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SPECIAL REPORT

Exploring Resin Modalities for Biotherapeutic Purification

BIO-RAD

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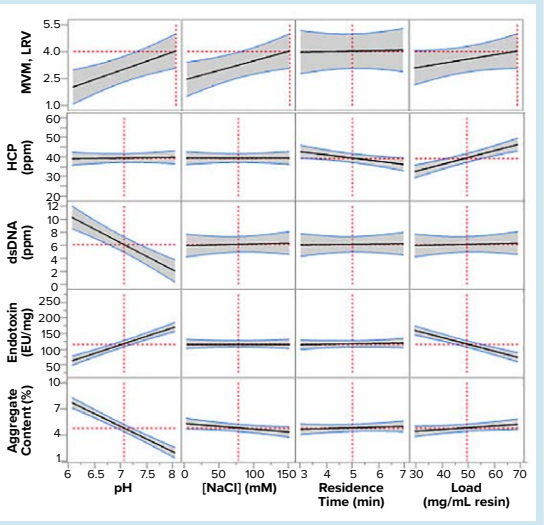
with Xuemei He, William H. Rushton, Andrew Lees, and Mark A. Snyder

Biologics have become important tools in treating a wide range of intractable diseases. Spurred on by game-changing advances in genomics and proteomics, gene editing, and cell line development, the biopharmaceutical industry is developing increasingly diverse product modalities. Now the industry needs equally critical advances in purification platforms to resolve those complex biomolecules productively and cost-effectively.

New chromatography supports must demonstrate improved selectivity, and bead technologies must be optimized for high binding capacity and product recovery. Drug manufacturers also need access to expertise and continued support from chromatography suppliers that can assist with method development and design of experiments (DoE) assessments. Working together, these industry groups could accelerate method development, increase process yield, reduce buffer consumption, minimize the number of unit operations, and improve overall process economies.

This report gleans insights that chromatography specialists from Bio-Rad Laboratories, Inc., shared during a series of *BioProcess International* Ask the Expert presentations throughout 2020. The speakers described lessons they have learned from purifying a wide range of monoclonal antibodies (MAbs) and vaccines using their company's chromatography supports. The presentations highlighted purification requirements and challenges for viral clearance study, removal of impurities from novel biomolecules, and column packing. Regardless of purification context, the speakers agreed that understanding a resin's functionalities can maximize yield and improve process economy, helping manufacturers to deliver low-cost biologics to patients with unmet medical needs.

Figure 1: Design of experiments (DoE) evaluation of impurity clearance during flow-through purification of a monoclonal antibody (pI 9.1) using Nuvia aPrime 4A resin; MVM = minute virus of mice, LRV = log reduction value

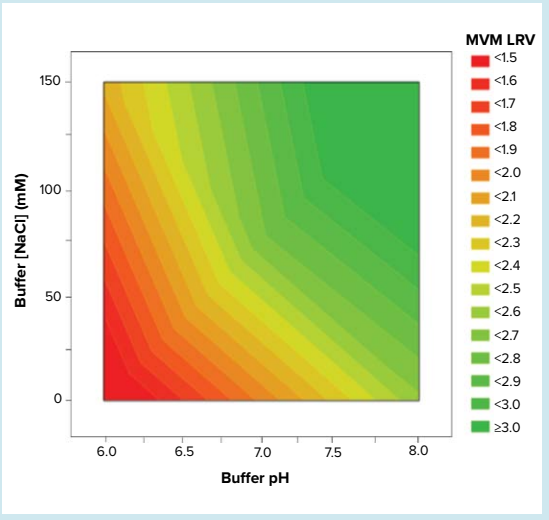


Mixed-Mode Chromatography Resins for Biomolecule Purification

In her April 2020 Ask the Expert presentation (**1**), Bio-Rad R&D manager of chromatography media chemistry **Xuemei He** explained how mixed-mode resins achieve unique selectivity. She recalled the importance of buffer pH and conductivity to conventional purification methods. For instance, a protein binds to an anion exchanger when the buffer pH is higher than the protein's isoelectric point (pI). In such cases, low-conductivity buffers facilitate binding, and buffers with high salt concentrations aid recovery. Mixed-mode media work according to that same principle but interact with proteins differently depending on changes in both buffer pH and conductivity.

