Facing the challenges in bio-pharmaceutical production: developments in ion exchange media to bring down cost of goods.

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As the bio-pharmaceutical industry matures, terms like "cost of goods" are becoming more and more important.

Up to now, strain optimisation for high productivity and upstream purification were the bottleneck for most bio-processes. However, with the progress made in recent years, titers in fermentation processes have increased significantly. Obviously, this increased volumetric productivity will help reducing the cost of goods, but it also has an impact on the downstream processing. Therefore, improved downstream processing media are required to process the increased product load in the same timeframe. Recently, new materials, based on fully synthetic polymer based matrices became available and show important advantages over traditional polysaccharide-derived media. In the following article the focus is on ion exchange chromatography (IEX) as an important step in the biopharmaceutical process.

Basics of Ion Exchange Chromatography

IEX has been used for many years for analysis and purification of bio-molecules[1]. Its simple concept of charge induced reversible binding has several important advantages, two of which are: binding is fast and media show a high capacity. Also, compared with other chromatographic methods, such as hydrophobic interaction chromatography (HIC) or mixed mode resins, method development is straightforward. The binding/elution behaviour can be described by a simple "on/off" mechanism. The molecules will bind to the chromatographic support at low ionic strength at a pH below (for cation exchange) or above (for anion exchange) its isoelectric point. Release will take place at increased ionic strength or by pH shift.

In both cases, there is a distinct and narrow zone of pH / salt concentration, which determines whether there is binding or not. This also means that isocratic elution is not possible with IEX. Simple salt (step) gradients are most commonly used for elution. Stationary phases are generally resistant to a wide range of pH. All these characteristics make the technique ideal for the two main process steps of capture and (intermediate) purification. In addition final polishing steps can also be performed with IEX. The differences between these three steps are summarised in table 1.

Process step	Important material characteristics	Typical Application
Capture	- Particle sizes between 45-200 µm, sometimes higher - High dynamic binding capacity at high flow rates (up to 1000 cm/h and more) - Good flow characteristics.	- Harvest of fermentation supernatants (capturing)
(Intermediate) Purification	- Particle size ca. 30-75 µm - Low non-specific binding - Narrow particle size distribution	- Purification of material up to 90+% purity, - Reduction of endotoxins
Polishing	Particle size between <10 – 30 µm	Purification of up to 99+%

Table 1 Media Characteristics for Typical Steps in Bioprocessing

In a capture step the target compound is extracted and concentrated from the (homogenised) fermentation broth where it is present in low concentrations. The main aim in this step is to concentrate the target compound, achieve complete recovery of the target compound and the removal of bulk impurities (including protease etc.). In this step, high purity of the resulting concentrates is an advantage, but is not essential. During (intermediate) purification the separation of the target compound from the main impurities is a key factor and purity aspects become more important.

Even though IEX is a comparatively simple method, there are still several parameters to keep in mind when developing a cost effective large-scale production process. In the following section, some key factors having an impact on the efficiency of an IEX-process step are discussed.

Capture step:

In a capture step, the target compound is extracted and concentrated from the (homogenised) fermentation broth, where it is present in comparatively low concentrations in the range from 1-10 g/L

fermentation broth for e.g. monoclonal antibodies and up to more than 20 g/L for e.g. interferon. Together with a range of other methods (e.g. precipitation, ultrafiltration, protein A affinity chromatography), IEX is frequently used for this task, where up to several thousand litres of fermentation broth are being processed. The advantage of using IEX over less discriminating methods, such as ultrafiltration, is that the amount of fermentation impurities (e.g. host cell proteins, unwanted proteins, proteases) present in the captured material can often be reduced significantly

Due to the fast adsorption/desorption mechanism it is possible to process the large volumes of fermentation broth in an acceptable amount of time. Traditionally cross-linked dextran and agarose gels (originally developed by scientists at Pharmacia[2]) have been used. New fully synthetic polymer-based materials, including YMC's BioPro IEX bulk materials, have favourable properties compared to the traditional media. The newer materials feature higher binding capacities, better pressure stability, lower non-specific binding and higher reproducibility due to their fully synthetic origin.

Because of the large volumes involved in a capture step, the flow rates should be as high as possible in order to get to acceptable process times. As a result, the most important factors impacting on the efficiency of a capturing step are the dynamic binding capacity (DBC) and the maximum flow rate achievable. The latter point is controlled by the fluid dynamic properties of the chromatographic column; DBC will be discussed in the next section.

As for every chromatographic application, particle size and its distribution, together with the mechanical stability of the chromatographic medium, limit the maximum flow. If the mechanical stability is inadequate, the stationary phase will collapse under the backpressure resulting from high flow rates. Modern fully synthetic polymer based materials, are generally sufficiently pressure stable. Obviously, the backpressure generated is influenced by the particle size and distribution. For new synthetic polymer based materials both parameters can be controlled more easily. However, the most efficient particle size for high flow rates will always be a trade off between binding capacity and backpressure. In principle, the binding capacity increases with decreasing particle size. At the same time backpressure increases. There are big differences between different materials with regards to flow properties and DBC at high flowrates.

