Materials Science and Engineering C 76 (2017) 130–138

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Spontaneous and efficient adsorption of lysozyme from aqueous solutions by naturally polyanion gel beads

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ARTICLE INFO

*Article history:*
Received 16 December 2016
Received in revised form 7 March 2017
Accepted 12 March 2017
Available online 14 March 2017

*Keywords:*
Alginate
Calcium
Electrostatic interaction
Lysozyme
Adsorption

ABSTRACT

In this study, polyanionic alginate gel beads crosslinked by $Ca^{2+}$ and glutaraldehyde have demonstrated a strong electrostatic interaction with specific proteins. Due to the naturally abundant carboxyl groups, the prepared alginate gel beads exhibited a relatively superior integrated adsorption performance toward lysozyme, including a superior adsorption capacity of 213 mg $g^{-1}$, fast adsorption equilibrium within 12 h, good selectivity, and good reversibility. Compared with other protein adsorbents, the as-prepared adsorptive beads have the advantages of excellent adsorption performance, easy to prepare, convenient, efficient, reliable and environment-friendly to apply, which can serve as a more sustainable material in protein separation and purification.

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## 1. Introduction

Highly purified proteins play critical roles in biotechnological and biopharmaceutical industry and are in great demand because of their wide range of applications involving immunotherapy, scientific research, foodstuffs, and cosmetics [1,2]. Adsorption has been used as the most effective method in the purification or recycling of proteins. For instance, various adsorptive nanofibrous membranes for efficient adsorption of proteins have been reported [3–5]. Bentonite tryptophan microcomposite [6], 2-mercapto-5-benzimidazolesulfonic acid modified $Fe_3O_4$/Au nanoparticles [7], cibacron blue F3GA incorporated poly(hydroxyethyl methacrylate) based magnetic beads [8], and graphene oxide [9,10] have been developed for efficient adsorption and purification of lysozyme. This research group has also developed poly(vinyl alcohol-*co*-ethylene) (PVA-*co*-PE) nanofibrous membranes with an immobilized metal chelate affinity complex for selective protein separation [11]. Currently, there are growing interests in the search for economically feasible, easily available, and environmentally friendly substances (e.g., biologically derived substances) appropriate for the efficient adsorption or purification of target species [12–14]. The search of a suitable natural product led to sodium alginate (SA), which possesses the supramolecular architecture needed for the preparation of polymeric beads, high affinity with charged species, high stability in organic solvents, biodegradability, and environment friendliness [15–17].

SA is a very important polysaccharide in nature, which is mainly composed of (1, 4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues, and can be arranged in the form of homopolymeric sequences (MMM or GGG) or alternating sequences (MGMG) along the polymeric [18–20]. SA has a unique property of crosslinking in the presence of multivalent cations, which is rather complex with GG sequences in the polymer chain to form the "egg-box" junctions [21–23]. Furthermore, with a carboxylate functional group per unit, SA is a negative polyelectrolyte making it a suitable agent to interact with positively charged groups of some compounds or materials. Therefore, increased attention has been focused on the use of SA as drug or protein carriers [24–27] and green adsorbents for removing dyes [28,29] or heavy metal ions [13,30,31] from wastewater. SA was utilized in interacting and adsorbing lysozyme, including with assistance of pectin [32], $Fe^{2+}$ ion [33], and nanocomplex structure [34]. However, divalent cations crosslinked alginate gels are destabilized when used in solutions containing high concentration of ions such as $Na^{+}$ or $Mg^{2+}$. In addition, the crosslinking with divalent cations would also sacrifice the carboxylate functional groups which are the preferable reaction site. To overcome such problems, some crosslinking agents such as glutaraldehyde (GA) [35–37] and epichlorohydrin [38,39] were used to provide the SA beads with good mechanical performance and stable chemical properties. Among these chemical crosslinkers, GA is the most widely used.

As mentioned above, the presence of negative carboxylate functions along alginate chains ensures its high affinity and binding capacity for charged species, which has been utilized as a naturally available low cost adsorbent for removing dyes or heavy metal ions from wastewater.

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http://dx.doi.org/10.1016/j.msec.2017.03.092
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However, few attempts have been done on alginate gel beads as an adsorbent material for proteins. Therefore, in this study, we aim to develop a simple approach to prepare naturally based protein adsorbents based on the interaction between proteins and alginate beads. The preparation and characterization of alginate gel beads and the application of these beads for the adsorption of lysozyme from aqueous solutions were investigated and discussed here.

## 2. Materials and methods

### 2.1. Materials

Sodium alginate (pharmaceutical grade, M/G = 6:4) was kindly donated by Qingdao Hyzlin Biology Development Co., Ltd. (China). Calcium chloride ($CaCl_2$) was of analytical grade and purchased from Spectrum Chemical Co., USA. Glutaraldehyde solution (25%) was purchased from Fisher Bioreagents Co., USA. Lysozyme powder purified from chicken egg white was obtained from Amresco Co., USA. Bovine serum albumin (BSA) was purchased from Fisher Scientific Co., USA. All other chemicals were purchased from Fisher Scientific (Fisher Scientific Co., USA). All reagents were used as received without any further purification. All water used in this study was deionized water.

