Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Full length article

Adsorption equilibrium and kinetics of Immunoglobulin G on a mixed-mode adsorbent in batch and packed bed configuration

Pedro Ferreira Gomes, José Miguel Loureiro, Alírio E. Rodrigues *

Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Dept. of Chemical Engineering University of Porto, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form
26 September 2017
Accepted 2 October 2017
Available online xxx

Keywords:
Immunoglobulin G
Mixed-mode chromatography
Adsorption equilibrium
Adsorption kinetics
Batch adsorption
Packed bed adsorption

ABSTRACT

It is commonly accepted that efficient protein separation and purification to the desired level of purity is one bottleneck in pharmaceutical industries. MabDirect MM is a new type of mixed mode adsorbent, especially designed to operate in expanded bed adsorption (EBA) mode. In this study, equilibrium and kinetics experiments were carried out for the adsorption of Human Immunoglobulin G (hIgG) protein on this new adsorbent. The effects of ionic strength and pH are assessed. Langmuir isotherms parameters are obtained along with the estimation of the effective pore diffusion coefficient (D_{pe}) by fitting the batch adsorption kinetics experiments with the pore diffusion model. The maximum adsorption of the IgG protein on the MabDirect MM adsorbent, $149.7 \pm 7.1\ mg \cdot g_{dry}^{-1}$, was observed from a pH 5.0 buffer solution without salt addition. Adding salt to the buffer solution, and/or increasing pH, decreases the adsorption capacity which is $4.7 \pm 0.4\ mg \cdot g_{dry}^{-1}$ for pH 7.0 with 0.4 M NaCl in solution. Regarding the D_{pe} estimation, a value of $15.4 \times 10^{-6}\ cm^2 \cdot min^{-1}$ was obtained for a pH 5.0 solution without salt. Increasing the salt concentration and/or the pH value will decrease the effective pore diffusion, the lowest D_{pe} ($0.16 \times 10^{-6}\ cm^2 \cdot min^{-1}$) value being observed for an IgG solution at pH 7.0 with 0.4 M NaCl. Fixed bed experiments were conducted with the purpose to validate the equilibrium and kinetic parameters obtained in batch. For a feed concentration of $0.5\ g \cdot L^{-1}$ of IgG in pH 5.0 buffer solution with 0.4 M NaCl, a dynamic binding capacity at 10% of breakthrough of $5.3\ mg \cdot g_{wet}^{-1}$ ($15.4\ mg_{IgG} \cdot mL_{resin}^{-1}$) was obtained, representing 62% of the saturation capacity. As far as the authors know, this study is the first one concerning the adsorption of hIgG on this type of mixed mode chromatography adsorbent.

1. Introduction

Protein purification has been performed for a long time. Monoclonal antibody technology appeared in 1975, involving the establishment of stable cell lines producing a single selected protein. This technique, in association with highly selective chromatographic media, made possible the conception of a large number of applications in research and pharmaceuticals production [1–4].

Immunoglobulin G (IgG) is a complex antibody composed by four peptide chains (with quaternary amine) with two heavy chains and two identical light chains arranged in a Y-shape typical of antibody monomers. IgG represents approximately 75% of immunoglobulins present in human serum. This protein has important functions on the immune system since it is the main antibody found in blood and extracellular fluid, having the function of controlling infection of body tissues. IgG antibodies have a molecular weight of 150 kDa and present four subclasses in humans (IgG1, IgG2, IgG3 and IgG4). IgG can be used for diagnostic purposes, where the measured IgG levels are generally considered to be indicative of an individual immune status to a particular pathogen. Also, it can be used in therapy, where it is extracted from donated blood plasma and used to treat immune deficiencies, autoimmune disorders and infections [5,6]. This antibody is the only class of Immunoglobulins that can cross the human placenta, and therefore convey protection to the newborn during the first months of life. Considering its abundance and the good specificity towards antigens, IgG became the main antibody to be used in immunological research and clinical diagnostics [5].

Efficient separation and purification of proteins to the desired level of purity is one bottleneck in pharmaceutical industries. Affinity chromatography, using Protein A and Protein G ligands, is commonly used for IgG separations. However, the use of these adsorbents presents potential issues, such as clean-in-place difficulty, ligand leakage and overall sorbent cost [7–10].

* Corresponding author.
E-mail address: arodrig@fe.up.pt (A.E. Rodrigues).

https://doi.org/10.1016/j.chroma.2017.10.003
0021-9673/

Mixed mode chromatography (MMC) appears as an alternative to these costly sorbents. MMC is a method of separation that uses more than one form of interaction between the stationary phase and the solutes in a feed stream. They provide a salt-tolerant, high capacity, easy elution and improvement of adsorption selectivity since they possess additional functional groups that will introduce new cooperative interactions, such as hydrophobic, electrostatic, thiophilic interactions and hydrogen bonds [11–13].

Adsorption techniques are commonly used for protein separation and purification. Adsorption can be carried out in batch or continuous stirred tanks, or using fixed bed or expanded bed technologies. Pros and cons of using each referred method are specified in a previous work [14]. Second generation expanded bed adsorption is a technology developed by Upfront Chromatography A/S [15,16]; it consists in the use of a high density material, in this case tungsten carbide in agarose matrix (MabDirect MM being an example), that allows high flow rates (300–600 $cm \cdot h^{-1}$) at a common expansion factor of two. Using this technology also allows a direct capture of the high-value biopharmaceutical products, reducing process cost and operating time without affecting product purity [11,17,18].

