CUSTOM CHROMATOGRAPHY SOLUTIONS FOR COMPLEX BIOTHERAPEUTICS

Nearly 40 years ago, recombinant human insulin became the first biotherapeutic to arrive in the clinic. Biotherapeutics have consistently increased their market share since then. In terms of sales, the share of the top 100 prescription drugs that were products of biotechnology rose from 34% in 2010 to 53% in 2018.[1] In 2019, 8 of the 10 top-selling drugs globally were biologics.[2]

Antibodies have historically dominated the biotherapeutic market, and while the overall number of this drug type in the pipeline continues to rise, other classes of biotherapeutics are growing faster.[3] "There's a lot more diversification in the molecule types that are getting entry to the clinic," says Scott Zobbi, the senior manager for custom chromatography resins at Thermo Fisher Scientific.

Instead of being chemically synthesized in a lab, biologics are harvested from microorganisms, such as bacteria or yeast, or animal cells. The need to extract them from the "biological soup" they are grown in and their large and complex structures make biologics inherently more challenging to purify than small-molecule drugs. Biotherapeutic purification methods vary, with chromatography being the dominant tool. Over the decades, effective chromatography methods for purifying many antibodies have been nailed down. That is less true for newer drug types like proteins, viral vectors, and cell therapies.

The growth of nonantibody biotherapeutics is exposing gaps in the chromatography product range on the market, according to Zobbi. This broadening of the types of molecules hitting the clinic is driving the need for more custom chromatography resins, he adds. Customization enables a resin to be designed to match the specific requirements for the biotherapeutic being purified.[4]

WHY CUSTOMIZE RESINS?

For many scientists in the biopharmaceutical industry, the first step when looking to purify a biotherapeutic candidate or improve the purification of an approved drug is to peruse the chromatography resin marketplace. The choice of off-the-shelf resins is extensive and constantly expanding. A 2020 market research report cited an estimate that the global chromatography resin market will grow from about $2 billion that year to $3.5 billion by 2027.[5]

Even so, suitable off-the-shelf chromatography resins aren't yet available for some biotherapeutics. This is when customization might be considered. "People look at off-the-shelf resins first and then, if there's an issue, go down the pathway of customization," says Frank Riske, a biotech consultant for the global accountancy and business advisory firm BDO.

Typical reasons for seeking a custom solution include not being able to achieve the required purity with off-the-shelf tools and a desire to streamline downstream processing. The recovery of a biologic from the bioreactor and its subsequent purification—stages collectively referred to as downstream processing—always requires multiple steps. A custom resin can, however, reduce the overall number of steps needed by achieving the same level of purity in a single step that would otherwise have needed a couple of sequential traditional chromatography columns to achieve.

This reduction in the number of required purification steps can save both money and time, and can result in a higher overall yield of the biotherapeutic. "Each step you add to a process decreases the recovery of your molecule of interest," Riske says. And Zobbi says the increased yield alone gives a boost in profits that can easily offset the cost of developing a custom resin.

Custom resins can be designed for all standard modes of chromatography. Customized depletion resins can also be developed; these contain a material that captures a specific process- or product-related contaminant on the column. The biotherapeutic flows through unhindered. Depletion resins can remove contaminants from biologics at the parts-per-billion level, according to Zobbi.

Thermo Fisher Scientific develops custom resins for the purification of biotherapeutics. The process starts with prototype resin development and ends with a scalable chromatography resin which can be used in commercial manufacturing.

Source: Thermo Fisher Scientific

Zobbi and his colleagues have developed custom resins suitable for the manufacture of several biotherapeutic types, including oligonucleotides, messenger RNA (mRNA), therapeutic proteins

(see box), peptides, viral particles, viral subunits, vaccines, and blood-derived products (such as blood factors and blood proteins).

A MULTITUDE OF OPTIONS

The most frequently used chromatography types for biomolecule purification are affinity chromatography, ion-exchange (IEX) chromatography, and hydrophobic-interaction chromatography (HIC). It is possible to customize all these types to match the requirements of a specific downstream process.

Affinity chromatography uses a unique interaction between a target molecule and a ligand coupled to the resin. The ligands bind with the target molecule as it flows through the column and trap it there. Everything else is then washed off and the binding is reversed to release the pure target molecule. IEX is a chromatography mode that separates molecules based on differences in overall surface charge, while HIC separates molecules based on differences in surface hydrophobicity.

When a custom resin is being developed, its ligand, surface charge, or hydrophobicity will be designed to exactly match the requirements of the chromatography process. The best type of base bead will also be considered, as will the most suitable coupling chemistry and way of attaching those chemistries to the beads. “There is a huge design space to play around in,” Zobbi says.

Popular base beads suitable for IEX, HIC, and affinity include soft agarose beads and rigid polymer beads. Thermo Fisher Scientific offers customization of its 50 µm poly(styrene-divinylbenzene) beads in its POROS™ product. Considerations in selecting a suitable base bead for a particular biomolecule include how the beads pack, the pore space between beads, and the size of the pores within the beads themselves. There are six POROS base beads, which vary in intrabead porosity, according to Zobbi.

