Process development on Freedom EVO® robotic workstations

Finite bath experiments and parallel chromatography

Introduction

The demand for biopharmaceutical products is increasing rapidly. Typically, new biopharmaceuticals have already undergone about 12 years of development before they enter the market, when little time is left to return on the investment before the patent expires. Fast process development and process optimization is crucial to speed up the market entry of new biopharmaceuticals. Upstream process has already been improved in recent years, so new methods to accelerate downstream process development need to be applied.

The purification of biopharmaceuticals is dominated by liquid chromatography in packed bed mode. Until recently, chromatographic process development has been restricted to a sequential time-consuming regime but, in recent years, methods have been developed to perform up to 96 parallel chromatographic experiments on a Tecan Freedom EVO® robotic workstation. This innovative approach has made it possible to design a complete chromatographic process, from initial batch binding and elution studies, up to dynamic binding and elution packed bed studies in an automated, parallelized and miniaturized mode. This saves time, cost and target molecule, which is especially important in preclinical studies.

This article gives an overview of chromatographic process development tools on a Freedom EVO robotic workstation. These tools include finite bath studies, which may include both adsorption and elution processes. A more sophisticated tool is further presented in the use of packed bed breakthrough / elution experiments with miniaturized chromatographic columns (MediaScout® RoboColumns from Atoll GmbH).

Besides this the high throughput process development portfolio which have been integrated on Freedom EVO robotic workstations includes batch binding / elution experiments in microtiter filter plates filled with chromatography resin (PreDictor™ Plates from GE Healthcare)[1], or disposable tips filled with chromatography resins (PhyTip® from PhyNexus).

Finite bath thermodynamic studies on Tecan Freedom EVO workstations

The chromatographic separation is influenced by multiple parameters ranging from a molecular basis such as protein-ligand interactions over protein transport in different adsorbent architectures to process parameters such as flow rates or column dispersion. On a molecular basis, where the distinct properties of the protein in respect to binding and transport is the governing factor, parameters such as liquid phase conditions (pH, IS), protein properties, protein-protein interactions and adsorber design span a multi-parameter space. This space can hardly be explored by simple sequential techniques.

Therefore, a rational approach for optimization of chromatographic parameters must consist of an initial screening procedure for resin capacity, uptake kinetics and elution performance by batch (infinite bath) experiments using robotic workstations. An example of the influence of different ligands and resin structures is given in isothermal equilibrium data in Figure 1. Adsorption isotherms were determined on a Freedom EVO robotic workstation for a monoclonal antibody in 50 mM MES (pH 6) on various CEX resins. The data shows that the dominant factor is not the protein-ligand interaction but the transport properties of the different resin designs. Resin capacity varies for adsorber with same ligands (SP Sepharose™ FF vs. SP Sepharose™ XL and Fractoprep® SO_3 vs. Fractogel® SO_3) and differences in adsorbent architecture, as well as chemical properties of the adsorbent[2].

Breakthrough and elution experiments with miniaturized columns

Large scale chromatographic processes are run in packed bed columns, either in bind and elute or flow through mode. Therefore, it is very important to study the dynamic binding, but especially elution characteristics of the target molecule in comparison to the contaminant molecules under different flow rates, salt type and concentration, pH values, resins etc. Miniaturized batch studies executed in early process development significantly reduce the experimental effort and amount of material consumed. The new opportunity to perform chromatography using any desired resin in correctly packed RoboColumns from Atoll on Tecan Freedom EVO platforms drastically improves the process information which can be gained in miniature scale, hence easing the following up-scaling procedure.

To determine and optimize the amount of target protein bound under dynamic conditions, RoboColumns from Atoll were operated on a Tecan Freedom EVO robotic workstation. In a simple breakthrough experiment (Figure 2), lysozyme solution was introduced into a RoboColumn packed with a strong cation exchange medium (Toyopearl® SP-550C). The ionic strength of the working buffer (20 mM phosphate buffer at pH 7) was varied by adding different amounts of sodium chloride. The resolution of the breakthrough curves obtained was sufficient to derive binding capacities at 10 % as well as at 5 % breakthrough for the different salt concentrations.

