Journal of Chromatography A, 1376 (2015) 53–63

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Silica-based strong anion exchange media for protein purification

Feng Gu[a,*], Kiran Chodavarapu[a], Dennis McCreary[a], Thomas A. Plitt[a], Edward Tamoria[a], Michelle Ni[a,1], Jennifer J. Burnham[a,1], Michael Peters[a,1], Abraham M. Lenhoff[b]

[a] W. R. Grace, 7500 Grace Drive, Columbia, MD 21044, United States
[b] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, United States

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form
24 November 2014
Accepted 27 November 2014
Available online 8 December 2014

Keywords:
Strong anion exchange
Wide pore silica
Protein purification
Bioseparation
Tentacle polymer attachment
Single use chromatography

ABSTRACT

The main objective of our research was to develop silica-based, polymer-functionalized ion exchange materials for single-use bioprocess applications, with the ultimate goal of achieving maximal binding capacity for target proteins. Herein we report the utilization of Grace® wide pore silica gel and bonding the silica with cationic polymers. The strong anion exchange materials have been prepared by a two-step process involving initial bonding with two trimethoxysilanes and subsequent aqueous solution radical polymerization with quaternary ammonium ion containing monomers and an azo initiator. Using the binding capacities for bovine serum albumin (BSA), a model protein for the evaluation of the new materials, we optimized the processes with regard to the median pore size of the silica gel, as well as polymer composition and ratios, which were determined by reagent ratios and reaction conditions. The products were also characterized by both chemical and physical methods. It has been found that higher binding capacities are associated with lower ligand density and higher molecular weight for the attached polymers, with over 20% higher in both static and dynamic binding capacity values with the same amount of attached polymers. The advantages of a large pore size distribution and optimal median pore size for the base silica are discussed. Optimal pore size range of 500–1500 Å and distribution of Span 90 for over 1.0 give the highest BSA binding capacities. Silica-based strong anion exchange materials showed excellent flow characteristics when packed into a column and were superior to commercial agarose-based strong anion exchange material with respect to dynamic binding capacity, elution of proteins, and baseline separation of a mixture of three model proteins.

1. Introduction

Ion exchange materials are widely used in separation and purification of proteins and other biologics. The binding of proteins to the ion exchange media or resin predominately occurs via charged amino acid residues located at the surface of protein molecules. Due to the large size of typical proteins, resins with wide pores are usually required to allow the functionalized surface to be fully utilized and for the separation media to have maximal dynamic binding capacity of proteins.

The most common chromatography media currently used in bioprocessing are functionalized natural polymeric resins such as agarose, synthetic polymers like poly(methyl methacrylate) (MMA) or polystyrene divinylbenzene (PS-DVB), among others [1]. Silica gel is more typically used in small molecule purification (e.g., reverse phase high performance liquid chromatography (HPLC), and is not as often used in protein purification, especially in large scale bioprocessing. This is mainly due to the common practice of utilizing sodium hydroxide solutions for cleaning-in-place (CIP) between process cycles and batches, and the fact that silica is labile to strong base. However, given the current industrial trend toward single-use technology in biopharmaceutical manufacturing [2,3], the application of silica gel in bioprocessing for single use, disposable media may add value to the process by eliminating CIP procedures and subsequent recertification requirements, completely removing the possibility of cross-contamination. Single use chromatography may be especially critical for the purification of antibody-drug conjugates (ADCs) given the high cytotoxicity of the drugs or drug candidates. Compared to polymeric resins, silica gel has the advantage of being incompressible and thus would allow high flow rates. Silica materials also have the benefits of low cost,

* Corresponding author. Tel.: +1 410 531 8328.
E-mail address: feng.gu@grace.com (F. Gu).
[1] Grace Analytical Group.

http://dx.doi.org/10.1016/j.chroma.2014.11.082
0021-9673/

wide availability of different particle sizes, shapes, pore structure and morphology, as well as extensive options of surface modification chemistries.

The so-called tentacle-type materials are those resin particles bonded with polymeric chains. The tentacle ion exchange concept was first realized by Müller [4–6] and discussed in detailed by Műller [7]. Essentially this is a type of ion exchange material with linear polymeric chains, consisting of ionic-group bearing repeating units attached to the porous supporting media. In Müller's work [4], a Ce^{4+} oxidation of surface diol groups was used to generate surface free radicals, and the polymerization proceeded with the surface radicals and monomers in aqueous solution. Both synthetic polymer (e.g., vinyl polymer)[2] and silica[3] based resins have been used and commercialized in these types of processes. The disadvantage of this particular chemistry is the high cost associated with the utilization of the expensive rare earth element cerium, and that multiple washes are required to remove Ce^{3+} byproduct from the reaction, generating a large volume of toxic waste.