A hydrophobic cation exchanger, Nuvia cPrime resin features ligands with benzene hydrophobic moieties and carboxylate functional groups. Purifying a biomolecule at a lower pH than the ligand's pK_a value alters its charge state. At pH 4.8, a significant population of the ligands are charge neutral, enabling them to engage with protein molecules via hydrophobic interaction. In more basic conditions (e.g., pH 7.0), the carboxylate groups ionize fully and carry a negative charge, making the resins behave as typical cation exchangers. Such versatility, He explained, "can give researchers a large window for proper manipulation to improve binding and elution selectivity of a protein."

Figure 2: Nuvia aPrime 4A resin retains minute virus of mice (capsid proteins, pI 6.1–6.2) most effectively at high pH and salt concentrations (LRV = log reduction value).



He compared Nuvia cPrime resin with a conventional cation exchanger for separation of ribonuclease A (pI 8.7) and cytochrome c (pI 10.7). A Bio-Rad team loaded columns and induced a gradient between buffers of 20 mM NaOAc with 150 mM NaCl (pH 5.5) and 20 mM NaPi with 1 M NaCl (pH 7.0). The cation-exchange resin did not separate the two proteins, although it retained cytochrome c slightly longer than it did ribonuclease A because of the former's strong electrostatic interaction. But Nuvia cPrime resin resolved these proteins. Cytochrome c eluted early, while elution of ribonuclease A was delayed, probably because of its stronger hydrophobic interaction.

Strong Selectivity: Mixed-mode media effectively enable fine-tuning of selectivity for improved purification. He demonstrated how to use a hydrophobic anion exchanger, Nuvia aPrime 4A resin. At neutral pH, this resin does not bind most high-pI proteins, but it can capture acidic proteins, nucleic acids, endotoxins, and viruses. Moreover, manipulating buffer pH and conductivity can enhance the recovery of target protein during Nuvia aPrime 4A chromatography while ensuring effective clearance of product- and process-related impurities.

A Bio-Rad team evaluated Nuvia aPrime 4A resin clearance of impurities, including minute virus of mice (MVM), during flow-through purification of a MAb (pI 9.1) (Figure 1). Changes in NaCl buffers and residence time did not influence impurity clearance significantly. However, MVM clearance required careful planning. According to DoE results, MAb recovery would be highest at pH 6.0, although MVM clearance would be best at pH 8.0 using 150 mM NaCl (Figure 2). The Bio-Rad team performed purification at pH 8.0 using 10 mM NaPi and 150 mM NaCl. That resulted in a viral log reduction value (LRV) >4.21 with 90% MAb recovery.

Reports from Bio-Rad clients suggest that purification using Nuvia aPrime 4A could streamline downstream MAb processing. One client compared its conventional three-step process involving protein A capture, anion exchange, and hydrophobic interaction with a two-step process using only protein A and Nuvia aPrime 4A resin. That workflow exhibited comparably high product purity (99.4%) and MVM clearance (>5 log) while shortening processing time from 29 hours to 15 hours and increasing overall yield from 49% to 70%.

Robust Resins: Hydroxyapatite calcium-affinity cation exchangers have been used for decades, but improved formulations such as Bio-Rad CHT Ceramic Hydroxyapatite media are strengthening purification steps. The ceramic component, He explained, adds "mechanical strength in spherical particles that can be packed easily into large columns used in downstream production." Purification scientists increasingly are purifying MAbs with hydroxyapatite calcium-affinity cation exchangers.

CHT media can bind MAbs from pH 6.5–7.5 with low-phosphate buffers. Target MAbs can be eluted

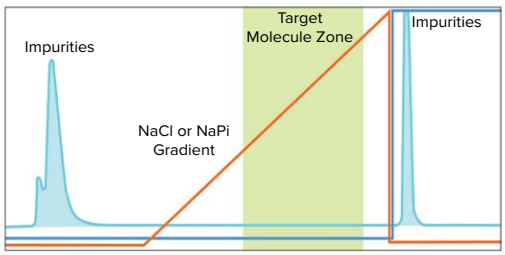
Questions and Answers, with Xuemei He

What besides changes to buffer pH and salt might improve selectivity and recovery? Consider using buffer additives. Arginine helps minimize nonspecific interaction, often facilitating target recovery. Glycine enhances hydrophobic interaction, enabling capture of targets without need for high salt concentrations. That can be useful for targets that are unstable in high-conductivity buffers.

