Lecture-4

ALTERNATIVES TO PACKED BED CHROMATOGRAPHY

Packed bed chromatography has been, and continues to be, the most widely used mode of chromatography used on both a large and small scale, industrially and academically. Despite this approach being on the whole **of high cost and low throughput**, it has predominated, largely due to **the very high resolution** that can be achieved and **a lack of alternatives that can deliver similar resolution**. However, the **high cost of this approach** coupled with **increased product yields/concentrations** in feedstocks and the **practicalities and limitations** that these bring mean that there is now a real need to investigate and develop alternatives to this traditional approach.

Such alternatives include, but are not limited to, **membrane chromatography**, **expanded bed adsorption**, **aqueous two-phase extraction** and **protein crystallization**.

1– Expanded Bed Adsorption

Expanded bed adsorption combines the essential product capture step with the separation of solids and liquids in one step, thus reducing the number of steps in a processThis approach can result in increased yield and reduced overall process time and cost.

The first stabilized **expanded bed adsorption column** was **developed in the early 1990s**, and this approach has now been used in the processing of whole cell mammalian cell culture broth and has found successful application in affinity chromatography. This approach has **also been used for the purification of monoclonal antibodies**, providing **higher recovery at lower cost** and **shorter processing time compared with packed bed chromatography**. Expanded bed adsorption has also been used as an **alternative process** for the **purification of an insulin precursor, MI3**. Despite the apparent advantages, expanded bed adsorption currently remains an alternative technique and has not yet proven to be a threat to the dominance of packed bed chromatography in the biopharmaceutical industry.

2– Aqueous Two-phase Extraction

The process of aqueous two-phase extraction has recently attracted the attention of investigators wishing to develop alternatives to traditional packed bed chromatographic procedures. This has been particularly evident in the **purification of plant-manufactured biopharmaceuticals**. Aqueous two-phase systems **integrate the steps of cell disruption with product recovery** using either **polymer-based systems**, **hydrophobic** and **affinity precipitation** or the use of **surfactants**. The theory is that the protein of interest and cellular debris and other material will exhibit differential solubility between two aqueous phases. The two phases are usually created by mixing solutions of polyethylene glycol with either dextran or salts to form two immiscible layers or phases. Despite the fact that aqueous two-phase extraction can be applied to very crude mixtures while providing high capacity and easy scale-up, its use to date has been limited to selected industrial applications. This is largely due to the high cost of the polymers used and a lack of fully developed design approaches. Novel polymers are currently being developed that may overcome such limitations, especially the development of low-cost starch- and cellulose-based polymers for some of the more attractive uses of aqueous two-phase systems such as affinity precipitation.

3– Membrane Chromatography and Filtration

A number of recent reviews on the use of membrane chromatography have suggested that theoretically this approach could have a number of advantages over traditional packed bed chromatography. Membrane chromatography can exhibit dynamic binding capacities of up to 10 times higher and flow rates of up to 100 times faster than packed bed chromatography, especially for selected applications such as the purification of large particles. Membrane chromatography is also significantly cheaper per cycle if hidden costs, including validation, cleaning and the manpower involved in packed bed chromatography, are taken into account. Despite these perceived advantages, membrane chromatography has not been widely adopted by the bioprocessing industry, mainly because it is limited by the throughput as a result of the available surface area per unit compared with packed bed chromatography.

Membrane filtration is also used as an alternative method of clarifying cell culture media and cell extracts and techniques such as ultrafiltration and high-performance tangential flow filtration (HPTFF) have been investigated as integral steps in downstream processing schemes. Indeed, there have been reports where HPTFF has been successfully used as a polishing step. Perhaps one of the more significant advances in this area has been the use of pH and charged membranes to manipulate the product. These approaches have been shown to be useful for the removal of viruses from mammalian cell systems and endotoxins from bacterial systems. Despite such advances, these approaches have not been fully adopted and membrane fouling remains a problem during such processing, and these approaches are usually used prior to chromatographic steps.

4 Crystallization

Crystallization as a purification technique has long been used by investigators and industrialists in the small-molecule field, but has largely been ignored until recently in the biologics field. With increasing product yields and concentrations, this approach is now attracting much interest as an alternative to some chromatographic steps, thereby reducing the number of these required. Crystallization of proteins has long been used in structural analysis studies where screens have been developed to find conditions that allow the successful crystallization of a target protein. Now the potential of this approach in downstream processing is being seriously considered and has been used during the purification of glucose isomerase and insulin. A number of investigators have now reviewed the principles of protein crystallization and the technical aspects of process development for its large-scale use. Despite the promise of this approach, there are still scale-up issues involved in further developing this technique and a lack of process development in the area. The development of novel microanalysis methods to determine large-scale conditions that will drive crystallization of the target protein of interest at high yield is currently under way and may help overcome such limitations. Hence there is the potential to develop low-cost methods of purification that utilize

crystallization for many biologics, although currently this potential is largely unrealized.

5– Monolith Columns

Monoliths are continuous stationary phases available in a variety of shapes and sizes and have shown potential to be developed with chromatographic ligands. Monoliths are polymerized directly into a single unit although, for biotechnology purposes, many regular monolith tubes are prepared and interlaced into a single column with a limited pore size of around 5 mm. Monoliths show great potential for providing high mass transfer through convective flow, thus enabling both capacity and resolution to be independent of flow rate. A further perceived advantage over conventional chromatographic beds is that they do not require packing and therefore reduce validation and labour costs. However, despite the fact that monoliths are being developed for affinity and ionexchange chromatography, they have as yet not been able to make significant inroads into industrial-scale protein purification processing, largely due to technical problems involved in developing large-scale monolith columns. One use of monoliths that has been commercially validated is for the purification of pharmaceutical-grade plasmid DNA. Success has been more readily achieved in this case as there are clear capacity advantages over conventional chromatographic resins which have porous internal structures designed for smaller protein-sized molecules. This capacity advantage may also translate to purifications of other very large molecular structures such as virus-like particles and viruses.