Our main objective in this research was to develop silica-based, polymer-functionalized ion exchange materials for single use applications, with the ultimate goal of achieving maximal binding capacity with model proteins for the new materials. In this article we explore the utilization of Grace® wide pore silica gel with polymer attachment, realized through aqueous solution polymerization, to prepare strong anion exchange (Q-Silica) materials. Herein we report an easy process for the preparation and excellent performance of our materials by fine tuning the polymer composition and matching silica properties. To achieve the performance goal, we studied the properties of silica, especially the optimal pore size and pore size distributions for higher protein binding, and on the chemistry side, we optimized the process with regard to the choice of monomers, the amount and the ratio of the reagents used in the initial bonding and subsequent polymerization, the amount of attached polymer, as well as polymerization reaction conditions. We also carried out comparisons against strong anion exchange materials based on other types of commercial silica materials and controlled pore glass beads, prepared by the same optimized process conditions. The adsorption binding capacities and elution of model proteins were compared between Q-Silica and agarose Q (GE Capto® Q).

2. Materials and methods

2.1. Reagents

Methoxysilanes were obtained from either Gelest, Inc. (Morrisville, PA) or Sigma–Aldrich Corporation (St. Louis, MO). (3-Acrylamidopropyl)trimethylammonium chloride aqueous solution (75%) was purchased from TCI America (Portland, OR). Diallyldimethylammonium chloride aqueous solution (65%) was from Sigma–Aldrich. 2,2′-Azobis(2-methylpropionamidine)dihydrochloride (V50 initiator) was obtained from Wako Chemicals USA (Richmond, VA) or Sigma–Aldrich. All other relevant chemicals were also purchased from Sigma–Aldrich or other chemical suppliers and were used without further purification. Proteins such as bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO).

2.2. Physical and chemical characterizations

The median particle sizes were determined by light scattering using a Malvern® Mastersizer® 2000, available from Malvern® Instruments Ltd. (per ASTM B822-10). Median pore diameter (size) distributions were measured by mercury intrusion using an Autopore® IV 9520, available from Micromeritics Instrument Corporation (Norcross, GA). Pore volumes referenced herein represent mercury intrusion into 100–10,000 Å size pores. BET surface areas (SA) were obtained from the nitrogen sorption analysis described in the literature [8]. Particle size (PS) is defined as median particle size by volume distribution. Median pore diameter (PD50) is defined as the midpoint at which 50% of the pore volume is contributed by smaller pores and 50% is contributed by larger pores.

Elemental analyses of carbon content of modified silica samples were conducted using a LECO® Carbon Analyzer SC-632 available from LECO Corp.

Inductively couple plasma-atomic emission spectroscopy (ICP-AES) (Shimadzu ICPE-9000) was used for the analysis of total elemental phosphorus of acid digested resins described in 2.8.

2.3. Bonding processes

Fig. 1 illustrates the process for making silica-based strong anion exchange material (Q-Silica). As shown, the bonding process was carried out and realized in two steps: (1) an "initial bonding" step involves a simple mix of two silanes, 3-(trimethoxysilyl)propyl methacrylate (vinyl silane) and 3-glycidoxypropyl)-trimethoxysilane (epoxy silane), with oven dried silica. The silane mix was added dropwise into silica in a round bottom flask and the flask was rolled slowly on a rotovap at room temperature overnight. After that, the silica was acidified with 0.5 M sulfuric acid (epoxy ring opening), and then washed with deionized water (DIW) several times; (2) aqueous solution, radical polymerization with quaternary ammonium salt-containing monomers and an azo initiator, in the presence of modified silica from the first step. After the polymerization, the mixture was diluted with 10% NaCl solution, filtered, and washed with NaCl solution and subsequently with DIW several times until the sample no longer contained free, unattached polymer.

2.3.1. Initial bonding

As described above, modified silica samples were prepared by treating the bare silica gel particles (dried in a 120 °C oven overnight) with two silanes. The vinyl and epoxy silanes were premixed prior to the addition. A 1 L, round bottom indented flask was charged with silica particles (100 g), and treating agent mix (equal weight for each) was added drop-wise into the flask while rotating. The mixture was allowed to roll on a rotovap overnight (16 h). 300 ml of 0.5 M sulfuric acid was then added. After 20 min of soaking, the silica was filtered. It was then washed with deionized (DI) water five times, filtered, and dried at 70 °C overnight. A small sample was taken and submitted for elemental analysis (LECO) for the percentage of carbon on silica.

2.3.2. Polymerization

A 500 ml, three-necked round bottom flask was equipped with an overhead mechanical stirrer with gas tight fitting, a nitrogen gas inlet and outlet, and heating mantle with thermocouple feedback. The initial bonded silica, monomers and DI water were first charged into the flask. The system was bubbled with nitrogen for 20 min. The azo initiator ((azobis-(2-amidinopropane)hydrochloride), or V50) was then introduced. Nitrogen was bubbled for another 20 min before the flask was gradually heated to 60 °C. The mixture was kept at 60 °C for 2 h with overhead stirring, then cooled down to room temperature. The viscosity of the reaction mixture increased appreciably with time. The mixture was cooled down to room temperature and was then poured into 5% NaCl solution in a beaker. The flask was rinsed with DI water to completely remove the residual silica inside the flask. After the mixture was stirred

[2] For example, Fractogel® TSK.
[3] For example, LiChrospher® 4000 DMAE.