre-buffer prep operations and prevent damage to single-use buffer tanks — Delivery and use of free-flowing powdered raw materials to eliminate de-clumping steps and prevent damage to single-use buffer tanks — Supply of pre-weighed cGMP powdered raw materials in single-use powder delivery systems to enable faster charging into tanks and quicker turnaround time — Implementation of rapid ID systems in the warehouse to speed up incoming material release into production — Hot WFI usage in dissolution to speed up dissolution in single-use tanks with poor heat transfer rate (cooling or heating)	— Extractable & Leachable (E&L) data on single-use packaging which enables longer shelf life — Single-use in-line dilution systems to reduce cleaning validations and enable faster batch changeovers — Stability studies on buffers made using buffer concentrates to analyze shelf life — pH/conductivity sensitivity to temperature of buffers for in-line dilution system (for example, TRIS buffers are extremely sensitive to temperature) to reduce rejected buffers — Harmonized concentrates/stocks across unit operations to improve flexibility of concentrates — Robust supplier agreements and forecasting of demand to prevent supply chain issues — Standardized single-use connectors for process use to enable more flexibility across unit operations	— Stability studies available on buffers to analyze shelf life (for example, 1x buffers are typically susceptible to pH/conductivity changes over time, leading to shorter shelf life) — Robust supplier agreements and forecasting of demand to prevent supply chain issues — Implementation of rapid ID systems including refractive index and Fourier transform infrared (FTIR) testing for quick release of buffer solutions

Table 3.