How does Nuvia aPrime 4A resin compare with other hydrophobic anion exchangers? Nuvia aPrime 4A resin is less hydrophobic than other commercially available hydrophobic anion exchangers. That can boost target recovery. However, successful method development requires careful consideration not only of recovery, but also of target purity and yield.

Can mixed-mode resins work for virus purification? The vaccine industry long has depended on Bio-Rad CHT media formulations to purify viruses and virus-like particles. Clients have used Nuvia cPrime resin to capture recombinant adenoviruses for gene therapies. We are working with clients to purify small viruses such as adenoassociated virus.

Figure 3: CHT media enables predictable chromatography performance and purification of monoclonal antibodies in a single polishing step.



with a gradient of 1.5 M NaCl or 5–200 mM NaPi; MAb aggregates, charged endotoxins, and basic species of host cell proteins, nucleic acids, and viruses will remain on a column until stripped with 0.4 M NaPi at neutral pH (Figure 3). Once that is accomplished, CHT media will have removed a wide range of impurities in a single polish step. An acidic MAb substance (pI 6.9) containing >30% high–molecular-weight impurities, for instance, can be eluted to contain only 3.1% product-related aggregate.

Rational Design: He concluded by touching on the intuitiveness of method development for mixed-mode resins. "Once we understand the interaction mechanism, we can use DoE and buffer gradients to come up with a chromatography method, just as we have been doing with traditional chromatography methods." Rational process design and mixed-mode resins can combine to reduce purification steps and minimize feed manipulation. "That," He concluded, "will help improve overall process economy."

Assessing Viral Clearance in Early Phase Process Development

In May 2020 (**2**), Bio-Rad chromatography support scientist **William H. Rushton** elaborated on He's remarks about viral clearance studies by explaining their importance to process development (PD). Regulatory agencies require viral clearance testing before phase 1 and 3 clinical trials. But Rushton suggested that assessing viral clearance during early PD stages "can assist in defining the design space of a process step that targets product recovery and impurity removal." Preliminary studies also enable downstream scientists to anticipate how process changes might influence viral clearance.

What to Test and When: Rushton pointed out that study parameters will depend on a drug's lifecycle position. Tests performed before submission of an investigational new drug application use two or more relevant/specific model viruses to establish a process's clearance potential. Subsequent development, characterization, and validation activities will spur on process changes, and once a process gets locked down, PD teams must ensure that it meets LRV targets.

Requirements become more exacting as a drug moves toward commercial manufacture. Scale-up and technology transfers often entail process modifications. Users must ensure that their clearance step maintains or exceeds LRVs from early phase work despite any such changes. Before phase 3 trials, developers also need to challenge their processes with at least four nonspecific viruses to demonstrate robustness.

Model virus selection depends on a drug's lifecycle position and expression system, Rushton observed. Early clearance studies of drugs produced in Chinese hamster ovary cells often use xenotropic murine leukemia virus (XMuLV) and MVM. The latter is considered a "worst-case model" because it has been known to contaminate bioreactors, it resists chemical inactivation, it grows to the same size as common biologics (18–24 nm), and it bears capsid proteins that interact with chromatography resins in the same way as many biopharmaceuticals.

Late-stage testing entails more comprehensive model virus selection. To XMuLV and MVM, PD teams often add pseudorabies virus and reovirus type 3 to ensure coverage of small and large, enveloped and nonenveloped, single- and double-stranded, and RNA and DNA viruses. Selected models also should represent distinct virus families and different levels of physiochemical resistance.

Studying the Process: Rushton described three case studies illustrating how to identify pragmatic strategies for viral safety in downstream processing. Bio-Rad teams evaluated how critical process parameters and media performance bear on viral clearance and target recovery. In one case, a team assessed viral clearance from samples bearing a target molecule with pI ~9.0. Samples were challenged with known quantities of MVM, then incubated in spin columns with Nuvia aPrime 4A mixed-mode resin. Process design considerations included buffer composition (pH and salt concentration), which influences drug product purity, as well as residence time and feedstream load level, which relate to drug product yield and process productivity. DoE results based on those parameters showed that at pH 6.0–8.0 and 0–150 mM of salt, binding capacity would be <25 mg/mL. Thus, clearance would be best for the drug product in flow-through mode.

Rushton pointed out that MVM capsid proteins have a pI between 6.1 and 6.2. Setting buffer pH

above that results in net negative charge. Under such conditions, Nuvia aPrime 4A resin should retain MVM most effectively at high pH and salt levels (Figure 2). Results from DoE screening using spin columns supported that hypothesis.