The objective of this work is to study a novel multimodal adsorbent, especially designed for expanded bed adsorption (EBA) operation, using batch and fixed bed operating modes in the adsorption of a polyclonal antibody protein, Immunoglobulin G (IgG), for a later use in EBA where the influence of different parameters will be investigated. First, batch adsorption experiments are conducted in order to determine the adsorption equilibrium isotherms and the effective diffusivity of the target protein in different conditions. The effects of pH and ionic strength (by addition of salt) are assessed. Fixed bed experiments are then performed in order to validate the batch adsorption results, previously obtained.

2. Materials and methods

2.1. Immunoglobulin G

Immunoglobulin G from Human serum (hIgG) was purchased from Sigma–Aldrich, USA. According to the product information sheets, human IgG was purified from pooled normal human serum, and purity was determined to be not less than 95% by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Product information sheet, Human IgG, Sigma-Aldrich, USA, Product Number I4506). Human IgG was shipped as salt free lyophilized powder. In this work, Human IgG is quantified as total and not its subclasses IgG 1–4, so the presence of confounding effects of these multiple subclasses on the protein adsorption and transport are not addressed.

2.2. Mixed mode adsorbent

MabDirect MM, from Rhobust Fastline series, is a mixed mode adsorbent composed by a base matrix of 6% cross-linked agarose with tungsten carbide particles [14,19,20]. Delivered in an aqueous solution containing 14% NaCl and 0.2 M sodium acetate, this adsorbent, with a tested ligand concentration of 35$mmol \cdot L_{adsorbent}^{-1}$ and adsorbent density of 2.9 $g \cdot mL^{-1}$, according to the manufacturer, was provided by Upfront Chromatography A/S. The mixed-mode ligand has a pKa of 5.0 and contains aromatic ligands with acidic substituents (benzoic acid) [14,19,20].

2.3. Equipment

Batch equilibrium experiments were conducted in a Sigma 203 model centrifuge and in a VWR himac CT 15RE centrifuge, while adsorption kinetics experiments were conducted in a Jeio Tech SI-300R shaking incubator. For fixed bed adsorption experiments, an Omnifit 6.6/11 column was used. A Gilson 117 UV detector equipped with a flow-cell was used to monitor IgG concentration at 280 nm wavelength for both batch and packed bed experiments.

2.4. Buffer solutions

Target protein is dissolved in different buffer solutions regarding the pH and salt conditions to be tested. A citrate buffer was used for pH values of 5.0 and 6.0 (citric acid, $C_6H_8O_7$, 192.12 $g \cdot mol^{-1}$, with tri-sodium citrate di-hydrate, $Na_3C_6H_5O_7 \cdot 2H_2O$, 294.10 $g \cdot mol^{-1}$, dissolved in deionized water). A phosphate buffer was used for pH 7.0 (sodium di-hydrogen phosphate di-hydrate, $NaH_2PO_4 \cdot 2H_2O$, 156.01 $g \cdot mol^{-1}$, with di-sodium hydrogen phosphate, Na_2HPO_4, 141.96 $g \cdot mol^{-1}$, dissolved in deionized water) and a Tris-HCl buffer was used for pH value of 8.5 ($C_4H_{11}NO_3HCl$, 157.64 $g \cdot mol^{-1}$, dissolved in deionized water). For all buffer solutions, a 20 mM concentration was used, except for the buffer in which the adsorbent was stored which had a 200 mM concentration. If necessary, the final solution pH was adjusted with 1 M NaOH or 1 M HCl. To study the effect of salt concentration, sodium chloride (NaCl, 58.44 $g \cdot mol^{-1}$) was added [14].

Sodium di-hydrogen phosphate di-hydrate and di-sodium hydrogen phosphate were purchased from VWR Chemicals, while citric acid, tri-sodium citrate di-hydrate, hydrochloric acid, sodium hydroxide, Tris-HCl, sodium acetate and sodium chloride were purchased from Reagente5 [14].

Before performing adsorption equilibrium and kinetics experiments, the adsorbent was conditioned with the same protein buffer solution. This procedure consists in placing some amount of adsorbent in a special glassware equipped with a proper filter connected to a vacuum pump where it will be cleaned with 1L of deionized water before flowing the chosen buffer [14].

2.5. Adsorption equilibrium isotherms by Batch experiments

Adsorbent samples (0.050 and 0.127 g – wet weight) are mixed with 1 mL of different protein concentration solutions for about 4 h at room temperature (20 °C) in a centrifuge (Sigma 203 model) at 1000 rotations per minute (rpm). After reaching equilibrium, the supernatant is collected by centrifuging (VWR himac CT 15RE) at 4000 rpm for 10 min. The equilibrium protein concentration in the liquid phase is measured by a UV spectrophotometer detector at 280 nm. The measurements were repeated three times, and the average value was obtained. The experimental reproducibility was in the range of less than 10%.

2.6. Adsorption kinetics by Batch experiments

An adsorbent sample (2 g–wet weight) is mixed with 40 mL of the chosen buffer with an IgG concentration of 0.7 $g \cdot L^{-1}$ at a controlled temperature (20 °C) in a shaking incubator (Jeio Tech SI-300R) at 270 rpm. Periodically, samples (20 μL) were collected and the protein concentration in the liquid phase was measured by a UV spectrophotometer detector at 280 nm. The measurements were repeated three times, and the average value was obtained. The experimental reproducibility was in the range of less than 10%.

2.7. Fixed bed adsorption

Before performing fixed bed adsorption experiments, 3 mL of adsorbent, measured in a graduated cylinder, was drained using a vacuum pump as previously described to account for the wet adsorbent mass. After this step, the wet adsorbent was hydrated in the chosen working buffer and the Omnifit 6.6/11 column was packed

Please cite this article in press as: P.F. Gomes, et al., Adsorption equilibrium and kinetics of Immunoglobulin G on a mixed-mode adsorbent in batch and packed bed configuration, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.10.003