Custom GMP Manufactured Raw Materials

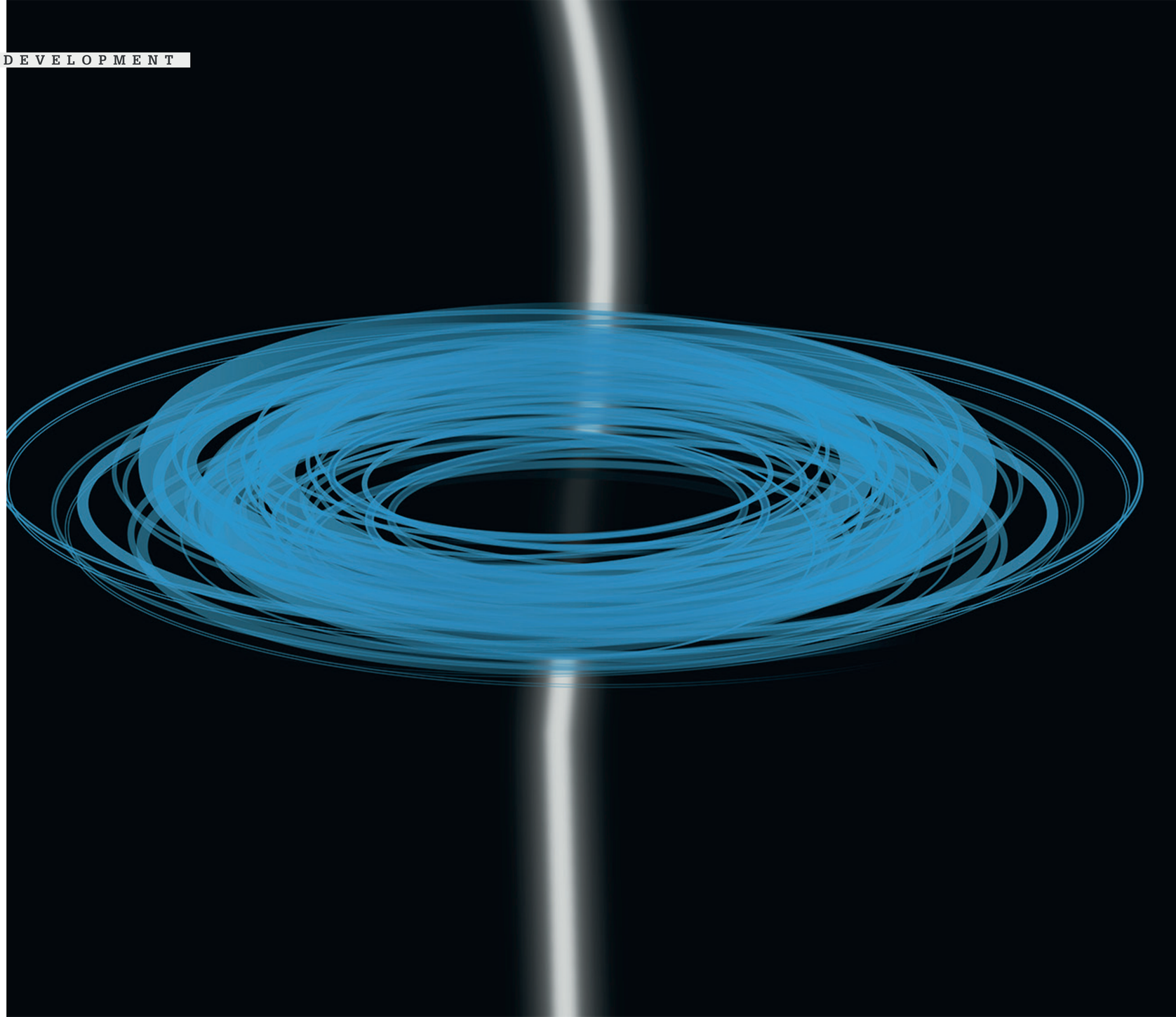
Visit us at
CPHI
Booth 821

actylis

INTERVIEW

Keeping Up To Date With X-Rays

We speak with the BPSA to find out why X-ray sterilization is being considered as an alternative to gamma irradiation



Gamma irradiation (using cobalt-60) is considered the standard method when it comes to sterilizing single use systems, but the high demand has caused supply issues. However, X ray sterilization can be used as an alternative. To help companies understand more, the Bio-Process Systems Alliance (BPSA) released a guide in 2021 titled: X-Ray Sterilization of Single-Use BioProcess Equipment, Part 1: Industry Need, Requirements & Risk Evaluation.

Now, the BPSA has released Part 2, which focuses on Representative Qualification Data. Readers can expect risk assessments, and data comparing X-ray and gamma irradiated components, generated by multiple component manufacturers for different types of single-use components and materials.

We spoke with Christopher Clark (Executive Director at the BPSA), James Hathcock (Director Regulatory and Validation Strategy at Cytiva), and Samuel Dorey (Principal Scientist Materials & Irradiations Product Development at Sartorius) to learn more.

"The continued success and rapid growth of single-use technologies in bioprocessing relies on a robust irradiation-sterilization supply chain."

What are the problems with gamma irradiation?

It takes years to produce colbalt-60 radioisotopes needed for gamma irradiation, not to mention the associated high costs. Gamma irradiation also requires replacing 12.3 percent of the globally installed base per year to account for radioactive decay, whilst navigating the challenges of regulatory approval.

Supply chain management and forecasting needed to meet industry demand has been impressive to date. However, rapid spikes in demand – such as those seen during the COVID-19 pandemic – have made it clear the industry needs alternative technologies that can supplement the growing need and strengthen the overall security of supply for irradiation sterilization.

The continued success and rapid growth of single-use technologies in bioprocessing relies on a robust irradiation-sterilization supply chain. Within our own companies, our sourcing partners suggested the only way to secure the irradiation capacity needed over the next 2-3 years was to embrace alternative (and now mature) technologies such as X-ray. An informed industry approach to qualifying alternative modes of irradiation sterilization may strengthen business continuity in the single-use industry, with the end goal of ensuring innovative patient therapies can be rapidly developed and delivered.

This is not to say that gamma irradiation will go away. Instead, it will continue to be a part of the holistic irradiation capacity solution moving forward, which can be strengthened and complemented by X-ray.

What are the most important points covered in the BPSA guide?

To support the risk assessments needed for implementation, multiple BPSA member companies have been working to generate and share supporting data aligned to the science-based protocol outlined in part one. Data not only help verify the understanding that X-ray and gamma are equivalent, but also show how different labs, components, and suppliers can be summarized and interpreted.

BPSA is not a standards organization; we do not set specific acceptance criteria for the testing results. However, we believe that sharing representative data and interpretations from different parties openly – as opposed to under confidentiality agreements – can help accelerate industry understanding and acceptance. The data include ISO 11137 standard requirements around the irradiation process, such as radioactivity (aka 'activation') and temperature effects, and the industry aligned test methodology to assess the suitability for use of single-use components.

What was covered in part 1?

A holistic approach to the assessment and qualification of X-ray sterilization entails a fundamental understanding of the impact of X-ray on single-use materials and components — as well as an overall assessment of the final packaged assembly.

In addition to establishing a cross-industry view on the types of testing that will best assess any potential risk, the working team identified specific tests (i.e., physical, functional, biological, and chemical) to be performed on representative components.

It is expected this risk and data-based assessment of materials and components used in the biotech single-use industry will support the strongly-touted arguments that X-ray is equivalent, or better, than gamma, thereby enabling much of the qualification data already in place for gamma to be leveraged as fully applicable to X-ray. For example, instead of performing animal-based USP <88> testing for biological compatibility, largely considered a requirement from which the industry is looking to move away, the BPSA team recommended non-animal-based cell culture testing associated with USP <87>.

Similarly for extractables and leachables evaluation, an extremely costly exercise that has been a major alignment challenge for the industry over the past decade, the team agreed to recommend a rigorous, but rationalized risk based approach using USP <665> moderate level testing, to verify the impact of the irradiation technologies are equivalent.