Dynamic binding capacity (DBC) and recovery:

The DBC is the capacity to bind the target molecule while the mobile phase is continually flowing through the IEX-column. It is expressed in mg target molecule bound per ml of resin in the column (mg target/ml resin) and depends on the flow rate which is being applied. It is determined at 10% breakthrough and is obviously different for every molecule and resin. For Sepharose FF, a widely used IEX material, a value of 120 mg BSA per ml resin has been reported [3]. For modern media DBC can be significantly higher. For example, YMC BioPro IEX material has a production specification to achieve 150% of this value and more (i.e. more than 180 mg BSA/mg resin[4]).

Theory predicts that increasing the flow rate will have a negative effect on the DBC. Even though the binding process is very fast, at high flows the molecules have less time to diffuse into the porous structure of the stationary phase and to bind the IEX ligands. Because of this and because of the mechanical problems outlined above, flow rates have been fairly limited in the past. However, new synthetic polymeric materials offer high binding capacity even at high flow rates. At the same time, the DBC at these higher flow rates is also higher than with traditional media (at lower flow rates). Increased mechanical stability and improved concepts of binding the ion exchanger functionalities to the support have made this possible. In Figure 1 the dependency of DBC to linear flow rate is shown for a YMC BioPro cation exchange material, which was tested up to 1000 cm/h without decrease of DBC.

In Figure 1, lysozyme was used as a model/test protein and the DBC was determined at 10% breakthrough. In this experiment, the dynamic binding capacity for the cation exchanger was in the region of 220 mg lysozyme per mg resin material and varied only slightly with increasing flow rates of up to 1000 cm/hr.

For anion exchange materials, several different materials were tested under the same conditions with bovine serum albumin (BSA) as the test substance. It is obvious from Table 2 that there are distinct differences in performance between the different phases. Both the DBC and recovery of the target molecule varies widely for the various media. For BSA, YMC BioPro QA (anion exchanger) shows the highest DBC of 187 mg/ml resin, which is more than 25% higher than some of the high performance materials from competitors.

A similar experiment was performed, using cation exchange materials and lysozyme as the test substance, see Table 3. Again there are distinct differences in performance between the different phases in terms of both the DBC and recovery. For lysozyme, YMC BioPro S (cation exchanger) shows the highest DBC of 186 mg/ml resin of the resins tested.

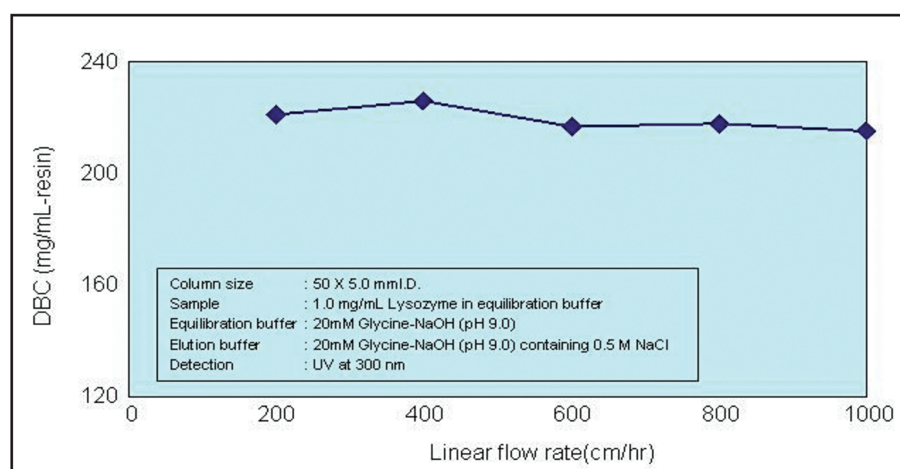

Figure 1 Dynamic binding capacity for lysozyme measured at different flow rates up to 1000 cm/hr using YMC-BioPro S75

	BioPro QA (75um) (YMC)	Gigacap Q-650M (Tosoh)	Super Q 650-M (Tosoh)	Capto Q GE
DBC [mg BSA/mL gel] (10%)	187	147	149	102
recovery (%)	100	93	32	127

Table 2 DBC of various anion IEX resins, at a linear flow rate of 180 cm/h and protein concentration of 1.5 mg/ml BSA

	BioPro S (75 µm) (YMC)	Gigacap S-650M (Tosoh)	Macrocap SP GE
DBC [mg lysozyme /mL gel] （10%）	186	182	81
recovery （%）	109	108	108

Table 3 DBC of various cation IEX resins, at a linear flow rate of 180 cm/h and protein concentration of 1.5 mg/ml lysozyme

The figures in Tables 2 and 3 were obtained using 50 x 4.6 mm ID columns filled with the corresponding bulk material.

