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Preparation of Novel Lectin Affinity poly(2-Hydroxyethyl Methacrylate-Ethylene Dimethacrylate) Hydrogel Membrane Systems for Investigation of Antibody Recognition

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The aim of the study: Immunoglobulin G (IgG) is the most important antibody species in the immune system and constitutes 75% of the immunoglobulins in human serum. The level of IgG in blood plasma is an indispensable marker for the detection of infection, cancer and other autoimmune diseases. Therapeutic, immunostaining and immunochromatographic applications require high purity IgG. For these reasons, the development of new generation systems is becoming important for the identification and purification of IgG. The aim of the study is recognizing IgG antibody with efficient, high amounted, fast, easily, with less toxicity, economically and purifying IgG in high ratios from its natural sources.

Material and Methods: In this study, poly(2-hydroxyethyl methacrylate-ethylene dimethacrylate) [p(HEMA-EDMA)] hydrogel membranes are synthesized with free radical photo-polymerization method. Then p(HEMA-EDMA) hydrogel membranes were activated with silanization agent (IMEO) and then attached to Con A as a lectin affinity ligand. p(HEMA-EDMA) hydrogel membranes are characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), swelling test and elemental analysis. After the characterization studies,IgG adsorption studies were carried out and calcuculated quantities of adsorption (Q value). In order to determine IgG adsorption condition on the p(HEMA-EDMA)-IMEO-ConA hydrogel membranes, adsorption time, pH, initial adsorption concentration, temperature, ionic strength experiments were performed. Also, reusability of p(HEMA-EDMA)-IMEO-ConA hydrogel membranes were investigated.

Results: In the characterization studies, hydrogel membranes are spherical structures according to the SEM analysis. Also, elemental analysis of p(HEMA-EDMA)-IMEO hydrogel membranes is found to be 10.85 mol/g hydrogel membranes from the nitrogen stoichiometry. And the amount of the Con A attached to p(HEMA-EDMA)-IMEO hydrogel membranes is found as 3.52 mg/g membrane. Highest swelling value is determined as 224.8%. In the adsorption studies, optimum conditions for IgG adsorption to membranes are; 1.5 mg/ml initial IgG concentration, 30 minutes of adsorption time, pH 4 citrate buffer 37 °C and without any different ion strength. Optimum adsorption capacity is determined as 26.81 mg/g (Q value) and it is also determined that this value is 4 times higher than nonspecific IgG adsorption to p(HEMA-EDMA) hydrogel membranes. IgG adsorption-desorption cycles (5 times) proved that product is reusable without losing its adsorption capacity. Consequently, alternative polymeric membrane system with high biocompatibility, fast and easy to apply for IgG purification, has been produced.

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Keywords: Lectin affinity chromatography, hydrogel membrane, silanization, Con A, IgG adsorption