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Review

Preparative chromatographic separations in pharmaceutical, diagnostic, and biotechnology industries: current and future trends

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Abstract

Chromatography is widely employed in industry for the purification of biotherapeutics and diagnostics. Regulatory requirements dictate that the chromatographic process be reproducible and provide the necessary product purity. Current trends in designing and implementing purification strategies take into consideration the requirements for both chromatography media and equipment. Future trends will require that cost containment is considered. New chromatography materials, new methods for equipment design analysis, and new technologies to ensure regulatory compliance will provide the biotechnology industry with tools to provide tomorrow's products.

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1. Introduction

Chromatography is the method most widely used to obtain the required purity for today's biotherapeutics and diagnostics. The needs of the industrial chromatographer vary in development and production. Requirements also depend on the nature of the product-diagnostic or thera-

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peutic; synthetic, as in the case of antisense drugs, or derived from fermentation, cell culture, or a natural source such as plasma.

In the first part of this review, current trends in industrial chromatography will be presented. The second part will present some potential future trends. Both equipment and chromatographic media, as well as new technologies that support chromatographic operations will be presented.

2. Current trends

2.1. Chromatography media

The current trends in media are broken down here into discussions of particle size, chromatography techniques, and media requirements.

2.2. Particle size

Today, most industrial chromatographers strive to maximize product purity and yield at a reasonable cost. One common approach to achieving these goals is to use different particle sizes at different stages of the process. These stages are often called 'capture', 'intermediate', and 'polishing'. Typical particle size ranges are shown in Table 1.

2.3. Capture

The capture step reduces the process volume and frequently removes the product from harmful contaminants such as proteases. On/off mechanisms are commonly used to bind and elute the product at a typical purity level of 70% of its final purity. By optimizing selectivity, recoveries in the range of 85 to 90% are common. Since capture steps are often used with starting materials that cause fouling, the media must be chemically stable to withstand rather harsh cleaning and sanitization methods. And they must be sufficiently stable to tolerate highly viscous feedstreams without losing flow capabilities.

Table 1
Particle sizes used at the different stages of the industrial chromatography process

Step	Typical particle sizes (μm)
Capture	100–300
Intermediate	30–100
Polishing	15–100

Capture steps used today employ large beads, sometimes called Big Beads and expanded bed adsorbents. Expanded bed adsorbents allow processing of feedstreams containing cells and their debris. The use of expanded bed adsorbents can reduce the number of steps by avoiding upstream filtration or centrifugation in addition to the first chromatographic step. This can provide a significant decrease in processing time and increase in product yields [1]. Chase has discussed existing operating formats for protein recovery by adsorption [2]. The advantages and disadvantages of packed columns, batch mode, fluidized beds, and expanded beds were compared. In expanded beds, grading of particle sizes and densities reduces mixing and stabilizes the bed.

2.4. Intermediate

Usually, one or two intermediate steps are employed to remove key contaminants, e.g., host cell proteins and endotoxins. To enhance resolution, slower binding flow-rates and sometimes gradient elution may be employed. After the intermediate steps, the product is usually at 99% purity. A range of particle sizes and functional groups are employed in intermediate steps. The number of optimal intermediate steps and their sequence are determined by the nature of the product and its intended use. Purification schemes for human leukocyte interferon [3] and tissue plasminogen activator [4] illustrate this point.

2.5. Polishing

Polishing steps are used to remove closely related contaminants from the product, e.g., a deamidated or aggregated form. This final step,

while not always required, provides the high degree of purity necessary for many therapeutic products. Smaller particles generating higher backpressures are often used. This final chromatographic step can also be used to transfer product into formulation solutions. Size exclusion chromatography is particularly useful for removing aggregates and changing solutions in one step [5,6].

Fig. 1 shows an application that illustrates the use of the capture, intermediate, polishing approach commonly used in industry today [7]. The product, annexin V, is a 34 kilodalton anticoagulant protein. It was produced by recombinant DNA technology in *E. coli* as an intracellular, soluble product. After high pressure homogenization, the unclarified feedstream was applied to the anion-exchange expanded bed adsorbent (2.8% solids). The product yield from this step was 95% measured by an anticoagulant assay and analytical reversed-phase chromatography. For the intermediate step (hydrophobic interaction chromatography), multifactorial analysis was used to optimize sample loading, loading flow-rate, elution flow-rate, and gradient volume. After the addition of ammonium sulfate to facilitate binding, the product from the anion-exchange capture step was applied directly onto the hydrophobic interaction step. Tangential flow filtration was used to reduce the volume and the final polishing step employed gel filtration. The product was greater than 99% pure measured by analytical reversed-phase chromatography.

