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IgG purification by bentonite–acrylamide–histidine microcomposite

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ABSTRACT

In this work, a new microcomposite composed of bentonite, acrylamide and histidine, as a pseudospecific ligand, was synthesized by bulk polymerization. The aim of this study was to improve IgG adsorption capacity of bentonite by incorporating histidine. The surface areas of the bentonite and bentonite–acrylamide–histidine microcomposites were 33.4 and 1.42 m^2/g, respectively. The amount of histidine was found to be 50 μmol/g bentonite via elemental analysis. Adsorption capacity was at the value of 100 mg/g from aqueous solution while adsorption capacity was 108 mg/g from human plasma with a purity of 90%. IgG biomolecules were able to be adsorbed and desorbed five times by using the same microcomposites without significant loss in their adsorption capacity.

1. Introduction

Monoclonal and polyclonal antibodies are important molecules in biotechnology and biomedical research. Human IgG, which is purified from human plasma, is used in therapeutical applications; for example, human IgG is used in managing genetic deficiencies like rheumatoid arthritis and lupus [1,2]. Many types of non-affinity chromatography antibody purification methods exist: size exclusion chromatography, ion-exchange chromatography, hydroxyapatite chromatography and hydrophylic interactions. Protein A, Protein G, Protein L, immobilized metal affinity chromatography and histidine affinity chromatography are types of affinity chromatography [3]. Among these methods the most useful one is Protein A chromatography. Despite its high selectivity, Protein A adsorbents have some drawbacks: (1) leakage of Protein A from the support, which causes contamination, (2) high cost of Protein A, (3) required tertiary structure for maintaining biological activity because of their protein nature and (4) inability to tolerate the harsh elution conditions [4–6]. Histidine as a pseudospecific ligand increases resistance to biochemical actions and has higher biochemical stability. Because of its functional properties (carboxyl, amino and imidazole groups) it can interact with proteins and be eluted in mild elution conditions by preserving catalytic activation of the antibodies. Histidine is cheaper than biospecific ligands, such as proteins and enzymes. Therefore, histidine as a pseudospecific ligand for IgG purification provides effective, economic and high adsorption capacity for antibody purification from a variety of biological fluids [7–9].

The interaction between IgG and histidine occurs because of hydrogen bonding, electrostatic and hydrophobic interactions; it also occurs because of the functional properties of histidine [5,10]. L-Histidine was loaded on various affinity carriers, such as poly(GMA-EDMA) [11], pHEMA monoliths [12], pHEMA beads [13], poly(ethylene vinyl alcohol [14], sepharose [15] and bentonite [7,16] from which montmorillonite clay, the most common mineral in the bentonite, is derived. Montmorillonite clay has an alumina octahedral layer between two silica tetrahedral layers that resemble a sandwich [17]. The aluminum in the structure is substituted by cations such as Mg^{2+} and Fe^{2+}. Therefore, a negative charge occurs on the surface. To balance the negative charge some cations such as Na^+, K^+, and Ca^{2+} are present [18]. Because of the presence of these properties, bentonite can be used as a cation exchange resin; this enables simple reactions between bentonite and polymers to produce composite materials. When low cost, high surface area and high hydrophylicity of the bentonite were associated with functional polymeric matrices, the results showed the high adsorptivity and mechanical properties [19–21].

Composite materials were synthesized by using bentonite and poly(sodium acrylate) [22], poly(vinyl chloride) [23], polypropylene [24] and acrylic acid in presence of cross linker [25], the first-time synthesized microcomposite adsorbents by bentonite–acrlamide–bisacrylamide–histidine, were prepared by bulk polymerization in this study.

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In the present work, sodium bentonite was mixed with acrylamide and bis-acrylamide as a cross-linker in the presence of initiator and histidine as a pseudospecific ligand. H-IgG adsorption studies on bentonite–acrylamide–histidinehistidine composites from aqueous solution containing different amounts of IgG at different pHs, temperature, ionic strengts and adsorption from human plasma were performed. Desorption of IgG and regeneration of these microcomposites was also tested.

2. Experimental

2.1. Materials

Na–bentonite in 98% purity (with a univalent cation exchange capacity [CEC] of 0.8 mol kg^{-1}), human immunoglobulin G (IgG, lyophilized), L-histidine, acrylamide, *N*,*N*′-methylenebisacrylamid, AIBN were supplied by Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade; these chemicals were purchased from available commercial sources and used as obtained. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D 3804 NANOpure organic/colloid removal and ion exchange packed-bed system.