"Our focus is currently on how to best track and share X-ray qualification datasets from a large number of suppliers – and for an even larger number of single-use components."

Do you expect X-ray technology to be used increasingly in the future?

The simple answer? Yes.

Investments from different service providers in all major geographies show this as a major trend to complement existing sterilization technologies – this includes new X-ray sites in Europe, the Americas, and Asia. Furthermore, with the recent challenges in ethylene oxide emissions, public perceptions, and pending new restrictions, there is a concern that this market, equal in size to gamma irradiation, could add pressure on other forms of contract sterilization. Overall there continues to be strong and increasing global demand for sterilization spanning from food irradiation, medical devices and consumables, single-use, and so forth.

What were the biggest challenges in developing the guide?

The challenges, especially around timelines and sense of urgency were daunting!

Testing single-use components can be very costly, and, in some cases, can require up to a year or more. Suppliers felt they could more readily justify the business case to generate data for their newest products on the market, which – in reality – represented fairly low irradiation volumes than products already on the market. In addition, the testing needed to include a direct comparison of X-ray and gamma-irradiated materials could nearly double the cost of already expensive testing measures. And since the relationships between X-ray and gamma-irradiating test materials at a specific dose were not yet established, this was especially challenging.

Many biomanufacturers wanted to see noteworthy data as soon as possible, whereas the rate at which testing was completed and available was incremental. We also found that having only one or two datasets to scrutinize can easily lead to overinterpretation of small statistically meaningless variations in the data.

I feel that we have addressed both of these with the most recent BPSA paper.

Additional concerns from biomanufacturers include regulatory acceptance requirements – with the subject of prior approval becoming increasingly concerning as the timeline progressed. In our case, we were very fortunate to be closely connected with BARDA. By working together with a small group of end users, suppliers, and industry subject matter experts, we were able to socialize the outputs of the first BPSA paper, representative supporting data, and end user risk assessment concepts with regulators, including the FDA Emerging Technologies Team, EMA Quality Innovation Group, and Japan PMDA. The feedback was largely supportive and has been shared with BPSA (1).

What else are you focusing on at BPSA?

Our focus is currently on how to best track and share X-ray qualification datasets from a large number of suppliers – and for an even larger number of single-use components. There is strong interest in monitoring successful implementations, as well as receiving additional regulatory feedback.

There will also be papers coming out from BioPhorum, which share an elegant risk evaluation strategy based on the types of data expected from the first BPSA X-ray white paper, and how the components are used in actual biopharmaceutical manufacturing processes. This should be a complementary paper illustrating how the outputs of BPSA dovetail and feed well into the other.

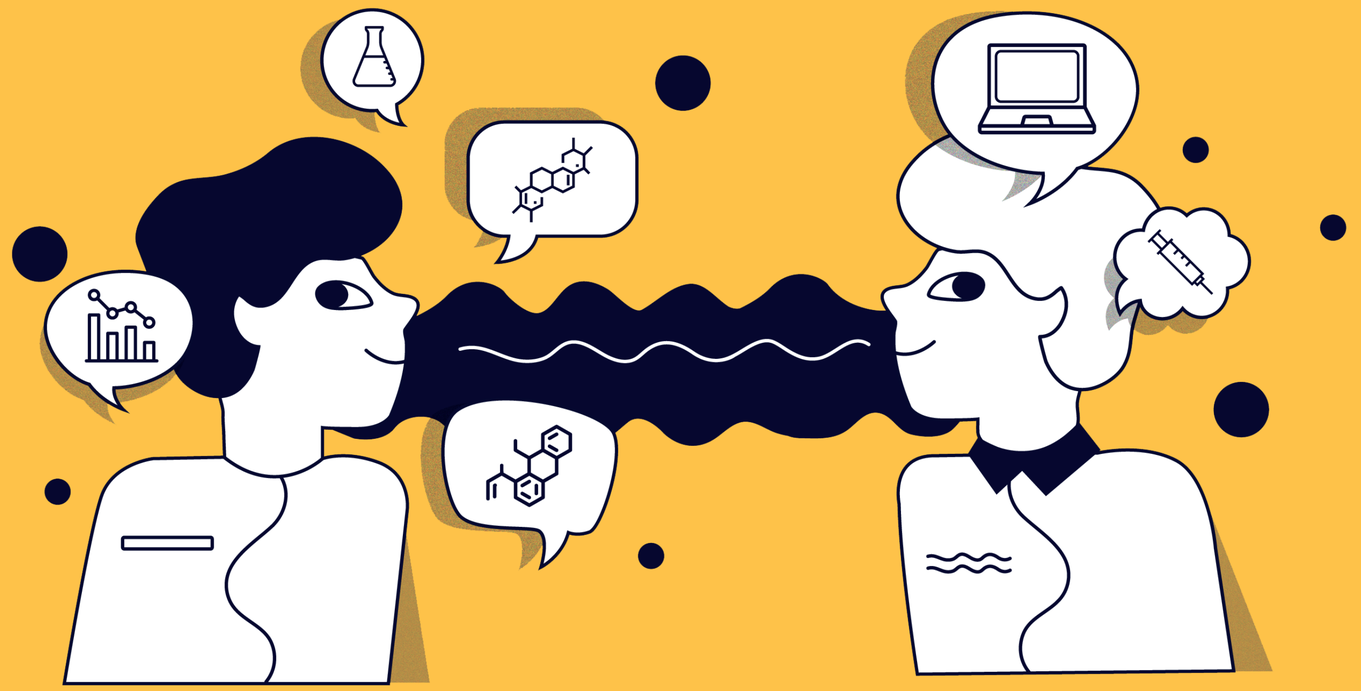
Certainly there are many other high-impact BPSA initiatives too, including responses to the REACH proposal to ban all PFAS materials – many of which are critical to the vast majority of medicines on the market (2). Other key initiatives include key guidance papers on integrity assurance for single-use systems, updates to the BPSA Quality Test Matrix, and efforts to ensure and improve sustainability in the biopharma sector.

