Journal of Chromatography A, 1216 (2009) 5039–5044

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Influence of ligand density on antibody binding capacity of cation-exchange adsorbents

Katarzyna Wrzosek, Michal Gramblička, Milan Polakovič*

Department of Chemical and Biochemical Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 7 December 2008
Received in revised form 22 March 2009
Accepted 23 April 2009
Available online 3 May 2009

Keywords:
Cation-exchange chromatography
Monoclonal antibody
Adsorption capacity
Ligand density
Ionic strength
pH influence

ABSTRACT

Adsorption properties of a set of polymethacrylate-based cation exchangers designed for purification of monoclonal antibodies were investigated. The materials differed significantly in the density of sulphoisobutyl ligand groups. The ligand density had a pronounced effect on the static adsorption capacity of a polyclonal human immunoglobulin G. An optimal ligand density was observed at any pH and NaCl concentration tested when sharp optima were observed at low pH and ionic strength values. This was caused by effective clogging of pore mouth at high ligand densities. An anomalous effect of ionic strength was observed for the adsorbents with the high ligand density when the adsorption capacity increased with the addition of NaCl at low pH.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Monoclonal antibodies belong to important pharmaceutical products. They find significant applications in treatments of diseases such as cancer, asthma, arthritis, transplant rejections, autoimmune, cardiovascular and infectious diseases [1,2]. Chromatographic techniques play an important role in industrial purification of monoclonal antibodies. Three main types of interactions are employed in protein binding—affinity, ion-exchange and hydrophobic. Intensive and selective binding on a given adsorbent, which can be adjusted by several process parameters such as pH, ionic strength or hydrophobic conditions, is necessary for a successful separation.

The most commonly used chromatographic step in antibody purification is protein A affinity chromatography. Numerous advantages of these chromatographic adsorbents include the highest selectivity for immunoglobulin binding, high efficiency, adsorption capacity and ability to bind antibodies from cell culture supernatants with a high ionic strength [3–5]. Naturally, protein A chromatography has some drawbacks, from which the most significant one is the cost several times higher compared to other chromatographic adsorbents. Further problems are the leakage of protein A and the need of antibody elution at pH as low as 3 which may cause changes in its conformation [3–7]. Since complete purification of antibodies cannot be achieved with protein A affinity chromatography alone, subsequent chromatographic steps are applied.

Ion-exchange chromatography is typically used as a polishing step after protein A affinity chromatography [1,8] that allows effective elimination of host cell impurities, leached protein A, high molecular weight aggregates and viruses [7,9]. A wide use of ion-exchange chromatography is substantiated by relatively low costs of ion exchangers, its simple methodology and mild conditions of purification. Recently developed ion-exchange adsorbents are more rigid, have better developed porosities and higher ligand densities [10]. If these improvements are combined with a proper process optimization, ion-exchange chromatography may find a more extensive use in antibody purification. Cation-exchange chromatography has been reported to be effective as a capture step that might efficiently replace protein A chromatography in some cases [1,11,12]. At least one commercial antibody purification process using cation-exchange chromatography as a capture step has been developed [10]. The limitation of the method is a high dependence of antibody binding capacity on the ionic strength. Dilution or diafiltration may be then necessary to lower the salt concentration.

The objective of this study was to investigate the influence of ionic strength and pH on the static binding capacity of a set of non-commercial strong cation exchangers for human immunoglobulin G (IgG) and human serum albumin (HSA) as a prototype impurity protein. The adsorbents were based on the same polymethacrylate

* Corresponding author. Tel.: +421 2 59325254; fax: +421 2 52496920.
E-mail address: milan.polakovic@stuba.sk (M. Polakovič).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.04.073

matrix, grafted polymer tentacles and sulphoisobutyl ligand but they significantly differed in the ligand density. The combination of pH, ionic strength and ligand density factors provided very interesting effects on the static adsorption capacity that were examined and the underlying phenomena were explained.

2. Materials and methods

2.1. Materials

Different non-commercial, polymethacrylate matrix-based cation exchangers developed for antibody purification, synthesized by Merck (Darmstadt, Germany), were studied. All adsorbents contained a grafted, swelling polymer layer called tentacles where antibodies were bound through sulphoisobutyl ligand groups. Two series of adsorbents were prepared which differed in the pore size of the original ungrafted particles. The mean size of the adsorbent particles of about 65 μm was determined by the producer using laser diffraction. In each series, adsorbents with different ligand density were prepared when the degree of grafting also varied. The ligand density was determined by titration and its values as provided by the producer were in the range of 90–979 μmol of sulphonic groups per g of dry adsorbent for Series I and 150–509 μmol/g for Series II (Fig. 1). The mean pore radius and porosity of the adsorbents (Fig. 1) were determined by a water desorption method [13] at the Laboratoire Chimie Provence of Universités d'Aix-Marseille I,II et III–CNRS (Marseille, France). The main advantage of this method is that less than 50 mg of adsorbent sample is needed for one measurement. The suitability of the method for these adsorbents was verified using a very similar commercial adsorbent, FractoGel EMD SE Hicap (Merck). The mean pore radius of 25 nm was obtained which is very close to the value of 24.1 nm determined by size-exclusion chromatography [14]. Fig. 1 shows that the grafting had no or minimal effect on the total particle porosity but the mean pore radius decreased with the ligand density, which is in a full agreement with the results obtained for grafted weak anion exchangers [15].