### 2.2. Preparation of alginate gel beads

Firstly, sodium alginate was dissolved in distilled water to give a final concentration of 2% (w $v^{-1}$). Then, the alginate solution was dropped through a peristaltic pump into 100 mL coagulation solution containing $CaCl_2$, GA and HCl under continuous stirring for 4 h (Fig. 1a). The diameter of needle used in this study is 0.8 mm and the flow rate of alginate solution is 100 mL $h^{-1}$. The GA and HCl concentration was controlled as 3 wt% and 0.1 wt%, respectively, and the concentrations of $CaCl_2$ were 0.005, 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mol $L^{-1}$, respectively (Table 1). The crosslinked beads were removed by filtration and then washed at least six times with 100 mL of distilled water to remove any free $Ca^{2+}$ and GA.

### 2.3. Characterization

The Fourier transform infrared (FTIR) spectra were collected by a Nicolet 6700 FTIR spectrometer (Thermo Electron Co., USA) at 4000–400 $cm^{-1}$ range with 64 scans and a 4 $cm^{-1}$ resolution. Thermogravimetric analysis (TGA) were conducted with a Shimadzu TGA-50 apparatus (Shimadzu Scientific Instruments Inc., USA) with a heating rate of 10 °C $min^{-1}$ from room temperature to 400 °C under a nitrogen atmosphere. Differential scanning calorimeter (DSC) analysis were carried out using a Shimadzu DSC-60 (Shimadzu Science Instruments, Inc., USA) at a heating rate of 10 °C $min^{-1}$ from room temperature to 260 °C under a nitrogen atmosphere. The water content was determined by weighing an amount of wet beads, then putting in an oven, and considering the residual (dry) mass after a stabilization period of 24 h at 105 °C. The diameter of alginate beads was measured with vernier calipers, and average diameter was obtained through the measurements of 20 beads out of approximately 200. Morphologies of dried alginate beads were observed by using a scanning electron microscopy (SEM) (SU8010, Hitachi, Japan) at 5 kV accelerating voltage on gold sputter coated samples. The swelling degree was calculated by using the following equation:

$$\text{Swelling degree} = (w_e - w_d)/w_d,$$

where $w_e$ and $w_d$ are the weights of the wet and dry beads, respectively.

The weight of dried beads was weighed to 0.0001 by a high precision balance (VP214CN, Ohaus Co., USA). The calcium content was determined by inductively coupled plasma optical emission spectrometer (Optima 8300, Perkin-Elmer Co., USA). The pH values of the lysozyme solution were measured by using a pH meter (XL600, Fisher Scientific Co., USA). The UV–vis spectra were recorded by a spectrophotometer

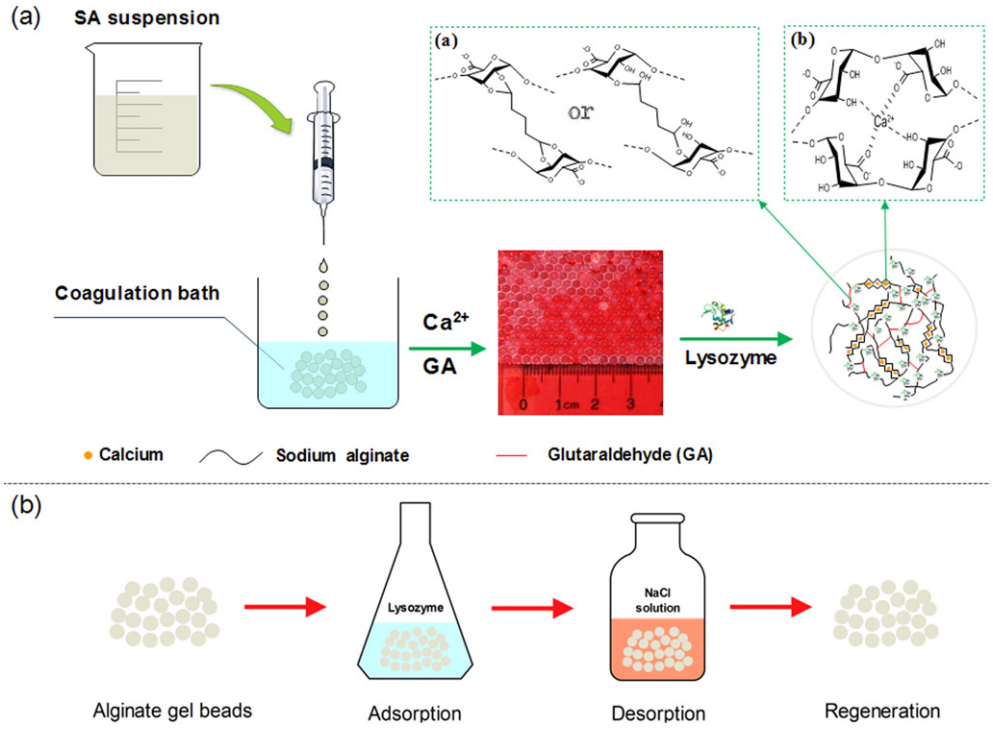


**Fig. 1.** (a) Schematic illustration for the preparation of alginate gel beads and the lysozyme adsorption process; (a) the crosslinking reactions between alginate and GA; (b) calcium ions crosslink the "egg–box" junctions on the guluronate residues in the alginate polymer; (b) Schematic illustration of the adsorption and regeneration procedures of the as-prepared alginate gel beads.

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