REFERENCES AVAILABLE ONLINE

FEATURE

The Case for MMC

The use of multimodal chromatography is increasing in biopharma purification processes. Here's why.



By Heidi Jones, Market Development Manager for Process Chromatography at Bio-Rad Laboratories

Multimodal (or mixed-mode) chromatography (MMC) is increasingly being used in the biopharma industry for purification. The technique allows for a broader range of ligand interactions with the target molecule, resulting in enhanced selectivity and purification capabilities compared with traditional chromatography methods.

In resin format, MMC uses ligands on the stationary phase that exhibit two or more interaction modes, such as hydrophobic, electrostatic, hydrogen bonding, and metal affinity. This approach enables the manipulation of the mobile phase to maximize multimodal ligand interactions with the target biomolecule and remove impurities, such as host cell proteins and DNA.

From a process development and business perspective, MMC has the potential to improve overall purification process efficiency by combining at least two interaction modes in one step. Ultimately, this improvement in efficiency can translate to reduced costs while increasing yields.

The rise of multimodal

Multimodal purification is not new. Indeed, many traditional monomodal resins exhibit multimodal interactions that are caused by attributes of the base bead matrix used to secure the ligand of interest (1).

"MMC offers a 'gentle' solution for the purification of sensitive molecules, minimizing the denaturation and aggregation of target proteins during the process."

Calcium hydroxyapatite (CHT), for example, is an inherently multimodal resin that has been widely used since the 1950s for a variety of bioprocessing applications, including mAbs, vaccines, separation of single-stranded from double-stranded DNA, antibody-drug conjugate (ADC), and bi-specific downstream processing (1, 2, 3, 4). CHT is a chemically synthesized ceramic bead composed of both phosphate and calcium groups, capable of both metal affinity (Ca2+) and cation exchange (CEX) interactions due to its phosphate residues. Manipulating salt and/or phosphate concentrations of the mobile phase enables selective elution of impurities and target biomolecules.

The predecessor of today's MMC resins were born from observations in the resolution differences of nucleic acid separation, which could not be explained by ligand chemistry alone. Differences were observed with different base matrices, despite using the same stationary phase ligand motifs; for instance, diethylaminoethyl cellulose (DEAE) bound to cellulose exhibited different resolution capabilities to DEAE bound to agarose. Other multimodal observations were also made, such as the effects on resolution due to free silanol groups on silica-based matrices in reverse-phase (RP) chromatography, retention affects caused by hydrophobic interactions in ion exchange (IEX) and affinity chromatography media, and the effects of electrostatic interactions on size exclusion chromatography (SEC).

In early nucleic acid separation using HPLC, anion exchange (AEX) resins were widely used, with alkyl amine-bound matrices enabling separation of nucleic acids (for example, plasmid DNA, viral DNA, or DNA restriction fragments) from biological samples based on interaction with negatively charged phosphodiesters. HPLC grade-silica-based bead technology was later developed and designed to withstand higher pressures and flow rates.

Secondary weaker matrix interactions were initially seen as detrimental in chromatographic separations. It wasn't until the 1960s that researchers made the first true attempt to make an MMC resin for HPLC applications, designed to enhance nucleic acid separation by using AEX attributes and the presence of lysine and arginine side chains, alongside secondary effects caused by methyl groups (2).

Bioprocessing benefits

MMC has now extended beyond its early nucleic acid applications into large scale bioprocessing, with advances in predictive screening processes enabling more rapid analysis of MMC resins for biomolecule separation. High throughput techniques, such as design of experiment plate screening and, more recently, advanced mechanistic modelling, can be used to predict the optimal mobile phase conditions for a particular biomolecule separation (1, 6).