The latter experiments used larger-volume Bio-Rad Foresight columns (0.8 cm inner diameter × 10 cm, 5 mL), a five-minute residence time, and drug-product load levels of 50–55 mg/mL of resin. Nuvia aPrime 4A resin achieved a MVM LRV of 2.23 in a buffer with 10 mM NaPi at pH 6.0. Increasing buffer pH and adding salt improved that value. Buffers of 10 mM NaPi and 150 mM NaCl (pH 8.0) generated >4.21 LRV; boosting salt to 200 mM resulted in >4.53 clearance. Excellent XMuLV clearance can be achieved under the same chromatography condition. A log reduction of >5.02 occurred when flow-through purification was operated with 10 mM NaPi and 150 mM NaCl (pH 8.0).

A Bio-Rad team also assessed clearance in bind–elute mode using Nuvia HR-S high-resolution cation-exchange resin and CHT XT calcium-affinity cation-exchange media. For both, the team spiked MAb samples (pI 9.0) with XMuLV or MVM, ran the columns, and measured viral titers in the product fractions.

The Nuvia HR-S resin study was run at typical conditions for a cation exchanger, with a buffer of 20 mM and 25 mM NaCl (pH 5.0). Product was eluted using a 0–100% B gradient over 10 column volumes (CV) with 20 mM NaOAc and 500 mM NaCl (pH 5.0). Flow remained at 300 cm/h throughout the process, and the end of the process featured a high salt hold to ensure full product recovery. For the CHT XT media study, researchers followed a similar process, keeping flow at 300 cm/h and instituting a salt hold toward the end of the process. But the team applied a buffer with 5 mM NaPi and 20 mM NaCl (pH 6.5) and a 0–100% B gradient over 10 CV to 5 mM NaPi and 1 M NaCl (pH 6.5).

Both media provided effective clearance of XMuLV. MVM proved to be more challenging, however. Product fractions from MVM-spiked columns exhibited limited virus reduction (<1 LRV). Rushton explained that — considered alongside the Nuvia aPrime 4A resin experiments — these results suggest that flow-through operations could offer more robust viral clearance than bind–elute steps for this MAb of pI 9.0.

Being Prepared: Those case studies also emphasize the importance of downstream preparedness. Rushton encouraged attendees to seek out chromatography resources from contract research, development, and manufacturing partners. By assessing viral clearance early in PD and leveraging expertise, chromatography specialists can adapt easily to process changes that might occur late in a drug's product lifecycle.

Questions and Answers, with William H. Rushton

How applicable are design of experiments (DoE) results to later viral clearance studies? DoE results tend to correspond well with findings from scale-up runs performed with prepacked columns. Therein lies an advantage. DoE studies use small sample amounts, so users can afford to adjust their processes until they are confident that they can achieve LRV targets in packed column runs.

When is it best to perform DoE clearance studies? Performing such studies with multiple samples comes at a high cost. DoE studies are most productive when researchers are applying new processes: for example, when a company has no historical data to anticipate how a resin will perform.

Do DoE studies require fresh media? Reductions in viral clearance can occur as resins age, so fresh media are recommended, even for preliminary testing.

An Efficient Purification Process for a Recombinant *E. coli*–Expressed Protein

Andrew Lees (owner and scientific director of Fina Biosolutions) delivered a webinar in August 2020 describing his company's application of Bio-Rad chromatography supports to purify a recombinant protein with complex requirements (**3**). FinaBio is a research and development company specializing in low-cost conjugate vaccines. Such vaccines leverage the immunogenicity of carrier proteins to induce immune responses to pathogens with poorly immunogenic antigens. Because conjugate vaccines require more chemical assembly than other modalities, they can be more difficult and costly to manufacture. Moreover, few carrier proteins are approved for clinical use: CRM197, a genetically detoxified diphtheria toxin, until recently had been too expensive to use in low-cost vaccines. As Lees explained, much of his company's expression and purification efforts are geared toward reducing costs for the protein component of conjugate vaccines.

Containing Costs: FinaBio has developed two novel technologies. The first is a proprietary strain of *Escherichia coli* that can express CRM197 as a soluble, properly folded, intracellular protein. This strain yields ~2.5 g of CRM197 per liter, which markedly reduces cost of protein production.