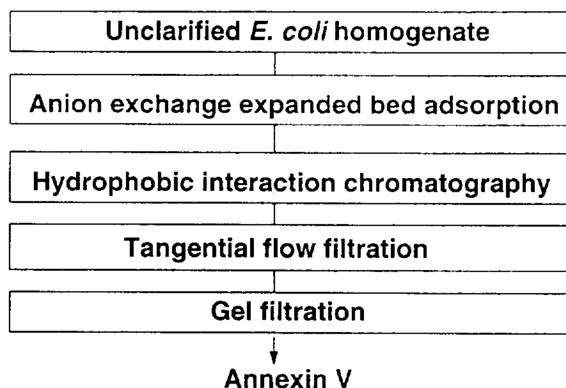

Fig. 1. The capture, intermediate, and polishing approach commonly used in industry today.

2.6. Techniques

A variety of selectivities are required to obtain the requisite product purity. Affinity chromatography is by far the most selective technique. The use of gene fusions to facilitate affinity purification has become more common and has been addressed by Stahl et al. [8]. Depending on the ligand, however, affinity may also be the most expensive chromatographic technique. The ability to replace multiple steps with one step can warrant this expense. Affinity chromatography is often a 'red flag' for regulatory agencies. If proteinaceous ligands are used, their leakage during product elution can pose a significant patient risk or interfere with a diagnostic assay. Such ligands may be toxic or immunogenic, and they may bind to the product or co-purify with it. For ligands such as monoclonal antibodies produced in cell culture, the risk of virus contamination and other impurities must be addressed, significantly adding to validation and purification costs for the ligand [9]. Sanitization and lifetime of the affinity media must also be considered. Non-protein ligands, such as chelating agents (immobilized metal affinity chromatography, IMAC), may provide good solutions for some separation problems [10]. The decision to use affinity chromatography in production is an economic one. Its use may allow a company to get to market faster than a competitor and achieve significant financial rewards that greatly surpass the cost of using this technique.

Ion-exchange chromatography remains the most widely used chromatographic technique [11]. Its broad specificity allows the removal of significant impurities such as deamidated forms, endotoxins, and unwanted glycoforms. Most of today's ion exchangers can be sanitized in place and used for hundreds of cycles [12].

Hydrophobic interaction chromatography is becoming more widely used as the industry has become more familiar with this technique (see Ref. [10]). Reversed-phase chromatography is widely used for smaller molecules, and may be

the only technique to resolve unwanted products, such as an oligonucleotide antisense molecule minus one nucleotide. For traditional pharmaceutical companies experienced in handling large quantities of organic solvents, this does not pose a problem. But for some start-up biotech companies, solvent handling, disposal or recycling, and environmental issues make this a problematic technique.

Reversed-phase chromatography and gel filtration are techniques used as polishing steps. Because of its capacity constraints, gel filtration (except in the desalting mode) is usually left for the end of a purification scheme when the volume is reduced [13].

2.7. Media requirements

Chromatography media used in industry today must be produced consistently. Lot-to-lot variability is not acceptable to the user who must produce a therapeutic or diagnostic that has to meet rigorous pre-determined specifications. Chromatography media must be reproducible in particle size range, porosity, degree of substitution, quality of functional group, and base matrix purity. In addition, these media must be available in large quantity for those products, e.g., insulin, that are supplied in large doses to a large patient population. Today's chromatography media must also allow for high throughput and tolerate stringent cleaning and sanitization routines.

The most commonly employed chromatography matrices in approved processes for biotherapeutics are agarose based. These materials have been available for a long time, and this is a conservative, regulated industry. Innovations are not readily accepted; safety comes first. Agarose-based materials have been proven to provide safe and efficacious products. Leachables occur at very low levels only under the most extreme conditions and are generally non-toxic. The highly cross-linked variants provide good chemical resistance and production flow-rates.

Synthetic polymer matrices are also being used in industrial processes. They offer the advantage of superior rigidity, but the toxicity of the leachables must always be addressed. (See Section 3).