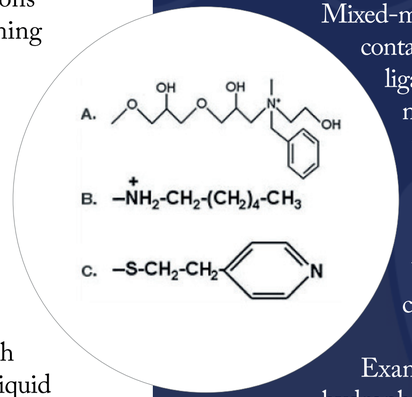
Because MMC allows for a broader spectrum of ligand interactions, it is particularly useful when purifying highly charged or polar molecules – or those exhibiting salt or pH sensitivity, which can make separation using reverse phase, ion exchange, or affinity liquid chromatography especially challenging. Additionally, the simultaneous manipulation of multimodal interactions can achieve increased biomolecule resolution, a valuable feature not only in bioprocessing, but also in HPLC applications for analytical scale bio-separations and impurity analysis (7).

SIDEBAR

Mixed-Mode Ligands

Hydrophobic, anionic ligand with hydrogen bonding – this ligand features hydrogen bonding, a quaternary amine, and a phenyl group.

Mixed-mode cationic ligand with hydrophobic binding – this ligand contains a secondary amine and is cationic over a wide pH range; therefore, it behaves as both a hydrophobic interaction resin and an anionic exchange media. At low ionic strength, it can bind acidic proteins as well as proteins with moderately high isoelectric points. However, hydrophobic interactions predominate, as binding capacity increases with temperature and salt.



Mixed-mode pH-controllable sorbents – this ligand contains a 4-mercaptoethylpyridine (MEP ligand. The pyridine ring is uncharged at neutral and basic pH. As the pH decreases, the pyridine nitrogen becomes positively charged, turning the resin into a mixed-mode media. MEP becomes a pH-controlled mixed-mode ligand, a property that has been termed "hydrophobic charge induction chromatography" (Burton and Harding, 1998).

Example of mixed-mode ligands. A is a hydrophobic, anionic ligand with hydrogen bonding. B is a mixed-mode pH-controllable sorbent. C is a mixed-mode cationic ligand with hydrophobic binding.

Figure 1. Examples of multimodal ligand chemistries (5)

MMC has several advantages over monomodal chromatography (1). Firstly, MMC offers multiple interaction modes, which provides greater versatility and high selectivity for a wide range of target molecules, such as bi-specific antibodies, acid- and salt-sensitive antibodies, and ADCs. Multimodal ligands may enable higher target binding capacity over traditional monomodal methods, leading to more efficient capture and purification.

MMC offers a 'gentle' solution for the purification of sensitive molecules, minimizing the denaturation and aggregation of target proteins during the process. The ability to purify multiple targets in a single step can reduce the number of purification stages, resulting in cost, time, and resource savings.

MMC: Why fix what's not broken?

Despite its advantages over current monomodal methods, MMC faces several barriers that make entry into the biopharmaceutical manufacturing space difficult.

For example, current manufacturing processes for therapeutic mAbs that rely on sequential monomodal purification are well developed, validated, and firmly established. Why change something that already works? Introducing multimodal methods would require an effective case to convince upper management and investors that the newer, less established technology is a superior alternative to standard purification processes.

Another drawback is that the selectivity of MMC varies depending on the target molecule and its interaction with the ligands, which can make purification unpredictable. Some MMC resins may also have limited stability under certain conditions, affecting their performance and reusability – and potentially increasing operational costs. Depending on the chromatography system, MMC ligands can also be prone to leaching from the matrix, resulting in contamination of the purified product.