The second technology is a carrier protein based on a tetanus-toxin heavy-chain (TTHC) fragment, which manifests as a 50-kDa doublet on sodium dodecyl sulfate–polyacrylamide gel electrophoresis

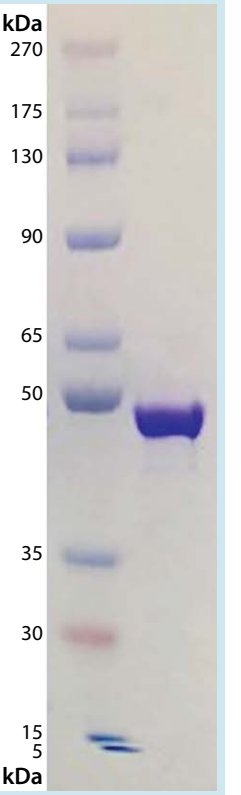
Questions and Answers, with Andrew Lees

How do you approach purification to ensure cost-effective vaccine production? We keep purification simple, reducing steps and minimizing processing. For instance, we load anion-exchange eluant directly onto hydrophobic-interaction columns, which eliminates need for tangential-flow filtration. We also maximize loading capacity and flow rate. Nuvia resins help us achieve those goals.

Why does Macro-Prep High Q resin provide lower dynamic binding capacity than Nuvia Q resin despite the former's higher ionic capacity? Nuvia Q resin features optimized pore sizes that increase usable surface area for binding.

What makes CRM197 so expensive to produce? CRM197 often is expressed in *Corynebacterium diphtheriae*. Because that microbe does not culture easily, CRM197 yields had averaged ~100 mg/L. Some companies have developed high-productivity strains, but such systems remain proprietary and expensive. However, FinaBio follows a different business model. Our strain of *Escherichia coli* can yield 2 g of CRM197 per liter, and we minimize the protein's price to enable low-cost production of conjugate vaccines in low-income countries.

Figure 4: SDS-PAGE rendering of purified TTHC, 4–20%



(SDS-PAGE) (Figure 4). This protein is slightly acidic and expressed within cells. FinaBio uses an *E. coli* lysate feedstock during fermentation, and yields average 1 g/L of purified protein. Lees explained that his company's goal is to produce TTHC at >95% purity with low levels of endotoxins and host cell protein and DNA. The company also aimed to achieve multigram yields.

Optimizing Capture: Early screening determined that anion-exchange and hydrophobic-interaction chromatography were best for TTHC purification. FinaBio evaluated three strong anion exchangers for binding and selectivity: Macro-Prep High Q resin, Nuvia Q resin, and Nuvia HP-Q resin. The Macro-Prep High Q resin features a methacrylate base and 50-µm beads that exert a high charge density. The other two media are derived from acrylamide and vinylic monomers. Nuvia HP-Q resin contains 50-µm beads with low charge densities, which facilitates binding of large molecules that can bridge ligands (e.g., IgM); Nuvia Q resin features 85-µm beads with surface extenders that are advantageous for capture steps.

FinaBio scientists ran each resin in 1-mL Bio-Rad Foresight columns at a 20-CV gradient to 1 M NaCl. All three media showed good binding, but the Nuvia Q and Nuvia HP-Q resins performed best. The former retained ~10 mg of TTHC per mL of media — nearly twice the capacity of the Nuvia HP-Q resin. Lees observed that such a result was promising considering the number of competing substances present in the unfiltered *E. coli* lysate broth loaded onto the columns. After applying a shorter, shallower gradient, converting that to a step elution of 0.15 and 0.4 M NaCl, and scaling up to 5-mL columns, anion-exchange eluant was at >90% purity.

Honing Purification: To purify the eluted TTHC, researchers evaluated an orthogonal binding step using Macro-Prep t-Buytl resin and Macro-Prep Methyl resin, both of which feature methacrylate bases and 50-µm beads. Columns with Macro-Prep Methyl media showed greater TTHC binding capacity, perhaps because the carboxyl groups in the Macro-Prep t-Butyl resin give it a negative charge, hindering binding with proteins that are negatively charged and slightly acidic.

After adjusting $(NH_4)_2SO_4$ levels, anion-exchange eluant was loaded directly onto the Macro-Prep Methyl resin. TTHC bound relatively weakly. When trying to improve binding, FinaBio process engineers observed that target proteins began to precipitate at 1.5 M $(NH_4)_2SO_4$ — but that purity otherwise was high. Thus, a step gradient was introduced instead, enabling early TTHC elution and increased purity.

