Advances in Affinity and Ion Exchange Chromatography

October, 2024 | Content supplied by Sartorius AG

Sartorius provides innovative solutions to the life science and biopharmaceutical industries. Our mission is to simplify progress, enabling scientists to accelerate the discovery and production of novel biological therapies that will improve human health. In this interview, we ask Product Manager, John Cashman, about the future of antibody purification.

"Efficiency is crucial when developing new antibody-based therapies and Sartobind® Rapid A Lab supports this in the early research and development phase."

John Cashman holds a PhD in biochemistry and has extensive experience in protein purification workflows. He joined Sartorius in 2019 and is now responsible for the success of a growing portfolio of separation technologies, including laboratory ultrafiltration and purification consumables.

Q: Thank you for joining us today. Could you start by explaining the importance of chromatography in life science research and how it works?

John: Of course. Chromatography is at the core of most biomolecule purification workflows, whether for small-scale screening to find the optimal expression construct or purification buffer, or for preparative purification for structural and functional studies when characterizing a new protein. The main components of a chromatography system are the stationary phase (a solid matrix), the mobile phase (the buffer system) and, in some methods, the ligands (designed to capture specific molecules). All these components work together to separate mixtures of biomolecules, such as those commonly found in cell culture media or lysates, into their individual components. The aim is to remove contaminants so that the purity of a molecule of interest (MOI) can be increased. High purity is important to ensure scientific accuracy and reproducibility of results, and for biotherapeutics it is critical to ensure safety and efficacy.

Q: Can you tell us a little more about the ligands that are used in chromatography?

John: There are many different methods used for chromatographic separations, but the most popular ones capture molecules based on either charge differences or a specific binding interaction. For example, quaternary ammonium and sulfonic acid are common ligands used in ion exchange chromatography (IEX), while Protein A is the ligand of choice for affinity chromatography (AC) when monoclonal antibodies (mAbs) and other Fc-containing molecules need to be purified.

Q: What are some of the limitations associated with chromatography in research laboratories today?

John: Many scientists use chromatography columns containing resins as the stationary phase. While this is a familiar technology that has been used for many decades, resin chromatography relies on microscopic beads with very small, dead-end pores containing low ligand densities. This means that mass transport of molecules occurs by diffusion and yields are limited, making purification slow and inefficient. Resin columns also have pressure limitations that make it difficult to reliably purify molecules without using a dedicated liquid chromatography (LC) system, which requires additional maintenance and set-up time. Matrix blocking and disruption by air bubbles are also common problems that limit reusability and make buffer degassing an essential preparation step, increasing cost per sample and process time. Finally, resin technology is not always available in formats suitable for GMP applications, so commercialization of a new biotherapeutic may require further time-consuming process development on a different platform.

Q: Those are some significant challenges. Is there an alternative technology that can overcome these limitations?

John: Yes, there is. Membrane chromatography is already an established alternative in biopharmaceutical applications and offers several advantages over resins. First, mass transport is primarily by convective flow, which allows for much higher flow rates and shorter processing times. This leads to increased throughput and productivity.

Membranes also tend to generate lower back pressures but can tolerate high pressures too, so they can be used for benchtop purification with a syringe or pump, where pressures would easily exceed the operating limits of resin columns. In addition, membranes are not susceptible to dissolved air making buffer degassing a thing of the past.

Q: That sounds promising. Can you tell us more about the Sartorius membrane chromatography portfolio and its specific advantages?

John: Absolutely. For lab-scale purification, we have a range of spin and pressure filter formats with Sartobind® membranes. All of these options are ready-for-use and support high flow rates and impressive binding capacities to reduce setup and process time and increase productivity. They also offer the flexibility to purify without a LC system, using standard equipment such as a centrifuge or a pump, or even without equipment, using a syringe. Another time-saving feature is Sartobind® Lab's Luer connectors, which allow for sample prefiltration — an essential step before chromatography — to be performed inline by attaching a Minisart® syringe filter directly upstream of the chromatography unit. Finally, the same Sartobind® membranes are available in process-scale capsules and cassettes, so scale-up for clinical trials and commercial production is a breeze.

Q: Can you tell us about any recent innovations in the Sartobind® Lab range?

John: Sartobind® Rapid A Lab uses a new membrane technology that represents a major step forward for antibody affinity purification. It has the fastest Protein A matrix on the market and is resistant to blocking, so it offers significant productivity improvements over resin columns and even some first-generation membranes. As with other Sartobind® Lab products, the new membrane is available in larger formats for a convenient transition to GMP production.

Q: Sartobind® Rapid A Lab certainly does seem like a big step forward. Can you elaborate on its performance improvements?

John: Sure. With our new membrane, effective antibody capture occurs within a two-second residence time. This means that we can use rapid flow rates and an entire purification cycle, from equilibration to regeneration, takes less than three minutes. Speaking of regeneration, we have shown that Sartobind® Rapid A Lab supports highly reproducible purifications over 100 cycles. When combining this high cycle count with the high dynamic binding capacity of the membrane, it is possible to purify as much as 17 times more antibody over the lifetime of a single Sartobind® Rapid A Lab unit compared to many resin columns, and three times the yield of alternative membrane chromatography consumables.

Q: Those are impressive figures. The sustainability of laboratory consumables is also becoming increasingly important to research scientists. Are there any notable improvements in this area with Sartobind® Rapid A Lab?

John: Yes, there are. In fact, all Sartobind® Lab products are already manufactured using 100% renewable energy. More specifically for our new consumable, protein-based ligands such as Protein A typically require cold chain shipping and refrigerated storage. To reduce these energy-intensive requirements, we have extensively tested the stability of Sartobind® Rapid A Lab in combination with its packaging to develop a solution that only requires passive cooling during transport, and each unit can be stored at room temperature throughout its shelf life.

Thank you. This has been a very informative discussion and we are looking forward to seeing further innovations in lab chromatography.

If you would like to contact John to discuss any unmet needs for your laboratory scale purification workflows, email labfiltrationpm@sartorius.com.

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