Proteins used were human normal immunoglobulin G (Gammanorm, Octapharma, Sweden) and albumin from human serum (Sigma, St. Louis, MO, USA).

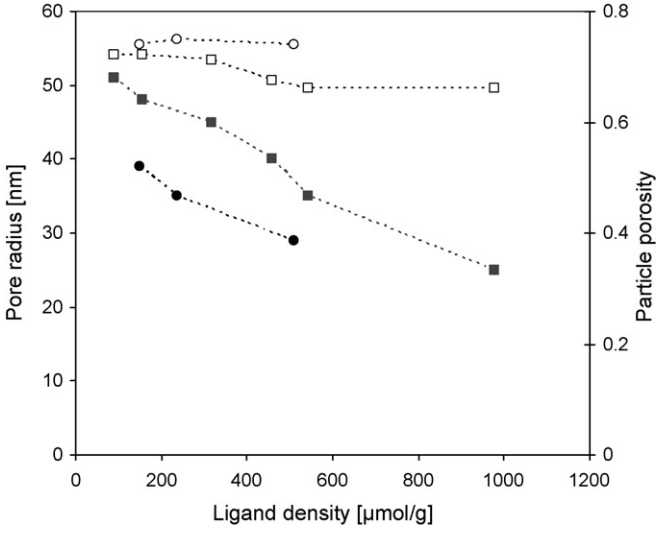

Fig. 1. Pore structure characteristics vs. the ligand density of investigated adsorbents measured in pure water, pH 7.0. Symbols represent the mean pore radius for Series I (■) and Series II (●) and porosity for Series I (□) and Series II (○). The connecting lines are made only for a better visualization of trends.

2.2. Methods

Chemicals used for buffer preparation (citric acid, dibasic sodium phosphate and sodium chloride) were of analytical grade. The buffer was prepared using double distilled water and filtered through a 0.45 μm cellulose-nitrate filter (Sartorius, Göttingen, Germany). Protein solutions were filtered through a low-protein binding 0.22 μm filter (Millipore, Consett, UK). The protein stock solutions were prepared directly before use.

The IgG static binding capacity was measured at pH 4–6.5 using a 50 mM phosphate–citrate buffer. The ionic strength was adjusted by addition of NaCl within the range from 0 to 300 mM NaCl. The protein concentrations in stock solutions were in most cases 4.5 mg/ml for IgG and 3 mg/ml for HSA. If the IgG binding capacity showed to be larger than 150 mg/ml, the protein concentration of 5.5 mg/ml was then used in order to safeguard the saturation of adsorbent binding sites in equilibrium. The measurements of HSA binding capacity were made in the same pH range with 75 mM NaCl and without salt addition. Before each experiment, adsorbent particles were conditioned in a buffer having the same pH and salt concentration as the corresponding protein solution. Extraparticle liquid was removed on a glass frit directly before use. An adsorbent in the amount of 0.030 g of was transferred into a prepared Eppendorf vial and 1.3 ml of a protein solution was added. The Eppendorf vials were placed in a horizontal position on a shaker GFL 1083 (Gesellschaft für Labortechnik, Burgwedel, Germany) and stirred at ambient temperature for 15 h. It was checked that this time was sufficient for reaching phase equilibrium. The samples were filtered through the low-protein binding filter and analyzed using a through-flow spectrophotometer equipped with a diode array detector at 280 or 310 nm (Agilent, Palo Alto, CA, USA).

The static adsorption capacity was determined from the decrease of protein concentration in the liquid phase,

$$q_a = \frac{V_s(c_0 - c^*)\rho_p}{m_p} \quad (1)$$

where q_a is the static adsorption capacity, V_s the solution volume, c_0 and c^* the initial and equilibrium concentrations of protein in the solution, m_p the mass of wet particles, and ρ_p their density. The standard deviation of q_a, obtained from duplicated measurements, was 5.2 mg/ml. The experiments were duplicated for the experimental conditions where a strong, non-monotonous change of adsorption capacity with the ligand density occurred.

3. Results and discussion

Human polyclonal antibodies are usually neutral or slightly basic compounds with the isoelectric point, p*I*, varying from 6 to 9 [2,12,16]. For that reason, they can be captured by cation-exchange chromatography at an acidic pH where they gain positive charge, protein–adsorbent interactions are stronger and the adsorption capacity is larger [2,8,12]. In this study, we investigated the static adsorption capacity at the pH values from 4 to 6.5. Strong cation exchangers are fully ionized in this range and the structure of antibodies remains stable. Fig. 2 shows that the adsorption capacity for Series I particles was as high as 150–200 mg/ml at the lower pH values of 4–5.5 whereas it reached only about 40 mg/ml at pH 6.5. This corresponds very well with that the protein net charge increases when the buffer pH moves away from the protein p*I*.

Fig. 2 also presents an interesting relationship between the ligand density and adsorption capacity that was observed at almost all pH values and salt concentrations. A maximum of adsorption capacity was found for the adsorbents at a medium ligand density from 318 to 543 μmol/g. The approximately linear increase of adsorption capacity up to the ligand density of 300 μmol/g can be interpreted so that the accessibility of ligand molecules did not change here.

Download English Version:

https://daneshyari.com/en/article/1206396

Download Persian Version:

https://daneshyari.com/article/1206396

Daneshyari.com