Next Steps and New Applications: The purification process that FinaBio devised — from harvest to centrifugation, to anion-exchange capture with Nuvia Q resin and hydrophobic-interaction purification with Macro-Prep Methyl resin — yielded product at >99% purity, with low endotoxin and host cell protein levels (Figure 5). Lees's company now intends to scale up TTHC production by increasing column volumes and optimizing binding during HIC.

FinaBio already is putting its purification process to the test, collaborating with researchers at Texas

Figure 5: Size-exclusion–high-performance liquid chromatography (SEC-HPLC) of purified tetanus-toxoid heavy chain (TTHC)

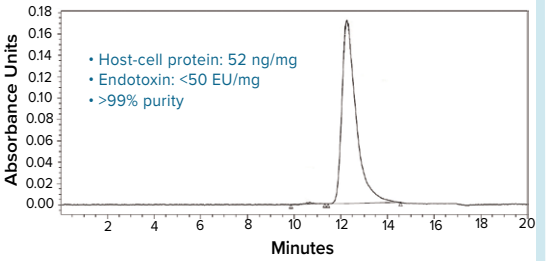
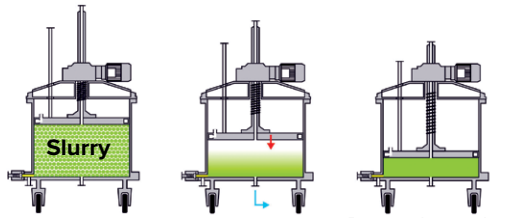


Figure 6: Example of slurry transfer and packing for incompressible resins



Slurry is transferred to a column by a pump or suction

Piston lowering is initiated. Speed should be greater than the media's gravity settling rate.

Piston is lowered to the bed height with a headspace of 1–5 mm. Precise consolidation is attained using a motorized piston.

A&M University in College Station, TX, to develop a low-cost vaccine for cattle tick fever disease. Lees expects that the purification process will enable kilogram-scale production of TTHC protein.

To Compress or Not to Compress: Packing Resins at Process Scale

Mark A. Snyder (manager of the Bio-Rad Process Applications R&D Group) explored practical considerations for packing resins at process scale during his October 2020 Ask webinar (**4**). By calling out differences between packing methods for compressible and incompressible resins, Snyder enumerated best practices for resin packing and explained how to evaluate columns to ensure their suitability for a process. He framed his remarks using CHT Ceramic Hydroxyapatite media, a incompressible support.

How Much, How Quickly: Tap-settled density, or the volume that a gram of resin will occupy in suspension, determines how much of an incompressible resin to load onto a column. Such resins also perform best when packed rapidly. "Bed consolidation equals bed packing," Snyder quipped. "As soon as incompressible resin particles fall to the bottom of the bed, you are done. Such resins settle rapidly, so you must work fairly quickly."

What amount of compressible resin to load depends on bed volume and how much compression must be applied once the particles have settled. However, most compressible resins do not settle rapidly.

Slurry Preparation and Transfer: A resin packing workflow comprises slurry preparation and transfer, packing, and column qualification. Most compressible resins come in premade slurries. Incompressible resins typically are formulated as dry powders that require rehydration. They require slurry tanks with low-shear impellers.

Regardless of resin type, columns must be level, and syringe transfers must be performed without introducing air. But pump style differs by resin type. Incompressible resins require diaphragm pumps to prevent particle damage, whereas compressible resins can handle peristaltic and rotary lobe pumps. For perspective on precolumn preparation, Snyder noted that column frits for CHT media (40-µm particle size) should be ≤10 µm in porosity.

Best Practices for Packing: Snyder emphasized prevention of particle stratification during packing. If packed improperly, small particles will remain at the top of a packed bed while large particles descend, generating poor height equivalent to a theoretical plate (HETP) results. Applying flow rates faster than the fastest-settling particle prevents stratification.

Compressible resins should not be packed with headspace. Thus, they require precise measurement of consolidated bed height to determine how much media must be compressed to reach a desired compression factor. Compression factors for compressible resins are typically 1.1–1.3, although factors as high as 1.5 have been observed in the biopharmaceutical industry. Compressible media usually are conditioned using a combination of up- and down-flow.

