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Preparative weak cation-exchange chromatography of monoclonal antibody variants I. Single-component adsorption

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ABSTRACT

The retention behavior of a monoclonal antibody has been characterized on a weak cation exchanger, Fractogel EMD COO^-(s). This new generation of resin materials comprise of a higher mechanical strength compared to softer gel-type matrices while maintaining elevated capacities, resulting in higher productivity and longer lifetimes. These parameters are extremely important when working with large bio-molecules such as proteins, and in particular monoclonal antibodies. In the first part of this work a parameter estimation strategy is presented to fully characterize the retention behavior of a single monoclonal antibody and determine suitable model parameters. Literature correlations were used for the estimation of mass transfer rates. The transport limiting parameter, pore diffusion, was regressed experimentally. Various methods for the adsorption isotherm determination have been applied, their combinations resulting in little experimental effort and accurate predictions of elution profiles. The process has been modelled with a complete pore diffusion model and the agreement between experimental and predicted profiles is good in general. However, a very marked sensitivity to changes in the effective pore diffusion coefficient has been observed. A correlation describing the effect of the separation conditions on the diffusion rate is therefore needed in order to have a fully predictive mathematical model.

1. Introduction

There are currently about 400 biotechnology drugs in development, of which monoclonal antibodies (MAb) constitutes an important fraction [1,2]. However, the development of biotechnological products becomes more and more expensive and the production costs for MAbs remain extremely high [3]. Downstream processing can account for about 50–80% of the total production costs for MAbs [4]. In addition, since the time from development to market of a MAb is critical in the pharmaceutical industry, purification processes have to be developed in an efficient and fast manner.

In the biotechnological production process of MAbs, structural variations may arise due to some enzymatic activity [5]. This may result in a distribution for example of C-terminal lysine and arginine groups which in turn may impact stability and activity of the protein [6]. For this reason, variations in the C-terminal end of the MAb molecule are nowadays important for diagnostic applications, but they may also play a role in future biotechnological applications. In particular, it cannot be excluded a need in the near future to produce pure variants for therapeutical applications. These cannot be distinguished by affinity stationary phases. In this work, we investigate the possibility of separating variants using preparative ion exchange supports [7].

Small changes in the isoelectric point (pI) of the proteins can induce largely different retention behaviors in ion-exchange chromatography. This is particularly true for example for MAb variants having different numbers of C-terminal lysine groups. The latter contains an amino group, and thus an increase in the number of lysine group increases the net positive charge of the MAb and its pI. Unfortunately, a predictive method for the design of chromatographic purifications of MAb variants on cation-exchange resins does not exist, yet [8].

Modelling and simulation in the design and optimization of a chromatographic process may help in reducing labor and time-consuming experiments and shorten the development time substantially [9]. The models have to be carefully chosen and validated by designed experiments to elucidate the basic physical mechanisms behind the process. If this is guaranteed retention behavior may be predicted and the process optimized efficiently. In the numerical modelling of a chromatographic process it is essential to carefully characterize mass transport phenomena and adsorption behavior of the solute. Mass transport comprises of axial

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dispersion in the mobile phase and the transport of the solutes to the intra-particle surface. This can be divided into two steps: film transfer to the external particle surface and diffusion into the pores of the particle (intra-particle mass transfer) where the adsorption/desorption process takes place.

In bio-chromatographic separations intra-particle mass transfer is particularly important since it is typically the major contribution to band broadening [10,11] due to very slow diffusion rates of large bio-molecules inside the pores of the stationary phase. It has been observed that the diffusion rate is not constant in a chromatographic process but it is a function of pH, protein concentration and salt concentration. Chang and Lenhoff determined effective diffusivities of lysozyme in a set of preparative strong cation-exchange stationary phases [12] based on different base matrices. In their study the experimental data from batch uptake data in a stirred vessel were fitted with either the pore diffusion model or the homogeneous diffusion model. The estimated pore diffusivities decreased with increasing protein concentration. The authors proposed that protein–protein interactions and pore constrictions resulting from protein adsorption contribute to this effect. Other authors have also reported diminishing diffusivity with increasing protein concentration on different stationary phases [13–15]. Controversial results with respect to the effect of ionic strength on pore diffusion can be found in literature. Axelsson et al. investigated protein diffusion in agarose gel and reported a decrease of the pore diffusion coefficient based on the neutralization of the electrostatic forces between protein molecules which results in a shielding effect of the protein charges [16]. This agrees with the results of protein diffusion in solution [17,18]. The opposite effect, i.e. an increase in diffusivity with increasing salt concentration was measured in other studies [12], and it is probably due to the increase of the pore size with increasing ionic strength.