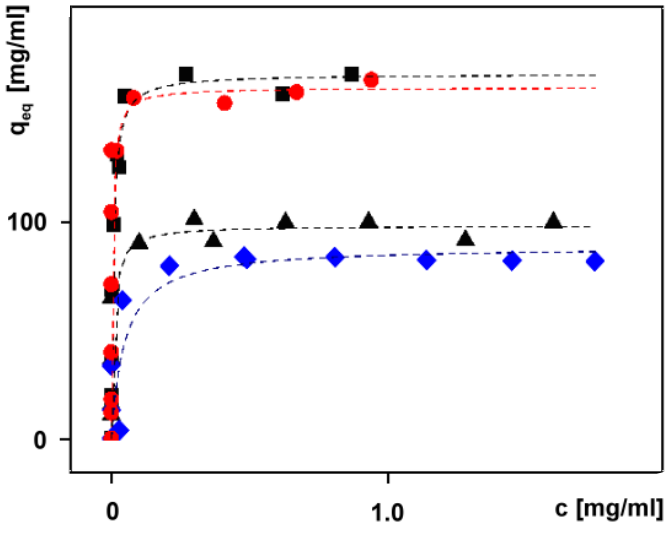

Figure 1: Adsorption isothermes on ▲ Sepharose™ FF, ■ SP Sepharose™ XL, ● Fractogel® EMD $SO3^-$, ♦ Fractoprep® SO3, showing that the adsorbent structure influences the binding.

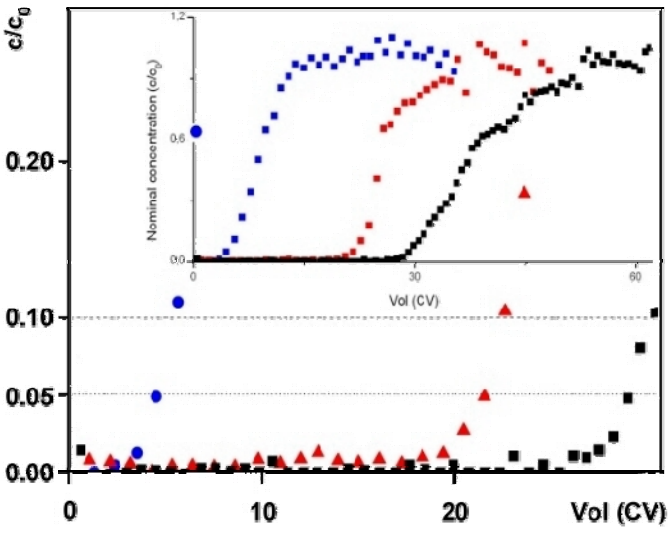

Figure 2: Dynamic capacities of lysozyme at ■ 40 mM, ▲ 80 mM or ● 160 mM sodium chloride. The 5 % and 10 % breakthrough values can easily be derived from the resulting graphs.

With a restricted parameter space, the elution behaviour of the target molecule compared to contaminant species was tested. The example of an elution experiment with a human growth hormone (hGH) and its precursor, Met-Glu-Ala-Glu-hGH (MEAE-hGH) is displayed in Figure 3. The RoboColumns (5/10 = 200 µl) were packed with POROS® 50 D (Figure 3, left) and Q Ceramic HyperD® (Figure 3, right) using sodium chloride as elution salt. It is shown that the separation process of a biopharmaceutical compound obtained on the Freedom EVO platform (Figure 3, bottom) compares well to standard laboratory scale (Figure 3, top). The observed shift of the peaks achieved using RoboColumns compared to the peaks achieved using ÄKTAdesign™ systems (GE Healthcare) relies mainly on the smaller dead volume when processing RoboColumns on the Freedom EVO workstation[3].