Incompressible resins are fragile and must be packed with headspace to prevent top-flow adapters from driving down into the beds. However, precise measurement of packed-bed height is unnecessary for this resin type because there is no need to calculate a compression factor, which always equals 1.0 in such applications. Care should be taken to prevent up-flow during bed conditioning.

Snyder added that 40-µm CHT incompressible media particles settle at 35–125 cm/h, necessitating packing at >150 cm/h. Eighty-micron CHT resins should be packed at 300 cm/h. Snyder recommended leaving 1–5 mm of headspace between a head plate and a settled bed (Figure 6).

Questions and Answers, with Mark A. Snyder

What packing strategies are recommended for scaling up from 50-mL to process-scale columns? Packing strategies are similar across column sizes, but manufacturing-scale hardware can be complex and time-consuming to assemble. Spare parts should be readily available, and teams should be trained thoroughly in how to set up and dissemble column hardware. Large columns also exhibit fewer wall effects than small columns do, so slower flow rates must be set for large columns.

What concerns arise in packing incompressible media into stainless-steel hardware? Stainless-steel columns prevent you from seeing the top of a bed. To negotiate that hurdle, estimate how tall your bed is based on how much media gets packed, let it consolidate, then lower your top flow adapter to ~2 cm above the estimated bed height. If your column does not pass qualification testing, lower the adapter by 0.5 cm and retest.

Incompressible resins can consolidate slightly after packing; for instance, during column transport between rooms or after a few buffer cycles. Snyder said that if bed settling occurs beyond a vendor-recommended headspace, then process operators can lower a flow adapter to the initial headspace. Then they should incorporate that step into their packing procedure to prevent testing discrepancies.

Evaluating Packing Quality: Compressible and incompressible resins share several principles for HETP and peak asymmetry assessment. Test-solute measurements are taken by assessing conductivity (usually with NaCl or NaOH) or absorbance (using acetone, caffeine, or vitamin B12). Injection volumes are 1–2% of total bed volume. Test solutes must not interact with chromatography media, and extra column volume must be removed from test hardware to ensure assay precision.

When using NaCl as a test solute for CHT media, the same level of phosphate should be used in the equilibration and injection buffers; CHT media will produce an artifactual phosphate peak after registering a NaCl peak.

Snyder emphasized that PD experience should guide establishment of HETP and peak asymmetry ranges. Because vendor data describe what ranges are achievable, acceptance criteria should be defined by what performance is required from a process step.

Snyder described ideal, acceptable, and unacceptable HETP and asymmetry ranges using data from a comprehensive evaluation of CHT media packing, which accounted for the influence of parameters such as packing reproducibility, slurry density, settling time, packing flow rate, headspace, differences between slurry-transfer and slurry-in-place operations, and packing method. Data were plotted comparing peak asymmetry factor (A_s) with the number of theoretical plates per meter (N/m).

A_s and N/m values for what usually is deemed effective column packing spanned 0.9–1.5 and 6,000–12,000, respectively. Suboptimal but acceptable packing yielded A_s factors of 1.5–1.8 and N/m values of 4,000–6,000. Snyder noted that Bio-Rad teams obtained such scores by letting packed columns settle overnight, allowing particle stratification to occur. Applying 10 mm of headspace rather than the recommended 1–5 mm generated similar results. Poor packing was simulated by applying 20 mm of headspace, generating A_s factors between 1.8 and 2.3 and N/m values of 2,000.

Ultimately, suboptimal values might be suitable for a process. Snyder concluded, "Look at your PD studies, and from them draw the data that will show your minimum HETP value and the asymmetry range that would be acceptable in your own process."

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1 He X. Ask the Expert: Mixed-Mode Chromatography Resins for Biomolecule Purification. *BioProcess Int.*, 1 April 2020; https://bioprocessintl.com/sponsored-content/mixed-mode-chromatography-resins-for-biomolecule-purification.

2 Rushton WH. Ask the Expert: Assessing Viral Clearance in Early Phase Process Development. *BioProcess Int.*, 13 May 2020; https://bioprocessintl.com/sponsored-content/assessing-viral-clearance-in-early-phase-process-development.

3 Lees A. Ask the Expert: Efficient and Rapid Purification of *E. coli*- Expressed Toxin Recombinant Protein Fragments. *BioProcess Int.*, 31 August 2020; https://bioprocessintl.com/sponsored-content/efficient-and-rapid-purification-of-e-coli-expressed-toxin-recombinant-protein-fragments.

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