It is obvious that a high DBC is only useful if the recovery is high at the same time. As the table shows, modern materials generally show both. Non-specific binding which causes losses in recovery has been reduced with newer media. For YMC BioPro non-specific binding reaches a very low level which warrants the high recoveries close to 100%.

Selectivity:

In chromatography, separation is dictated by the selectivity of the stationary phases for the given molecules. Because of the electrostatic nature of the separation principle and the resulting "on/off" mechanism, the selectivity is very similar for media from different manufacturers. Generally speaking, under otherwise identical conditions, the elution order is the same, only the peak shape can change. Figure 2 shows a comparison of three cation exchange materials. In the example shown, a mixture of three proteins was separated with a generic salt gradient.

The elution order is the same and also the retention times are similar for all three materials. Only the peak shape varies between the different materials due to differences in particle and pore size. Obviously, decreasing particle size increases the number of theoretical plates and this, in turn, yields sharper peaks. As can be seen from the chromatogram for GE's Macrocap resin (particle size of 50 µm as compared to 75µm for the Tosoh and the YMC materials), particle size has a strong influence on the peak shape, with smaller particles giving sharper peaks. On the other hand, smaller particles will normally generate higher backpressures.

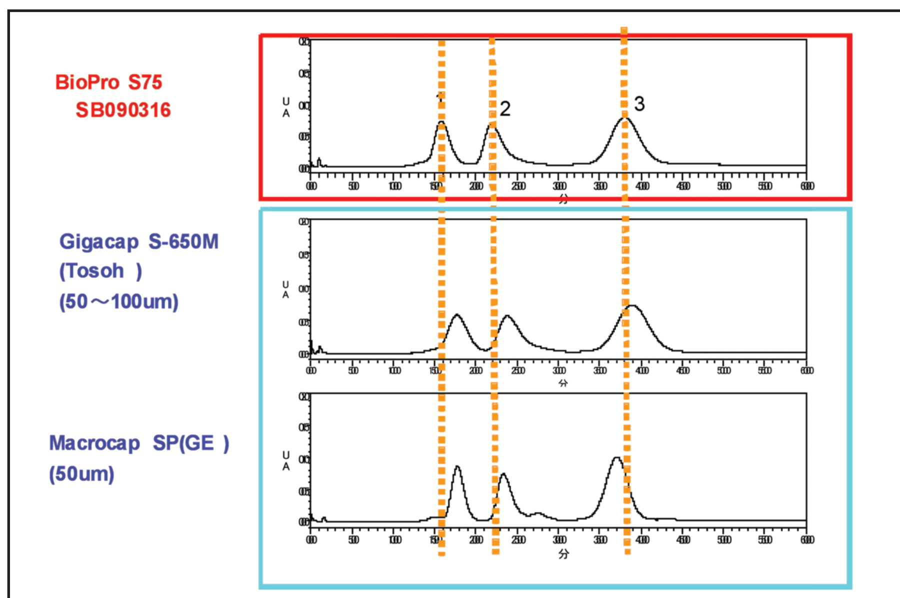

Figure 2 A comparison of three cation exchange materials used to separate a mixture of three proteins using a generic salt gradient. (1) ribonuclease A (MW：13,700) (2) cytochrome C (MW：12,400) (3) lysozyme （MW：14,300)

Summary

Using the optimal IEX material for a specific application can results in a significant decrease in the costs of the process. Due to the charge-based mechanism, selectivity is similar with different media. However, there are big differences in dynamic binding capacity, recovery and backpressure. As shown above, new media exhibit DBCs of 150% and more compared to traditionally used media. Also new synthetic media can be engineered to show less non-specific binding of the target molecule to the media, which increases the recovery. These increases are highly dependent on the molecule used, but can also be in the region of 10 to 50% higher recoveries. By improving particle size distribution and homogeneous pore size distribution, sharper peaks and lower backpressure have been obtained. This not only aids in more selective isolation of the target molecule but, together with high DBC at high flow rates, enables faster processes. As a result columns will be smaller and lower buffer volumes are required which will have a great impact on the overall processing costs.

References

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2 Porath, J., Flodin P. (1959). Gel filtration: A method for desalting and group separation. Nature 183, 1657.

3 Data file: Sepharose Fast Flow ion exchangers, Code number: 18-1020-66 AB, 2003-09, GE Life sciences, Upsala, Sweden.

4 Product information YMC BioPro 75 µm, YMC Japan. Product information (2009) YMC BioPro 75 µm, YMC Japan.