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Adsorption of serum albumin and γ-globulin from single and binary mixture and characterization of pHEMA-based affinity membrane surface by contact angle measurements

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Abstract

In this study, an affinity membrane was synthesized using 2-hydroxyethylmethacrylate (HEMA) via UV-initiated photopolymerization. A dye-ligand (i.e., Procion Red HE-3B; Red-120) was covalently immobilized onto membrane. Human serum albumin (HSA) and human γ-globulin (HIgG) adsorption onto pHEMA-Red-120 membrane were studied using bare poly(hydroxyethylmethacrylate) (pHEMA) membrane as a control system. The information about surface energy, hydrophobicity and chemical heterogeneity of the affinity membrane was obtained by contact angle measurements. The contact angle values of the affinity membrane were determined by sessile drop method using water, glycerol and diiodomethane as test liquids. Component and parameters of the surface free energy of all the investigated samples were calculated from measured contact angle values using the acid–base method of the van Oss. The adsorption of HSA and HIgG significantly changed both the contact angles and component of surface free energies of the affinity membrane. The reversible HSA and HIgG adsorption on the pHEMA-Red-120 followed the Freundlich and Langmuir–Freundlich isotherm models. Selectivity of the affinity membrane was tested at different pH values to HSA and HIgG and the protein concentration of in the binary system was determined by HPLC. The affinity membrane was stable when subjected to sanitization with sodium hydroxide after repeated adsorption–elution cycles.

Keywords: Microporous membrane; Affinity chromatography; Dye-ligand; Adsorption; Separation; Albumin; γ-Globulins; Contact angle

1. Introduction

The recently introduced affinity membrane chromatography offers some apparent advantages over conventional bead-packed column chromatography in bio-separation such as higher flow rate, low-pressure drops, faster binding kinetics, and simple scale-up [1,2]. Affinity separation relies on the highly specific binding between a target protein in solution and an immobilized ligand to achieve a high degree of protein purification. Different types of affinity ligand molecules have been introduced on the membrane surfaces for protein purification such as protein A [3,4], triazidine dyes [5–7], immobilized metal [8–10] and amino acids [12–14]. The dye-ligand affinity separation has become a popular technique for purifying proteins. For example, Arıca et al. [11] reported the purification of hydroxypyruvate reductase from bacterial homogenised solution using the poly(HEMA-EGDMA) microspheres with Cibacron Blue F3GA as a dye-ligand. Suen et al. [15] reported the effective adsorption of lysozyme from aqueous solution using Cibacron Blue F3GA immobilised cellulose membrane discs. Reactive Red-120 and Reactive Yellow-86 have been used as dye-ligands for the purification of high-Mr glutenin subunits from wheat flour [16]. This affinity technique makes use of immobilised dye molecules that interact with the protein sites by electrostatic and hydrophobic force. The three-dimensional surface properties of the protein and the ligand-polymer structure, the surface ligand density, the type and concentration of salt, pH of the medium and temperature influence the dye-ligand affinity separation of proteins. [17,18].

The performance of an affinity membrane depends on its surface properties. The surface properties of an affinity membrane may affect the adsorption capacity and its behaviour by

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controlling adsorption of proteins present in the fluids [3,6,7]. In particular, protein adsorption depends on the surface composition and morphology of the affinity membrane [8–13]. There are reports that the amount of adsorbed proteins on a chromatographic matrix surface are quantitatively changed, depending on the type of ligand molecules used. Selective adsorption of a protein on a chromatographic material is a very complex process, which can be determined by several factors. Among these, the hydrophobic and polar character, the chemical structure of immobilised ligand and the electrostatic interactions of the protein molecules with surface and with each other are the most important. These interactions between adsorbents surfaces and proteins are usually long-range and due to the large size and the shape of the proteins, the interaction between the adsorbed protein on the surface are non-coincidental and can be strongly influentiated by the fact that the proteins may undergo conformational changes upon adsorption [19,20]. Therefore, the surface properties of an affinity matrix should be characterized to explain its interactions with proteins. The hydrophobic and polar character can be determined by measuring contact angles of different test liquids on membranes.