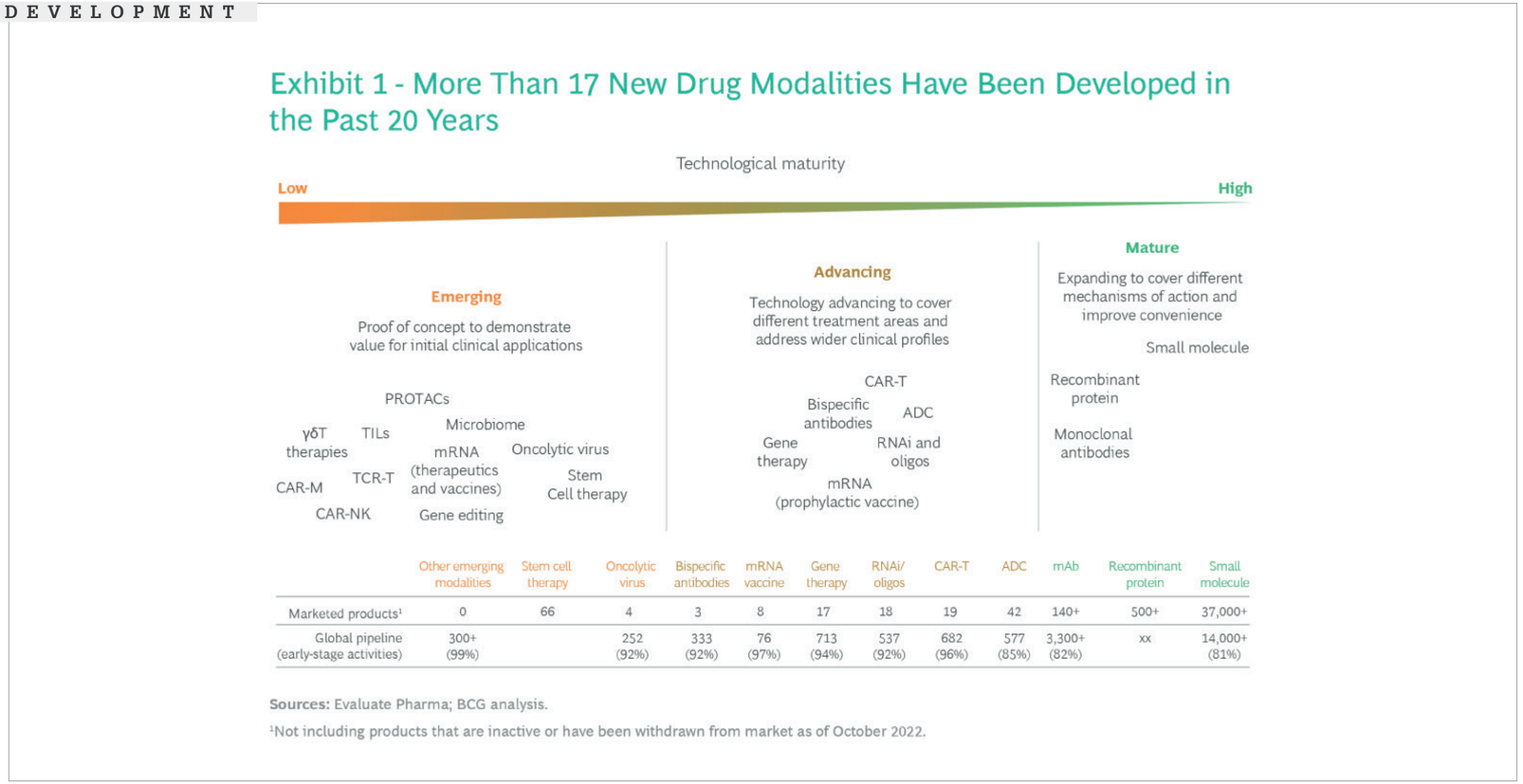


Figure 2. Benefits and risks of new drug modalities (8)

These are not insignificant issues; however, in a world where mAbs dominate the therapeutic landscape – and with continual boosts to upstream processing – MMC looks like an increasingly attractive solution. After all, with increased protein production comes increased levels of aggregation and HCP impurities, which can make high purity and high yield more difficult to achieve (1).

In addition, many new drug modalities are coming to fruition (see Figure 2), which creates an exciting space for researchers to design unique downstream processes. Indeed, there has been a surge not only of new MMC resins, but also new media technologies (for example, multimodal membrane and multimodal monolith technology; see sidebar) that aim to deliver greater selectivity at a faster rate than current resins.

Downstream bioprocessing typically involves an orthogonal purification approach; for example, a mAb platform process can comprise an affinity capture step followed by viral inactivation and additional chromatography steps to ensure high product purity is achieved along with sufficient reduction in process impurities (see Figure 3). MMC has the potential to reduce this downstream processing workflow by combining two or more steps into one,