The importance of relative pore size and connectivity as influencing parameters on diffusivity has been extensively described in literature [19,14,20,12]. Various experimental methods have been described to determine mass transfer rates. A very comprehensive overview can be found in [21]. These methods can be divided into macroscopic measurements, in which the different mass transfer resistances are estimated from macroscopic quantities as the elution peak moments, and microscopic methods [22] by which the intra-particle concentration profiles can be directly estimated. Since the implementation of microscopic methods is rather complicated and it often requires labelling of the protein, which might then alter its transport characteristics, in this work we adopted a suitable combination of macroscopic methods.

The adsorption isotherm can also be determined by a number of different methods. Fernandez and Carta [23] have shown that stirred batch experiments are well suited to measure uptake kinetics and adsorption isotherms at different eluent salt concentrations. They are run under highly nonlinear conditions, i.e. high solute concentrations. Column methods may give more precise measures of the adsorption isotherm parameters than batch experiments. In particular, isocratic pulse experiments under linear adsorption isotherm conditions, i.e. using very diluted samples, allow the direct determination of the Henry coefficient as a function of the modifier concentration in the eluent. However, also in this case care must be taken since only pure samples can be applied, so as to avoid peak tailing due to unknown impurities [24], and the eluent salt concentration has to be controlled very carefully since small changes may significantly alter the retention times. A more complex method, the so-called inverse method [25] in which the adsorption isotherm parameters are regressed by direct peak fitting of gradient experiments, requires the *a priori* selection of an isotherm model [26]. On the other hand, each run contains many information since it spans a whole composition interval and thus it only requires a few overloaded experiments (with no pure samples), thus minimizing solute and solvent consumption [27]. Increasing computational power has reduced simulation times and thus made peak fitting a very convenient tool. Frontal analysis has been claimed to be the most accurate dynamic method for the determination of adsorption isotherm parameters [28]. The results can be directly used for characterization and optimization. The problem with this method, besides the fact that the adsorption isotherm model must be known *a priori*, is that solute consumption is very high, and the experiments are time-consuming [21]. The above described methods have been all applied in this work to the adsorption of MAb variants and their results will be discussed in the following.

Purity, yield and productivity represent the key factors in determining the cost of biomolecule purification process [29]. Polysaccharide-based polymers and other gel-type sorbents generally exhibit poor mechanical properties and hence do not withstand high pressure drops. Synthetic sorbents can withstand much higher pressure drops while maintaining high capacity and improved kinetics. In this work, we consider Fractogel EMD COO^-, which is made from a synthetic methacrylate based polymeric resin with a hydrophilic surface. The functional carboxy-groups are located on so-called tentacles, that is long polymer chains bound to the surface [30]. The high selectivity and capacity of this material, where non-specific solute–matrix interactions are minimized, makes it promising with respect to the separation of very similar compounds with very small charge differences.

In this work the retention behavior of C-terminal MAb variants on Fractogel EMD COO^-(s) has been characterized. The characterization of the MAb retention behavior on a non-porous analytical resin, Propac WCX-10, is discussed in detail in [31]. The results of this study are extended here to explore adsorption under diluted and overloaded conditions on the porous preparative cation-exchanger, Fractogel EMD COO^-(s). A systematic approach for the determination of transport and adsorption isotherm parameters is developed. This is carried out using a single-component (variant) system. This is a unique situation since the presence of many variants in the same monoclonal and the presence of other impurities typically affects significantly the reliability of the estimation procedures for the transport kinetics and, in particular, for the adsorption isotherm. The objective of the design procedure is to minimize both, solute consumption and experimental effort. A complete pore diffusion model of the chromatographic column has been developed and validated by comparing simulated and experimental elution profiles. In the second part of this study the competitive separation of the three MAb variants on the same cation-exchanger will be studied.

2. Theory

2.1. Mathematical model

The chromatographic column has been modelled through a detailed pore diffusion model [24], where it is assumed that the convective flow takes place in the interstitial space between the particles. The solute molecules are transported into the particle pores by diffusion, and adsorption and desorption take place on the particle surface within the pores, where equilibrium conditions are assumed. The differential mass balance over the solute in the mobile phase can be written in dimensionless form as follows:

$$\frac{\partial C}{\partial \tau} + \frac{\partial C}{\partial \xi} + \epsilon_p \frac{1-\epsilon_b}{\epsilon_b} St\,(C - c_p|_{\rho=1}) = \frac{1}{Pe_{ax}} \frac{\partial^2 C}{\partial \xi^2} \quad (1)$$

where C is the solute concentration in the bulk liquid, τ the dimensionless time, ϵ_p and ϵ_b the particle and the bed porosity, respectively, ξ the dimensionless space coordinate and $c_p|_{\rho=1}$ is the