2.2. Preparation of bentonite–acrylamide–bisacrylamide–histine (BABH) microcomposites

To prepare the BABH adsorbents, 1 g of bentonite [Na–montmorillonite (bentonite) in 98% purity (with a univalent CEC of 0.8 mol kg^{-1})] and 1 g L-histidine in 20 mL of water was stirred for 20 min. For obtaining a homogeneous suspension, 0.2 g/mL of acrylamide solution to provide a mass ratio 2:1 was added into the suspension; additional stirring was performed for 5 min at 60 °C. 0.2 g of *N*,*N*′-methylenebisacrylamid and 50 mg ammoniumpersulphate dissolved in 10 mL distilled water was poured onto the suspension. Finally, 50 μL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was added to propagate the polymerization. A washing procedure was applied after polymerization with distilled water to remove any possible unreacted L-histidine from the BABH composites. Then, BABH composites were dried with a vacuum oven at 60 °C. Finally, BABH composites were screened by using Retsch Standard sieves (ModelAS 200, Retsch Gmbh & Co., KG, Haan, Germany). In this study, BABH composites with a size range of 10–1000 μm were used as a solid matrix for h-IgG adsorption. The leakage of the L-histidine from the BABH composites was followed by the incubation of the fully wetted adsorbents with 10 ml of phosphate buffered (pH 7.4) solution for 24 h at room temperature (25 °C). The leakages experiments were carried out a stirring rate of 50 rpm. L-Histidine released after this incubation was measured in the liquid phase spectrophotometrically. When not in use, the resulting sorbents were kept in 0.02% NaN_3 (+4 °C) solution for preventing microbial contamination.

2.3. Characterization of BABH composites

The degree of L-histidine immobilization in the synthetized BABH composites was determined by measuring the C, H, N, O contents with a Leco Elemental Analyzer (Model CHNSO-932) Monosorb analyzer.

X-ray diffraction (XRD) patterns were recorded by Rigaku 2000 (USA) automated diffractometer using Ni-filtered Cu Kα radiation. The crystallite size was obtained from Scherrer's formula [26,27].

Surface area measurements were performed by BET analysis by using N_2 sorption system (Quantochrome Instruments); scanning electron microscopy (SEM; jeol/jsm-6335F) analysis was used for predicted modification of the produced composites.

2.4. Effect of initial concentration and pH on IgG adsorption

Adsorption of immunoglobulin G (IgG) on the BABH composites were incubated with 10 ml of IgG solution at 25 °C for 2 h (i.e., equilibrium time), in flasks agitated magnetically at 100 rpm. To observe the effects of the initial concentration of IgG on adsorption, the initial concentration of IgG was changed between 0.3 and 2.0 mg/ml. At the end of the pre-determined equilibrium period, the BABH composites were separated from the solution by filtration. IgG concentration was determined by measuring the absorbance at 280 nm, with a molar absorptivity of 14.0 for a 1% (w/v) solution of IgG. For obtaining adsorption isotherm, adsorption capacity was determined as follows:

$$Q = (Co - C) \times \frac{V}{m} \quad (1)$$

Here, *Q* is the amount of adsorbed IgG per gram of BABH microcomposites (mg/g), Co and C initial concentration (before the adsorption process) and final concentration (after adsorption process) of IgG (mg/ml), respectively. *V* is volume of the medium (ml); *m* is the mass of sorbent used in experiment (g).

To study pH on coupling of IgG to BABH composites, the pH of the solution was varied between 4.0 and 8.0 by using different buffer systems (0.1 M CH_3COONa—CH_3COOH for pH 4.0–6.0, 0.1 M K_2HPO_4–KH_2PO_4 for pH 7.0–8.0). The effect of temperature on IgG adsorption was carried out in 0.1 M K_2HPO_4–KH_2PO_4 for pH 7.0 containing 1.0 mg/ml IgG at four different temperatures. The effect of ionic strength was investigated containing NaCl in the range of 0.03–1.0 M.

2.5. Desorption and repeated use studies

To determine the reusability of the BABH composites, adsorption and desorption cycle was performed five times by using the same composites. Desorption of IgG was studied in aqueous solution containing 1.0 M NaCl. The IgG-adsorbed BABH composites were placed in this desorption medium and stirred continuously (at a magnetic stirring rate of 100 rpm) for 1 h at room temperature. The IgG concentration within the desorption medium was determined by the previously described method. The desorption ratios of IgG were calculated from the amount of IgG adsorbed on the bead and the final IgG concentration in the desorption medium. Adsorption–desorption cycle was performed five times by using the same composites.

When desorption was achieved, the BABH composites were finally cleaned with 50.0 mM NaOH solution to remove the remaining IgG and to regenerate them and then re-equilibrated with the starting buffer.

2.6. IgG adsorption from human plasma

Human blood was collected into EDTA-containing vacuum containers; red blood cells were separated from plasma by centrifugation at 4000 × *g* for 30 min at room temperature, then filtered (3 ml Sartorius filter) and frozen at 20 °C. Before use, the plasma was thawed for 1 h at 37 °C. Before application, the viscous sample was diluted with 25 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). Dilution ratios were 1/2, 1/5, 1/10 10 ml of the human plasma with a IgG content of 12.5 mg/ml stirred by BABH composites at a rate of 100 rpm for 2 h. The amount of IgG adsorbed on the BABH composites was determined by a solid phase-enzyme-linked immunosorbent assay method (ELISA). Human anti-IgG (Sigma, I-9384) diluted 1/1000 in 50 mM $NaHCO_3$, pH 9.6, was adsorbed to