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Effect of spacer-arm and Cu(II) ions on performance of L-histidine immobilized on poly(GMA/MMA) beads as an affinity ligand for separation and purification of IgG

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Abstract

In this study, the beads were prepared from glycidiyl methacrylate (GMA) and methyl methacrylate (MMA) via suspension polymerization and, the used beads fractions were between 75 and 150 μm. The epoxy groups of the beads were converted into amino groups by the reaction of ammonia or 1,6-diaminohexane as a spacer-arm. L-Histidine ligand was immobilized onto both beads. Cu(II) ions were chelated onto spacer-arm attached and L-histidine immobilized beads. The IgG adsorption capacity of the spacer-arm attached and Cu(II) chelated affinity beads led to higher adsorption capacities about 1.64- and 2.94-fold, respectively. The adsorption equilibrium studies showed that the adsorption isotherm of IgG obeyed the Langmuir isotherm model. The experimental data was well described by the second-order equations. Purification data of IgG with spacer-arm attached and Cu(II) ions chelated (i.e. poly(GMA/MMA)-SAH-Cu(II)) beads indicated that 87.5% of IgG was removed from human serum with a purity of 90%.

Keywords: Affinity beads; Histidine; IgG; Adsorption; Purification; Thermodynamic parameters

1. Introduction

Chromatography has been widely used as a powerful technology for the separation and purification of biological macromolecules in both analytical and preparative scales. As a new and alternative technology in affinity separation, the affinity chromatography has proven its efficiency and time stability [1–3]. One of the most important factors in affinity chromatography is the identification of suitable support materials. Synthetic polymers, such as glycidiyl methacrylate, methyl methacrylate, nylon, polystyrene and polysulfone are suitable from a mechanical point of view [4–6]. Porous hydrophilic copolymers beads based on glycidiyl methacrylate are almost ideal ones to perform easy immobilization of ligands. On the other hand, a ligand is coupled directly to the polymeric support, steric hindrance between the support surface and the target biomolecules will occur [7,8]. For this reason, the ligand molecules critical in the interaction with a biomolecule should be moved away from the support surface to some extent using a spacer-arm [9,10].

Most biospecific ligands in affinity separation of IgG, such as protein A or protein G, are expensive and unstable in chromatographic systems. To overcome this drawback, pseudo-specific ligands (such as textile dyes, chelated metals, amino acids and thiophilic ligands) are used for the purification of IgG from plasma [11–13]. Single amino acids have been reported to be effective ligands for the purification of variety of proteins [14–16]. L-Histidine incorporated membranes were demonstrated to be an alternative to protein based ligand for purification of IgG from human plasma [17].

Immobilized metal affinity chromatography is a powerful separation technique that uses covalently bound chelating compounds on solid chromatographic supports to immobilize metal ions, which serve as affinity ligands for various proteins, making use of coordinative binding of some amino acid residues (such as the imidazole group of histidine, thiol group of cysteine, indolyl group of tryptophan and hydroxyl group of tyrosine) exposed on the surface [18–20].

Human immunoglobulin G is an important serum protein produced worldwide on a large scale. The purification of human IgG is essential, for diagnostic and therapeutic applications. They

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also serve as bioaffinity ligands for purifying other high value proteins of pharmaceutical importance, such as cytokines and blood-clotting factors [21,22].

In this work, porous beads were prepared from glycidiyl methacrylate (GMA), and methyl methacrylate (MMA) in the presence of cross-linker ethyleneglycol dimethacrylate (EGDMA) via suspension polymerization. Three different affinity supports based on poly(GMA/MMA) beads were prepared and used for adsorption and separation of IgG from aqueous solution and human serum, respectively. The effect of spacer-arm and chelated Cu(II) ions on the performance of L-histidine ligand was tested in separation of IgG from solutions. The effects of solid/liquid ratio, pH, ionic strength, initial concentration of IgG, equilibrium time and temperature were investigated in a batch system. Finally, the adsorption isotherm and kinetics of IgG on the affinity beads with different functionalities were studied to characterize of the surface complexation reaction.

2. Materials and methods

2.1. Materials

Human immunoglobulin G (IgG), 1,6-diaminohexane, polyvinyl alcohol (PVA), toluene, acetonitril (AcN) and trifluoroacetic acid (TFA) were supplied from Sigma Chemical Co. (St Louis, MO, USA) and used as received. Methyl methacrylate (MMA), glycidyl methacrylate (methacrylic acid 2,3-epoxypropyl isopropyl ether; GMA) and ethyleneglycol dimethacrylate (EGDMA) and α-α′-azoisobisbutyronitrile (AIBN) were obtained from Fluka AG (Switzerland), and the monomers distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C until use. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). The water used in the present work was purified using a Barnstead (Dubuque, IA, USA) ROpure LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure organic/colloid removal and ion exchange packed-bed system.