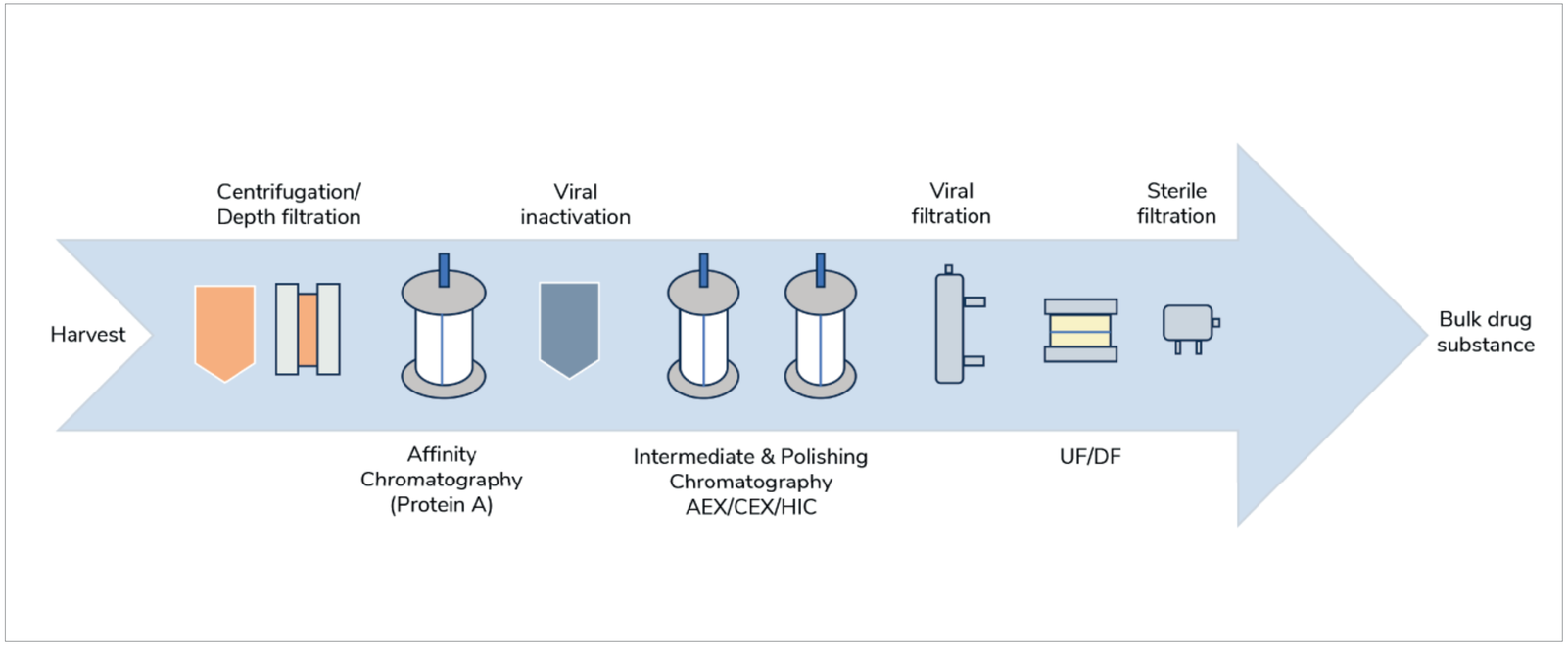


Figure 3. Overview of a typical mAb manufacturing process

improving purification efficiency whilst simultaneously reducing processing time and cost.

It also seems likely that there will be a rise of MMC specifically designed to enhance mRNA and other oligo therapeutic separations. Although various resins have been developed over the last ten years or so years that preferentially bind to the poly A tail of in vitro transcribed mRNA to enable straight forward capture steps, secondary polishing steps are still required for additional purity enrichment.

The future of MMC is promising. As research and innovation continues, the theoretical basis and practical applications of MMC will become even better understood, broadening its use.

I believe that improvements in resin design will overcome issues related to ligand stability, leakage, and selectivity variation, improving the overall performance, reliability, and, therefore, adoption of MMC. Additionally, with a deeper understanding of multimodal interactions and ligand design, personalized purification strategies tailored to specific biomolecules or complex mixtures may become more common.

With advances in automation and robotics also enabling large-scale and high-throughput purification processes, MMC undoubtedly has huge potential as a powerful and versatile tool across biotech, pharma, and beyond.

REFERENCES AVAILABLE ONLINE

SIDEBAR

Membrane and Monolith Technology

Recent advances in ligand and polymer technology have resulted in the development of single use membranes with higher dynamic binding capacities and improved selectivity, which can be used for the purification of a wide range of biomolecules in flowthrough mode, including viruses, mAbs, and endotoxins. Membranes have the advantage of significantly higher throughput over resins because of their flow properties.

A key obstacle in mAb bioprocessing is salt sensitivity. Load material must often be diluted to reduce conductivity before proceeding to the next step in a process. Multimodal membranes seek to bypass this processing bottleneck through the combination of IEX ligands (usually AEX) with hydrophobic, coulombic, and/or hydrogen bonding attributes to enhance salt tolerance while maintaining high binding capacity and selectivity (9).

Multimodal monolith technology has recently emerged that uses hydrogen bonding and AEX modalities (10). Specifically designed for the downstream processing of mRNA and virus separation, these technologies have the potential to meet demands for efficient high throughput processing and high selectivity.

However, both membrane and monolith technologies come with several drawbacks that may make wide scale adoption challenging. For monoliths, an inherent lack of homogeneity of pore size distribution throughout the column creates scaling issues, complicating GMP validation processes. Single use membrane technology is more expensive than other chromatography resins that can be cycled multiple times before discarding (11).

SITTING DOWN WITH

Making Miracles

After serving as the Head of Strategic Research Development and Director of the Office of the Dean of Research at Trinity College Dublin, Fiona Killard-Lynch was appointed Director of Research and Innovation at NIBRT

"If you want to maintain a forward momentum, you can't get bogged down by convention."

