NOVEL BIOTECHNOLOGICAL APPROACH FOR RECOGNITION AND PURIFICATION OF ANTIBODY: LECTIN AFFINITY MEMBRANES

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1. Introduction

Immunoglobulin G is a glycoprotein structured molecule that is produced by the immune system and protects organism from harmful effects of antigens. Ig G amount in the blood plasma is an appropriate indicator of; infection, cancer, diabetes, cardiovascular diseases, Alzheimer and other autoimmune diseases. Besides, purification of Ig G used in the treatment of these diseases from naturel sources is carried out at high costs on the World market. It is hard to obtain Ig G in high amounts and without any decomposes, that's why it is important to develop new systems that will help to recognize and purify Ig G antibody.

2. Method

In this project, my purpose was; recognizing Ig G antibody with efficient, high amounted, fast, easily, with less toxicity, economically and purifying Ig G in high ratios from its natural sources. For this purpose p(HEMA-EDMA) membranes are synthesized with free radical photo polymerization method and characterized according to SEM images, swelling behaviors FTIR analysis and elemental analysis. In order to adsorb Ig G to polymeric membranes; polymeric membranes are activated with silanization agent (IMEO) and derivatized with Con A which is a lectin affinity ligand. Optimization of Ig G adsorption conditions to the p(HEMA-EDMA)-IMEO-Con A polymeric membranes, the effects of time, pH, temperature, ionic strength and the initial Ig G concentration on adsorption were examined. In order to investigate the reusability of Ig G adsorption to p(HEMA-EDMA)-IMEO-Con A polymeric membranes, Ig G adsorbed polymeric membranes were desorbed with desorption agent. Finally I performed the electrophoresis experiment for demonsration.

Figure 1. Synthesis of p(HEMA-EDMA) polymeric membranes

3. Results

In the SEM results it is examined that membranes are in spherical structures. Highest swelling value is determined as 224.8%.Binding of IMEO was demonstrated with FTIR and Elemental Analysis.

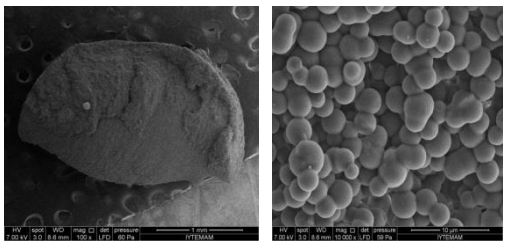

Figure 2. SEM Images of p(HEMA-EDMA)-IMEO-Con A polymeric membranes

Optimum conditions for Ig G adsorption to membranes are; 1.5 mg/ml initial Ig G concentration, 30 minutes of adsorption time, pH 4 citrate buffer 37°C and without any different ion strength. Optimum adsorption capacity is determined as 253.8 mg/cm^2 and it is also determined that this value is 7 times higher than nonspecific Ig G adsorption to p(HEMA-EDMA) membranes. Ig G adsorption-desorption cycles (5 times) proved that product is reusable without losing its adsorption capacity. According to the electrophoresis, Ig G could be desorbed in pure form without any denaturation to its structure.

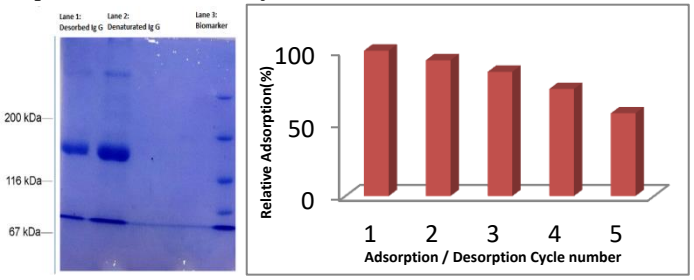

Figure 3. Electrophoresis Experiment and Reusability of the Polymeric Membrane System

4. Conclusion

The system that I have developed for Ig G recognition which makes it efficiently, fast, easily and purifying Ig G in high ratios from It's natural sources. With the regeneration capability of polymeric membranes, the product can be used over and over again, also it reduces cost which is an important factor in recognizing and purification process so that the widespread use of these systems will be quite favorable.

5. References

1. Lis, H., Sharon, N., 1998, Lectins: Carbohydrate-Specific Proteins That Mediate Cellular Recognition, Chem. Rev., 1998, 98 (2), pp 637–674.
2. Öztürk N., Akgöl S.,Denizli A., Bereli N., 2008, High capacity binding of antibodies by poly (hydroxyethylmethacrylate) nanoparticles, Volume 67, Issue 1, Pages 14–19.