2.2. Preparation and modification of poly(GMA/MMA) beads

Porous beads were prepared from glycidiyl methacrylate and methylmethacrylate via suspension polymerization. Ethyleneglycol dimethacrylate was used as a cross-linker. The aqueous continuous phase was comprised of 0.1 M NaCl (400 ml). The organic phase contained GMA (7.5 ml), MMA (7.5 ml), EGDMA (5.0 ml) and 5.0% polyvinyl alcohol (20 ml, as stabilizer) were mixed together with 0.2 g of AIBN in 20 ml of toluene. The polymerization reactor was placed in a water bath and heated to 65 °C. The reactor was then equipped with a mechanical stirrer, nitrogen inlet and reflux condenser. The polymerization mixture was placed into a dropping funnel and was introduced drop wise into the reactor in about 10 min during stirring at 250 rpm under a nitrogen atmosphere. The polymerization reaction was maintained at 70 °C for 2.0 h and then at 80 °C for 1.0 h. After the reaction, the beads were filtered under suction and washed with distilled water and ethanol. The product was dried in a under vacuum oven. The beads were sieved and 75–150 μm size of fraction was used in further reactions.

The epoxy groups-carrying poly(GMA/MMA) beads was aminated with 0.5 M ammonia or 1,6-diaminohexane solution (i.e. spacer-arm) at pH 10.0 and at 65 °C in a reactor containing 10 g beads and was stirred magnetically for 5 h. After the reaction, the aminated and/or spacer-arm attached poly(GMA/MMA) beads were washed with distilled water.

The aminated (poly(GMA/MMA)-A) and/or spacer-arm attached (poly(GMA/MMA)-SA) beads (10 g) were equilibrated in phosphate buffer (20 ml, 50 mM, pH 7.0) for 6 h, and transferred to the same fresh medium containing glutaric dialdehyde (20 ml, 0.5% (v/v)). The activation reaction was carried out at 25 °C for 12 h, while continuously stirring the medium. After the reaction period, the excess glutaric dialdehyde was removed by washing sequentially the beads with distilled water, acetic acid solution (0.1 M, 100 ml) and phosphate buffer (0.1 M, pH 7.0). The resulting both beads were used for the immobilization of L-histidine ligand.

The activated (poly(GMA/MMA)-A) and poly(GMA/MMA)-SA beads were swollen in phosphate buffer (0.1 M, pH 8.0) for 4 h, was then transferred to the L-histidine solution (2.0 mg/ml L-histidine; in 25 ml same phosphate buffer solution). The immobilization of L-histidine was carried out at 22 °C in a shaking water bath for 6 h. Physically bound histidine was removed first by washing the supports with saline solution (40 ml, 1.0 M) and then phosphate buffer (0.1 M, pH 7.0) and was stored at 4 °C in same fresh buffer until use.

The amount of immobilized histidine on the poly(GMA/MMA)-AH and poly(GMA/MMA)-SAH beads was determined by measuring the initial and final concentration of amino acid within the immobilization medium, at 230 nm by using a double beam UV/vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601). A calibration curve constructed with L-histidine solution of known concentration (0.02–0.50 mg/ml) was used in the calculation of amino acid in the solutions. L-Histidine leakage from the beads was investigated in phosphate buffer (pH 8.0, 0.1 M) for 48 h and L-histidine leakage was determined as described above.

In order to prepare poly(GMA/MMA)-SAH-Cu(II) complexed beads, a 50–300 ppm solution of the Cu(II) ions was prepared from sulphate salts in distilled water at constant pH 4.1 (adjusted 0.1 M HCl). The poly(GMA/MMA)-SAH beads were placed in the metal ion solution while stirring 100 rpm at 25 °C for 1.0 h. After this period, the poly(GMA/MMA)-SAH-Cu(II) beads were washed several times with phosphate buffer (50 mM, pH 7.0).

The concentration of the Cu(II) ions in the resulting solution was determined with a flame atomic absorption spectrophotometer [(AAS), Shimadzu AA 6800, Japan]. The Cu(II) ions desorption experiments were performed in a buffer solution containing 25 mM EDTA at pH 4.9. The Cu(II) ions chelated poly(GMA/MMA)-SAH beads were placed in a medium for 60 min and, the final Cu(II) ion concentration in the medium was determined by AAS. Cu(II) leakage from the poly(GMA/MMA)-SAH-Cu(II) beads was investigated with