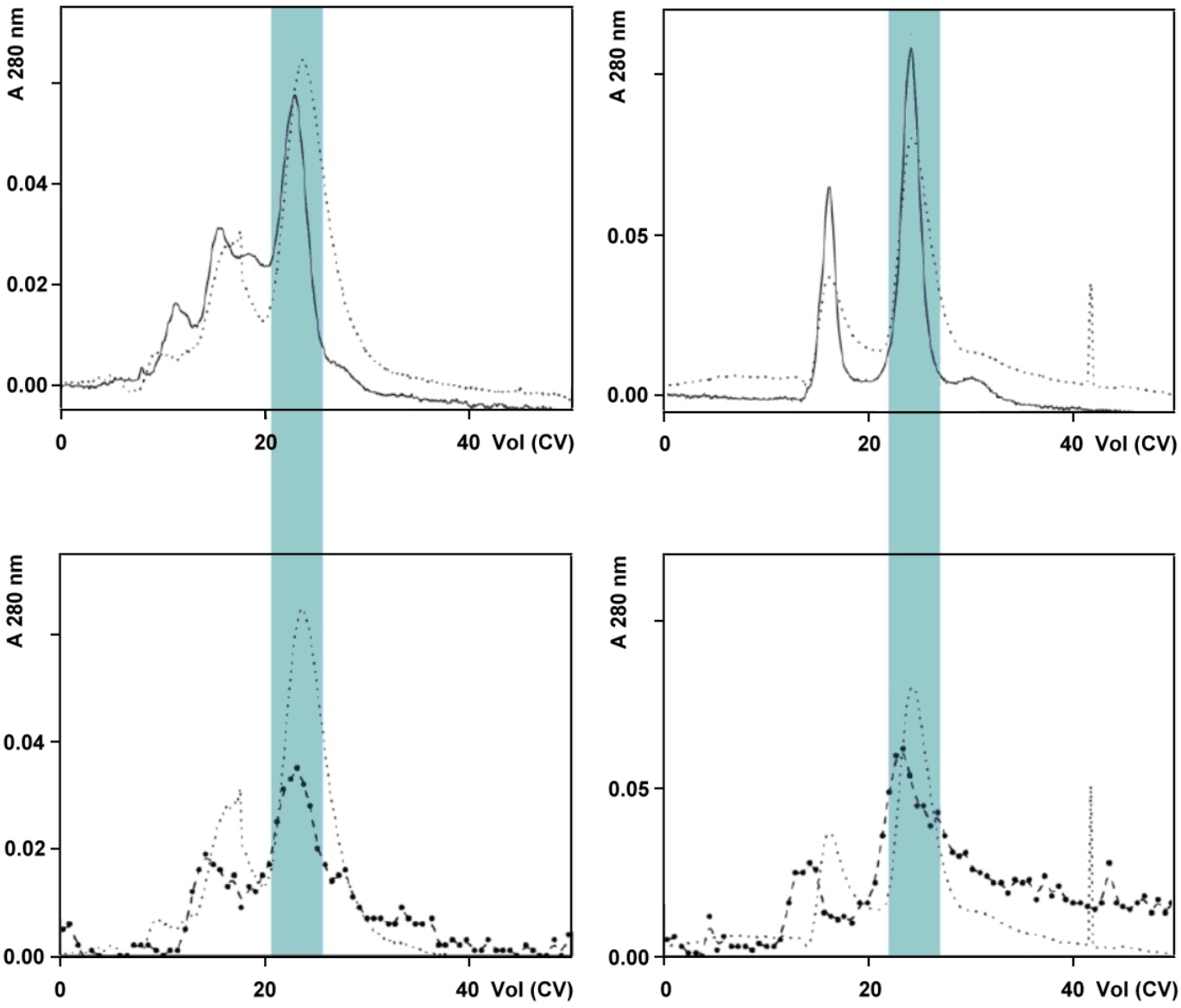

Figure 3: Separation of hGH and MEAE-hGH on RoboColumns filled with POROS® 50 D (left) or Q Ceramic HyperD® (right) UV 280 signal measured with — HR 5/100 column on ÄKTAdesign™; ···· RoboColumns on ÄKTAdesign™; ●- RoboColumns on Freedom EVO; ■ position of second, major peak. The results show that the separation of the target protein obtained on Freedom EVO is easily transferable to larger scale.

Summary

The combination of Tecan Freedom EVO workstations and screening procedures evaluating chromatographic process parameters bears the potential to shorten development time of biopharmaceutical purification processes significantly. The optional modules range from simple batch adsorption techniques performed under high throughput mode to miniaturized chromatographic columns such as RoboColumns from Atoll. Future fields to be tackled are in the use of such systems for Process Analytical Technologies (PAT) and Quality by Design (QbD) applications.

References

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