In the present work, the surface free energy parameters were calculated from the contact angles of sessile drops of apolar and polar liquids on membrane samples using the acid–base method of van Oss [21]. The adsorption conditions (i.e., concentration of human serum albumin (HSA) and human immunoglobulin G (HIgG), medium pH and ionic strength) were varied to evaluate their effects on the performances of the affinity membranes. Selectivity of the affinity membrane to different proteins (i.e., HSA and HIgG) from binary mixtures was studied under different pH conditions. The adsorption isotherms were measured in order to evaluate the discrepancy between the experimental data and the theoretical equilibrium capacity predicted from the kinetic equations.

2. Experimental

2.1. Materials

Human serum albumin and human immunoglobulin G were supplied from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. 2-Hydroxyethylmethacrylate (HEMA) was obtained from Fluka AG (Switzerland), distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C until use. α-α′-Azoisobutyronitrile (AIBN), Procion Red HE-3B (Reactive Red-120), glycerol and diiodomethane (DIM) were obtained from Sigma Chemical Co. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). The water used in the following experiments 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. Synthesis of poly(hydroxyethylmethacrylate) (pHEMA) membrane

The membrane synthesis was achieved by mixing a phosphate buffer solution (0.1 M, pH 7.0, 3.0 ml) with 2-HEMA monomer (2.0 ml) containing 20 mg AIBN. Following the nitrogen flash, the solution was poured into a round glass mould (diameter: 9.0 cm), sealed and exposed to UV for 1.0 h at ambient temperature. The nitrogen atmosphere was maintained during the UV irradiation. After polymerisation period, the resultant product was washed with distilled water. The pHEMA membrane was cut into circular pieces (diameter: 0.75 cm) with a perforator and left in the wet state at 4 °C.

2.3. Dye-ligand immobilisation onto pHEMA membrane

Red-120 was covalently immobilised onto pHEMA membrane via the nucleophilic substitution reaction between the chloride of its triazine ring and hydroxyl groups of the pHEMA under alkaline conditions. Red-120 (300 mg) was dissolved in distilled water (10 ml), and transferred to NaCl solution (1.0 M, 60 ml) in which pHEMA membrane disks (6 g) were equilibrated for 1 h. After this period, sodium carbonate solution (3.2 M, 30 ml) was added to the medium (about pH 10) and heated at 80 °C for 4 h in a sealed reactor. After the reaction period, the solution cooled down to the room temperature and pHEMA membrane disks were washed several times with distilled water, 2.0 M NaCl and 10% methanol. pHEMA-Red-120 membrane was stored at 4 °C until use.

2.4. Adsorption experiments

Adsorption of HSA and HIgG from single and binary solutions on the pHEMA-Red-120 membrane were studied at various pHs', in acetate (7.5 ml, 50 mM, pH 4.0–5.5), in phosphate (7.5 ml, 50 mM, pH 6.0–7.0), in Tris–HCl (50 mM, pH 7.5–8.0) or carbonate buffer (7.5 ml, 50 mM, pH 8.5–9.0). Adsorption experiments were conducted in stirrer cells at 25 °C, for 2 h while continuous stirring. The effect of temperature and ionic strength on both HSA and HIgG adsorption were carried out in acetate buffer (50 mM, pH 5.0) and phosphate buffer (50 mM, pH 6.5) for pHEMA-Red-120 membrane at four different temperatures (i.e., 4, 15, 25 and 37 °C) and at three different KCl concentrations (i.e., 0.1, 0.25 and 0.5 M), respectively. All experiments were conducted in triplicates with 0.26 ml affinity membrane and initial concentration of HSA and/or HIgG was 1.0 mg ml^{-1} in each set experiments.

In order to determine the adsorption capacities of membranes, the concentration of HSA and HIgG in the medium were varied between 0.5 and 3.0 mg ml^{-1}. For single systems,