2.8. Chromatography equipment

It is important to distinguish the needs of the industrial process development scientist from those of production engineers. Process developers need flexibility. Equipment must be used for multiple functions, and it should be automated and capable of a high level of data handling. The production engineer needs, above all, reliability. Equipment is generally dedicated, but may be campaigned. The equipment must be compatible with hygienic routines and stringent cleaning regimens. Automation should add to the reproducibility of chromatographic processes and provide data handling that securely documents all chromatographic run parameters and results.

Today, most industrial process development chromatographers use glass and plastic chromatography columns and plastic tubing. The on-line and off-line monitoring devices and fittings are often designed for laboratory use and are generally not optimal for meeting the needs for sanitary design. In production, stainless steel is more commonly employed. Downstream processing equipment has been described by Brocklebank [14] and Johansson et al. [15]. The industry has come a long way since the beginning of the 1980s, when the only sanitary components available came from the dairy industry and were generally too large to maintain the resolution achieved by the chromatographic media.

3. Future trends

What will the future bring? The biotech industry will probably need to produce products of higher purity as a result of the development of more sophisticated analytical methods, able to detect higher and higher levels of impurities. This is really a "Catch-22" situation. More companies will be producing carbohydrates and nucleic acid products, such as oligonucleotides for antisense therapeutics and gene therapy. Peptide mimetics that are not susceptible to

proteolytic breakdown and active sites of antibodies and receptors may require the industrial chromatographer to break out of the current ways of approaching purification. We believe, however, that whole proteins will still be required for many therapeutics and diagnostics, since carbohydrate moieties and tertiary structures appear to play a significant physiological role in a large number of applications.

3.1. Chromatography media

In all probability, tomorrow's chromatographer will be faced with a much greater choice in base matrices, many of which will be synthetic polymers which offer the advantages of superior rigidity, faster flow-rates, greater resistance to pH extremes, and more tolerance to in-place cleaning methods.

Still to be worked out are the disadvantages of these polymers. They are generally more hydrophobic which sometimes causes non-specific binding. While they are more rigid, they are also more fragile and may, for example, shear during mixing. The greatest issue for therapeutics production remains the leachables which are potentially toxic.

Future trends in chromatography media include the employment of ligands derived from peptide and phage libraries and oligonucleotides, as well as non-toxic dyes already on the market but in limited use. These ligands will require preactivated chromatography media which are easy to use. While some industrial users will rely on vendors to perform ligand coupling, we believe that if the coupling technology is simple and readily available, most users will prefer to do their own coupling. The coupling chemistry should be stable to cleaning reagents and, of course, non-toxic.

3.2. Chromatography equipment

Tomorrow's chromatography equipment will be made out of inert, cost-effective materials. An important need today is a listing of available materials that are compatible with biological feedstreams and cleaning agents. The medical devices industry has a wealth of confidential information from implantable devices, which is very difficult, if not impossible, to obtain. Performing studies for Class VI implantable devices is an extremely expensive undertaking for a chromatography systems' manufacturer or the end user. And the data from these studies does not include the effect of harsh cleaning agents and storage conditions on the equipment surfaces.

More sophisticated techniques may be employed for assisting in equipment design. NMR can now be used to evaluate column performance for glass and plastic columns. This technique is currently being used to evaluate column distribution systems.

There is a definite trend toward automation of chromatography systems. These systems can provide reproducible chromatography and minimize the risk of operator contact with the product. Data handling and especially data storage will be easier with these systems. Regulatory issues for automated systems such as ensuring system security and software validation will continue to be issues for some time to come.

Barrier isolator systems with their own controlled environment may provide a solution that ensures manufacturing of safer products at a lower cost than conventional clean rooms [16]. These systems are particularly promising for small start-up companies with limited facility space and for multi-use facilities. Perhaps, in the future, we will even see robots operating industrial chromatography systems.

4. Conclusions

There is ample room for further development of novel chromatographic media and equipment. But for today, and probably tomorrow, as stated by Pfund [17], "there are no miracle media".

The industrial chromatographer will also have to face the issue of cost containment. While one group of process economists has determined that the cost of chromatography media is only 0.1% of production costs [18], this is not widely accepted. In a recent article [19], one estimate

was that purification, including ultrafiltration, represents 60% of the operating costs of the entire process.

The head of a purification process may only look at downstream processing costs and see chromatography without the whole picture, which includes: raw materials, cell culture, isolation steps, personnel, air and water systems, quality control, documentation, and validation costs. Perhaps it is up to this group to ensure that we develop some good process economic analyses for chromatography operations.

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