A Decade of BioProcess

In 2012, *BioProcess International* will celebrate its 10th year of publication. During this decade, we have provided more than 30,000 readers with the opportunity to read, learn about, and implement the biopharmaceutical industry's most impactful scientific breakthroughs and process improvements, while spotlighting the brilliant people making it all happen. To recognize, honor, and celebrate the most significant accomplishments in bioprocessing over the past 10 years, BPI has created the **2012 *BioProcess International* Awards: Honoring A Decade of BioProcess.** This unique awards program will recognize outstanding products, services, companies, and people that have had the greatest positive impact on upstream production, downstream processing, and manufacturing — the three pillars of bioprocessing. Four awards will be granted under each category:

- **Technology of the Decade**
- **Collaboration of the Decade**
- **Technical Application of the Decade**
- **Thought Leader of the Decade.**

EXTENDED DEADLINE: Nominations are open until 30 June 2012. For all award details, rules and deadlines, visit www.bioprocessintl.com/awards.

All nominations will be reviewed and evaluated by BPI Editorial Advisory Board members and other expert judges. Following tabulation of results by an independent agency, finalists for each award category will be announced in September 2012. Winners and runners-up will be unveiled at a live awards ceremony taking place in October 2012 during the 2012 BPI Conference and Exhibition at the Rhode Island Convention Center in Providence, RI.

In June 2012, *BioProcess International* will publish a supplement highlighting "A Decade of BioProcess." BPI's editorial staff will offer perspectives on how technologies, applications, collaborations, and individuals have advanced the industry over the past decade — and how their contributions have enabled the biopharmaceutical industry to improve the quality of life on a global scale. And here in the "Spotlight" section of every issue this year, we'll focus on a particular aspect of bioprocessing: how it has changed, where it has come from, and where it is going.

http://www.bioprocessintl.com/awards

Ten Years of Chromatography

by Cheryl Scott

Chromatographic separations are vital both to the analysis of biological macromolecules and to their manufacturing. When properly applied, chromatography provides exquisite specificity in separating different molecules from solution based on their size, electrical charge, or other physicochemical properties. Large liquid chromatographic (LC) columns remove host-cell nucleic acids, endotoxins, viruses, and process intermediates from harvest material. Combine high-pressure liquid chromatography (HPLC) with mass spectrometric (MS) or ultraviolet–visible (UV–vis) spectroscopic detection, and you can qualify and quantify macromolecules in such complex biological mixtures. Apply Fourier-transform infrared (FTIR) detection to gas chromatographic analysis, and you convert data into wave form, making it easy to compare spectra for confirming the identity of raw materials or determining leachable/extractable components in single-use equipment.

Speaking of disposables, this is one technology that has thus far defied conversion from reusable to single-use formats. Glass or stainless steel LC columns are regenerated a number of times to offset their cost (1–3). And it's not just the columns themselves but also the chromatographic resins packed inside them that can be very expensive depending on their construction and chemistry.

Protein A and Beyond

When it comes to expensive media, the most notorious (and celebrated) are protein A affinity resins. Because of the strong affinity that certain antibodies have for binding protein A (due to their role in immune response), it is so useful that despite its cost, high-yield protein A affinity capture is the first in line for a purification platform (4, 5). That means it handles the largest, least-refined process stream (after clarification removes debris) — and delivers 99% purity in a single step (6).

Even so, the expense combined with engineering and practical difficulties (6, 7) are making some process developers in this era of high-titer production wonder whether some alternative might be a better choice than protein A. Among those are mercaptoethyl-pyridine ligand–based affinity resins (7). Thus far, however, protein A remains king (8, 9).

We call them *resins*, but chromatographic media can be made from a number of materials. These began with cellulose and over the years have expanded to include carbohydrate polyethers, ceramics, silicates, and various polymers (10, 11). Different vendors sell different types.

Membranes and Monoliths

Although traditional column chromatography doesn't lend itself easily to single-use mode, two variations on a theme have emerged that do offer a disposable option: membrane adsorbers (12–14) and monoliths (11, 15, 16). The former are not really chromatography per se; they use binding chemistry, but not in a flow-through, bind-and-elute mode. More accurately, they function as a specialized form of tangential-flow or depth filtration.

Monoliths, however, "are chromatography media cast as a single integrated unit" rather than as porous particles packed in a column (12). They offer many attractive features: an ability to maintain high resolution and capacity regardless of flow rate and molecular size; and low-shear fractionation for shear-sensitive products such as DNA plasmids, live viruses, and labile proteins (15).

Process Development

Like every other aspect of biotherapeutics production and processing, development of chromatographic capture and polishing steps is changing due to the quality by design initiative (17, 18). New statistical and other modeling approaches are speeding optimization while advanced analytical methods help process engineers find and fix problems better and than ever before (19–29).

For example, column packing procedures can affect results in both analytical and process chromatography (19–22). Different media/supports require different methods (19). Larger companies and those performing high-throughput analytics can make use of automation (20). Too-high sample loads or flow rates can push solutes through too fast, blurring the unstable interface between fluids of different viscosities (21). And packing is just one aspect of chromatographic operations that's subject to scale-down optimization (22–29).

Anything But . . . ?

A few years ago, we began to hear a strange rallying cry that would have been unthinkable when *BioProcess International* began: "ABC" for "anything but chromatography" (30). As downstream process groups faced the challenges of high-titer expression and highly concentrated product streams, they began to look "outside the box" for answers. But not only is chromatography familiar to regulators and well known for its power, it also has many years of engineering and optimization behind it. Other techniques face an up-hill road trying to replace it.

Meanwhile, chromatographers haven't stopped improving their technologies. Expanded-bed adsorption is already used in a number of market-approved processes (31). And perhaps the most exciting recent advancement has been in combined chemistries for multidimensional separations (32–37).

Sometimes thought of as an "alternative to protein A," multimodal resins put two different chromatographic chemistries to work in separating biotherapeutics from their contaminants: typically hydrophobic interactions and ion exchange (32). They provide "unique selectivities that are not achievable by single-mode sorbents used sequentially, so they enable some proteins to be purified when single-mode sorbent combinations fail" (33). Some can even reduce endotoxins to clinically acceptable levels (33).

Multimodal (mixed-mode) chromatography is no new idea. Hydroxyapatite (HA) was the first, combining cation exchange and metal affinity in the 1950s. "HA's selectivity was recognized as unique from its introduction, but a lack of practical knowledge concerning its binding mechanisms long delayed the development of scouting pathways that fully revealed its abilities. That discouraged process developers who might have benefited from its capabilities. As those pathways were defined, it became possible to control each binding mechanism, and HA has emerged as the most broadly capable process option for removing fragments and high levels of aggregates from antibody preparations" (36).

The development of HA mirrors that of chromatography overall: It is a powerful method that's been around the block, improving and expanding its applications as time goes by. Don't expect its importance in bioprocessing to fade any time soon.

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