The customization process starts on paper, with the design of some initial bespoke resin prototypes using all available information about the biomolecule and its performance with off-the-shelf resins. These prototypes are then made up and tested at the customer’s manufacturing facility. “Once the customer has finished that prototype resin evaluation, they’ll come back and present that data to us,” Zobbi says.

The resin vendor assesses the data to see whether the resin design requires any optimization. Typically, a few rounds of prototype development are needed to create the best possible resin. Once the vendor and customer agree upon the final resin design, the viability for large-scale manufacturing is confirmed. The custom resin is then ready to be produced at research-grade quality.

To produce a resin that complies with current good manufacturing practices (cGMP), the vendor must make three consecutive large-scale batches for manufacturing process validation and compilation of a regulatory support file.

CUSTOMIZING AFFINITY RESINS

The ligand design is the most vital part of the process for developing a custom affinity resin. There are many factors that dictate the suitability of an affinity ligand. It must bind well to the target molecule and do so selectively so that nothing else is captured. The binding must also be easily reversed.

"There is a balance," says Neal Gordon, a biotech consultant and colleague of Riske's at BDO. "Too high an affinity might mean not being able to get the molecule off completely, or you might denature the product when you elute it."

Riske says finding the right ligand reminds him of the fairy tale Goldilocks and the Three Bears: "The affinity has got to be somewhere in the middle: not too strong, not too weak."

The first step toward identifying a ligand is to select a suitable ligand library to screen the target biomolecule against. These can contain antigen-binding fragments, single-chain variable fragments, monoclonal antibodies, or camelid-derived single domain (V_HH) antibody fragments. If no suitable library is available, a new library can be created.

Thermo Fisher Scientific, for example, uses V_HH antibody fragments for its CaptureSelect™ affinity ligand range. The Camelidae family includes llamas, alpacas, and camels that—thanks to an evolutionary quirk—have a unique set of antibodies far smaller than standard mammalian Y-shaped antibodies. To create a new ligand library, "we give a llama the target of interest or a cocktail of targets of interest to elicit an immune response," says Pim Hermans, Thermo Fisher Scientific's director of ligand discovery. "We isolate the cells that produce these antibodies from a small blood sample and then selectively clone the V_HH antibody fragments to make expression libraries."

Multiple factors are taken into account when screening for the most suitable ligands. These include best fit in terms of ligand selectivity to the target biomolecule (over any other molecules present in the mixture), ensuring that the biomolecule adequately binds to and releases from the ligand, and the stability of the ligand. "When you look at all those different parameters, you'll find there's only a few that are really going to be able to achieve all of that," Zobbi says.

WHAT ELSE TO KNOW

Resin customization processes take about a year from start to finish. Vendors allow the option to stop or pause resin development at various milestones. Typical reasons for doing so include drug candidates' failing clinical trials, cash flow concerns, or changes to the drug development timeline.

The customer benefits from being as transparent with vendors as possible, says Hermans, adding that the more information provided about the biomolecule targets—including previous purification problems—the smoother the process of

customizing a resin. “The more they share, and the more we know, the better we can design a product for them,” he says.

Once developed, custom resins can be provided on an exclusive and ongoing basis. To reduce ongoing purchasing costs, however, custom resins are often converted into off-the-shelf products. “Exclusivity comes with a price because you have no economies of scale,” says Ingeborg van Gemeren, vice president of business development and marketing at Thermo Fisher Scientific. Many of the company’s off-the-shelf resins started out as custom projects, she adds.

Through in-house R&D projects, vendors are also bringing to market chromatography resins suitable for different types of biotherapeutics. But the myriad types of biomolecules hitting the clinic means customization of resins

CUSTOM AFFINITY RESINS FOR NOVEL FUSION PROTEINS

Thermo Fisher Scientific is currently designing two custom affinity resins for Shattuck Labs, a biotechnology company based in Austin, Texas, and Durham, North Carolina.

The resins are intended for use with a new class of fusion proteins—dual-sided fusion proteins—being developed by Shattuck. These proteins contain both an immune checkpoint receptor and a tumor necrosis factor ligand in a single biomolecule. The hypothesis is that a biotherapeutic with both these entities will be more effective against a range of cancers and autoimmune diseases than other biologics directed to either single target. “These are highly unusual biologics,” says Shattuck CEO and cofounder Taylor Schreiber, and therefore, the off-the-shelf chromatography options for them are limited. Shattuck has two of these dual-sided fusion proteins in Phase 1 trials against various cancer indications.

Two custom affinity resins are being developed for Shattuck—one as a standard affinity column and the other as a depletion resin. “We are having built one resin that has a specific task of capturing the drug and another resin that has a specific task of removing a process-related impurity,” Schreiber says. Both custom resins will be suitable for multiple dual-sided fusion proteins.

Both projects are completing the final steps to produce a cGMP-suitable resin. For the Phase 1 trials, Shattuck’s dual-sided fusion proteins have been manufactured using off-the-shelf resins. The current process works but Shattuck saw room to innovate early in development. The plan is that the custom resins will speed up and improve the efficiency of the dual-side fusion proteins’ purification and therefore make the biologics economically viable when manufactured at scale, Schreiber says.

will continue as these bespoke products offer pharmaceutical and biotechnology companies a way to stay ahead of the game.

For more information, watch a webinar here.

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