What kicked off your career in bioprocessing?

Even as a child, I was always interested in health, diseases, and drug development. Interests like this become a passion in childhood, setting you on a particular path. Then, life unfolds in front of you and the pathway becomes serendipitous. The spark was always there, it just needed to be developed.

At the same time, I also knew that I wanted to be involved in something meaningful and impactful – I wanted to be engaged. As I progressed in my career, I developed a real passion for research and how important it is to open debates. Emerging research in bioprocessing has a real world impact on people's lives. Seeing therapies developed for diseases, such as multiple myeloma, and seeing people help babies with neurodegenerative disorders were considerable drivers for my trajectory into the sector.

My current role at NIBRT is a great environment for research because the Irish government has invested significantly in biopharma R&D.

How do you keep your research team interested and focused?

Enabling researchers to engage in whatever they are passionate about is important – it means they need no motivation! That said, we do need to make sure talented people are properly funded. Notably, we've made progress in ensuring our PhD students receive a living wage. At NIBRT, they also have the infrastructure and a dynamic community

to work within. The entire research community should always be working to help people reach their potential, while removing barriers.

What's your greatest achievement in research?

During the pandemic, there was a real risk of research being shut down. Along with my team, we were able to secure almost 10 million euros from the Irish government, and we worked very hard to disseminate it as quickly as possible so that as much research as possible could continue. Researchers were given assurance that they could continue and their career wouldn't be impacted by COVID-19.

The funding went across Trinity and was given to researchers from countries where COVID-19 had been particularly devastating. In some cases, researchers had family members who had died from COVID-19. The funding allowed them to continue their careers. I'd say it had an immeasurable impact.

How important are relationships with policymakers when it comes to research and training?

You need policymakers to have successful research programs, and those policymakers need to be integrated at all stages of that pathway. Part of NIBRT's success is a result of involving policymakers from the very beginning – in turn, they saw the huge need for a facility like NIBRT, which was established to train talent for the biopharma industry.

What factors are detrimental to research?

Knowledge security is one. There needs to be a balance between what you're doing and what the industry wants – improvements in manufacturing processes, for example, and building on knowledge that comes from other research groups. There is also a lack of clarity around research careers. Some researchers remain on precarious contracts – it's not a stable landscape for them, so there's a risk of a brain drain from academia into industry.

Academic research is sometimes viewed by the outside world as a very comfortable lifestyle, but from the inside I can attest to the fact that they are struggling. In Ireland, the third level sector is very underfunded; second level students are funded at a greater level, but PhD candidates are not on a living wage. When you look at the biopharma sector and how strong it is in Ireland, it should be much more tempting to stay in academia rather than move into manufacturing where there are so many opportunities and so many great companies.

What challenges have you faced in your career?

As a woman in science, meetings tend to be dominated by males, and whoever is speaking will speak with the guys in the room. You could see it during the pandemic, in particular, where I was, maybe naively, a little surprised by how many childcare responsibilities still fell on women. The impact affected grant applications and scholarly outputs from female academics. However, if you start to dwell on that topic too much, you can start to drown in it. I've been extremely fortunate in the Irish system because most of the presidents or provosts of Irish universities are inspirational female leaders. The former Dean of Research is now the provost at Trinity. I worked with her for years and she's just an incredible person. She taught me to just keep moving forward.

If you want to maintain a forward momentum, you can't get bogged down by convention. People have many different challenges, so it's important to persevere (even when it frustrates you).
Where I get a lot of satisfaction is in people saying, "It was hard!" Research is hard, but when you break through to the other side you commit to making it easier for the people coming behind you. Empower and enable them – especially other females – to have as positive an experience as possible. Converting all that stress and frustration into energy used for developing somebody else's life and career is what I try to do. Yes, there may be glass ceilings, but let's just keep breaking through them.

So you enjoy mentoring female researchers…

When you look at two grant applications, you can often tell which was written by a male and which by a female just from the language and the tone used. Having the opportunity to motivate and inspire women to sell themselves, because they have the skills and capabilities to match anybody in their field, gives me a real sense of satisfaction. What better legacy could I hope to leave behind?

What do you see in biopharma's future?

We're on the cusp of something incredible. I'm not one for hyperbole, but some of the new therapies emerging are nothing short of miracles. We all remember a time when cancer was (and still is) a frightening thing. There is now a war against cancer and a real future for cancer patients is within touching distance. There has been a huge surge in developments in cell and gene therapy, too. We are going to see a world where people live longer and better lives. NIBRT's CONCEPT, a core facility for early-stage biotherapy, could be a key enabler of these developments where researchers and industry can generate optimized cell lines and biological material for advanced therapeutics and biologics experiments.

Now, the onus is on the nations of the world to work together to realize these miracles. And it begins by recognizing and supporting the incredible work of researchers – the young people dedicating their lives to enabling these changes.