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Role of the ligand density in cation exchange materials for the purification of proteins

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ABSTRACT

The performance of functionalized materials, such as cation exchange resins, is dependent not only on the ligand type and ligand density, but also on the pore accessibility of the target molecule. In the case of large molecules such as antibodies this latter parameter becomes crucial, because the size of such molecules falls somewhere inside the pore size distribution of the resin. The influence of the ligand density and accessibility on the overall performance of the material is explored systematically. Five different materials, having the same chemistry as the strong cation exchange resin Fractogel EMD SO_3^- (M) , have been analyzed. These materials only differ in the ligand density. It is shown that the ligand density directly influences the porosity of the materials as well as the pore diffusivity and the dynamic binding capacity. For a given purification problem an optimal ligand density can be found. Based on the above results a new material is proposed, showing superior properties in terms of dynamic binding capacity. This is achieved by an optimization of the ligand density and by a decrease of the particle size of the stationary phase. The material properties are modeled with a general rate model. Further simulations were conducted to evaluate the performance of the new material in comparison with a conventional resin.

1. Introduction

Within the pharmaceutical industry, the production of monoclonal antibodies is the fastest growing sector [1]. For 2009, an annual production of 14 t of monoclonal antibodies is forecasted [2]. The upstream process made great progress within the last couple of years, so that it is possible to reach antibody titers as high as 10 g/l. As a result, the downstream process arrived to account for 50–80% of the production costs [3]. The classical approach for the purification of immunoglobulin G (IgG) typically starts with a capture step by Protein A affinity chromatography followed by a number of chromatographic and non-chromatographic steps [4]. The main advantage of the affinity step is the extremely high selectivity towards antibodies. The drawback of this method is the high resin price. Furthermore, Protein A is highly toxic [5]. Therefore, the removal of leaking Protein A must be proven in subsequent purification steps. In order to overcome this problem, alternative purification strategies have been proposed [6]. Promising alternatives include ion exchange and hydrophobic interaction materials [7,8]. Furthermore, new stationary phases have been introduced that combine both previously mentioned effects, the so-called mixed mode materials [9–12]. In this regard, ion exchange resins appear particulary promising due to their comparably low cost and their ability to both capture and resolve different proteins [13].

Many authors evaluated and compared stationary phases in order to find the best material for a given separation problem. Staby et al. [14–19] measured the properties of anion as well as cation exchange resins extensively. Ghose et al. [20] compared the properties of hydrophobic charge induction materials to Protein A mimetic and Protein A resins. Pore size and retention factors of hydrophobic interaction materials were studied for a set of proteins by To and Lenhoff [21]. However, in all cases materials from different suppliers were compared. As differences in the synthesis and manufacturing of the resins might lead to significant differences in the performance for a certain kind of application, a fair comparison of those materials is difficult. In particular, it is hard to link certain performance data directly to a material property, because the information about each material is incomplete.

Historically, ion exchange resins were developed for the purification and analysis of small molecules [22]. Their application to the purification of proteins introduces a manifold of new problems due

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to the fact that antibodies such as IgG has dimensions comparable to the pore sizes. This leads not only to high mass transfer resistances, but also to limited accessibility of the protein into the pores of the stationary phase. As it will be discussed in detail in this work, some other factors, such as the particle size and especially the ligand density of the support, play an important role here. The influence of the latter on the performance of ion exchange materials has already been studied by Wu and Walters for silica supports in 1992 [23]. However, they chose rather small proteins such as lysozyme and cytochrome c. Zhang and Sun [24] studied the behavior of bovine serum albumin and bovine hemoglobin on affinity resins with different ligand densities. Langford et al. [25] studied the mass transfer of lysozyme on a set of cation exchange resins with varying ligand density.

In this work, a set of custom made cation exchange resins are studied regarding pore size distribution, ligand density and particle size in order to find the effect of those parameters on mass transfer resistances and static and dynamic binding capacity for IgG. To our knowledge, these parameters have not been analyzed systematically for large biomolecules such as IgG. In the first part of the work, all analyzed materials have the same polymeric support as the commercial material Fractogel EMD SO_3^- (M).

Based on the obtained results, a new cation exchange material, tailor made for the purification of IgG, i.e. FractoAIMs, is developed in the second part of this work. The behavior of this material and the benchmark material Fractogel EMD SO_3^- (M) is simulated with a general rate model. This model considers the concentration distribution of the solutes in the axial direction along the chromatographic column as well as along the radial direction in the stationary phase [26]. Even though the model needs to solve a large number of differential equations, its application is needed in the case of systems with dominating mass transfer resistances [27]. The objective here is to verify the possibility of such a model to predict the dynamic binding capacity of a given stationary phase. This could help tremendously in the screening phase of operating conditions and stationary phases by avoiding the dynamic column breakthrough experiments which require time and materials not always available in the early stage of process development.

The combination of a rational design of a new material, based on experimental data, with the simulation of the behavior of IgG on this material gives new insights in the mode of operation of ion exchange resins for preparative protein purification. Accordingly, this paper provides a new design strategy for preparative stationary phases for the purification of large molecules.

2. Materials and instrumentation

Strong cation exchange resin Fractogel EMD SO_3^- (M) , that was chosen as a benchmark material, was provided by Merck (Darmstadt, Germany). It has a crosslinked polymethacrylate matrix with sulfoisobutyl as functional groups. These are bound to the matrix with linear polymer chains, the so-called 'tentacles' [28]. The particle size of Fractogel EMD SO_3^- (M) is $d_p = 40 - 90$ μm (average $d_p = 65$ μm) with a pore size of about $r_p = 400$ Å. For large scale operation, Fractogel EMD SO_3^- (M) is operated at velocities up to $u_{lin} = 350$ cm/h. As the columns used in this work are much shorter, it was possible to flow pack the resin at a velocity of $u_{lin} = 800$ cm/h. The maximum velocity for chromatographic experiments is set to a linear velocity of $u_{lin} = 400$ cm/h.

Based on the same support used for Fractogel EMD SO_3^- (M), Merck (Darmstadt, Germany) synthesized materials with different ligand densities (Series 1, see Table 1). Furthermore, a new material called FractoAIMs, was developed by Merck within this work. This material has an average particle size of 40 μm. It was designed in order to have a high rigidity which leads to a much higher operating flow rate than Fractogel EMD SO_3^- (M), also at preparative conditions. Therefore, FractoAIMs was flow packed with a velocity of $u_{lin} = 1100$ cm/h in this work. The ligand density of all materials was evaluated by the supplier by titration as summarized in Table 1.

Gammanorm, which is human normal immunoglobulin G (IgG > 95%) in solution ($c_{IgG} = 165$ g/l), has been chosen as an model protein to investigate the behavior of antibodies on ion exchange materials. The polyclonal antibody mixture was purchased at Octapharma (Lachen, Switzerland). Human serum albumin and myoglobin from equine sceletal muscle were purchased at Sigma–Aldrich (Buchs, Switzerland). Sodium chloride and acetic acid (glacial) were purchased at J.T. Baker (Deventer, the Netherlands), sodium acetate trihydrate and sodium hydrogen phosphate dihydrate were purchased at Merck (Darmstadt, Germany). Sodium phosphate dibasic dodecahydrate was obtained by Acros Organics (Geel, Belgium). Pullulan standards were purchased at Polymer Standards Service (Mainz, Germany). Dextran standards were purchased at Fluka (Buchs, Switzerland). Deionized water was purified with a Simpak 2 unit by Millipore (Zug, Switzerland). All used chemicals were of analytical grade. All buffer solutions were prepared using a precision balance METTLER AT250 (Mettler-Toledo, Greifensee, Switzerland). The buffer composition was calculated according to the recipes by Beynon and Easterby [29].

For chromatographic measurements, a HPLC 1100 Series by Agilent Technologies (Santa Clara, CA, USA) was used. The instrument is equipped with an UV-detector and a refractive index detector.

Resins were packed into Tricorn columns purchased at GE Healthcare (Chalfont St Giles, United Kingdom) and Superformance columns by Goetec Labortechnik (Mühltal, Germany). Tricorn columns have a volume of $V \approx 2$ ml and a diameter of $d = 5$ mm. Superformance columns have a volume of $V \approx 20$ ml and a diameter of $d = 16$ mm. 20% mechanical compression of the bed was applied to the resin in those columns. Furthermore, columns by Infochroma (Zug, Switzerland) with a diameter of $d = 4.6$ mm and a volume of $V = 1.0$ ml and columns by YMC (Kyoto, Japan) with a diameter of $d = 7.5$ mm and a volume of $V = 2.2$ ml were used. The latter two columns could not be compressed mechanically.

3. Methods and model

3.1. Pore size distribution

The pore size distribution was measured by inverse size exclusion chromatography (ISEC) [30,31]. In comparison with other techniques for the determination of the pore structure, such as mercury porosimetry or BET measurements, this method has a number of advantages. Particularly, drying of the sample is not necessary. Furthermore, the measurements can be performed directly in the packed column.

ISEC measurements with various dextran and pullulan tracers, as well as protein tracers, were executed under nonadsorbing conditions. The ratio of the accessible liquid volume $V_{t,i}$ for a generic tracer i of specific molecular weight and the total column volume

Table 1
Synthesized Fractogel materials and FractoAIMs and corresponding ligand density. Series 1 includes Fractogels A–E.

Material	ρ_{lig} [μmol/g]
Fractogel EMD SO_3^- (M)	380
Fractogel A	144
Fractogel B	236
Fractogel C	338
Fractogel D	400
Fractogel E	485
FractoAIMs	385