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L-Lysine Imprinted Nanoparticles for Antibody Biorecognition

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The aim of this study was to prepare L-lysine-imprinted poly(HEMA-MAAsp) nanoparticles which can be used for the adsorption of IgG from aqueous solutions. L-lysine was complexed with MAAsp and L-lysine-imprinted poly(HEMA-MAAsp) nanoparticles were synthesized by miniemulsion polymerization. Also, non-imprinted nanoparticles were synthesized without L-lysine for control purpose. L-lysine-imprinted poly(HEMA-MAAsp) nanoparticles were characterized by means of elemental analysis, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM).

Keywords: Molecular imprinting, Nanoparticles, Poly(HEMA), Aspartic acid.

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1. INTRODUCTION

Advances in nanobiotechnology have resulted in the several novel colloidal carriers such as nanocomposite, nanorobots, nanocrystals, nanoparticles, killing effect during the tumor growth phase, and to protect the surrounding healthy cells from unwanted exposure to the excess cytotoxic agent. Polymeric nanoparticles are the most attractive colloidal carriers owing several merits such as the ease of purification and sterilization, drug targeting possibility, and sustained release action[1].

Molecularly imprinting is a novel technique to create recognition sites for target molecule. Molecularly imprinted polymers show high selectivity for a target molecule[2]. Functional monomers form a complex with the template via covalent or noncovalent interactions and then in the presence of cross linking functional monomers are polymerized. After polymerization, the template is removed, and specific cavities that complementary to the template in size, shape, and position of the functional groups in the polymeric matrix are occured. Molecularly imprinted polymers (MIP) are easy to prepare, stable, inexpensive and capable of molecular recognition. Molecular recognition-based separation techniques have received much attention in various fields because of their high selectivity for target molecules. One of the many attractive features of the molecular imprinting method is that it can be applied to a diverse range of analytes [3]. The imprinting of small, organic molecules (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars) is succesfully reported. Somewhat larger organic compounds (e.g., peptides) can also be imprinted via similar approaches, whereas the imprinting of much larger structures is still a challenge. If a short peptide or amino acid residue (lysine) representing only a small exposed fragment of a protein structure is used as a template, then the resultant macroporous MIP recognizing the imprinted peptide or amino acid will also be able to recognize the protein molecule.

2. EXPERIMENTAL

2.1 Materials

Template molecule L-lysine immunoglobulin G (IgG), albumin (human serum), hemoglobulin, poly(vinyl alcohol) (PVA), sodium dodecyl sulfate (SDS), ammonium persulfate, sodium bicarbonate and sodium bisulfite were obtained from Sigma Chemical Co. (St. Louis, USA). Ethylene glycol dimethacrylate was purchased from Fluka A.G. (Buchs, Switzerland). All other chemicals were of reagent grade and purchased from Merck A.G. (Darmstadt, Germany).

2.2 Synthesis of N-methacryloyl-l-aspartic acid (MAAsp monomer)

N-methacryloyl-L-aspartic acid (MAAsp) was chosen as a pseudospecific ligand and synthesized by using methacryloyl chloride and L-aspartic acid [4].

2.3 Preparation of L-lysine imprinted poly-(HEMA-MAAsp) nanoparticles

L-lysine imprinted poly(HEMA-MAAsp) nanoparticles were prepared by two-phase mini-emulsion polymerization method. The first aqueous phase was prepared by dissolving of PVA (200 mg), SDS (30 mg) and sodium bicarbonate (25 mg) in 10 mL deionized water. The second phase was prepared by dissolving of PVA (100 mg) and SDS (100 mg) in 200 mL of deionized water. Functional monomer [MAAsp, 25 mg was dissolved in monomer (ethylene glycol dimethacrylate, 2.1 mL) to form oil phase. The oil phase was slowly added to the first aqueous phase. In order to obtain mini-emulsion, the mixture was homogenized at 25 000 rpm by a homogenizer (T10, Ika Labortechnik, Germany). After homogenization, the template molecule [L-lysine, 22.7 mg was added to mini-emulsion to establish the ratio between monomer and template as 1:1 in mole basis. Then, the mixture was slowly added to the second aqueous phase while the phase has been stirring in a sealed-cylindrical polymerization reactor (250 mL). The reactor was magnetically stirred at 300 rpm (Radleys Carousel 6, Essex, UK). The

polymerization mixture was slowly heated to 40°C, polymerization temperature. After that, nitrogen gas was bubbled through solution for 5 min to remove dissolved oxygen. Then, initiators, sodium bisulfite (125 mg) and ammonium persulfate (125 mg), were added into the solution. Polymerization was continued for 24 h. The obtained L-lysine imprinted nanoparticles were washed with water and water/ethyl alcohol mixtures, in order to remove unreacted monomers, surfactant and initiator. The solutions were centrifuged at 30 000 rpm for 30 min (Allegra-64R Beckman Coulter, USA) for each washing step and then the nanoparticles were dispersed in fresh solution. After last washing step, the nanoparticles were dispersed in deionized water containing 0.5% sodium azide to prevent contamination and stored at 4°C. The non-imprinted nanoparticles were synthesized by applying same procedure except addition of template molecules, L-lysine.

2.4 Characterization of L-lysine imprinted poly-(HEMA-MAAsp) nanoparticles

Nanoparticles were characterized by Zetasizer (NanoS, Malvern Instruments, London, UK), FTIR and TEM (FEI, Tecnai G2 F30, Oregon, USA). In zeta-size measurement, the light scattering was carried out at incidence angle 90° and 25°C. For TEM analysis, imprinted nanoparticle sample was dropped onto carbon coated copper grid and then dried at room temperature. TEM photographs were taken at 200 kV by TEM microscope.

2.5 Adsorption of IgG on poly (HEMA-MAAsp) nanoparticles

Adsorption of IgG on the oly (HEMA-MAAsp) nanoparticles from aqueous solutions was investigated batch-wise. The adsorption experiments were carried out at 25°C at stirring rate as 100 rpm for 2 h. Effects of pH of the medium, IgG concentration, temperature, salt type and ionic strength on the ad sorption capacity were studied.

3. RESULTS AND DISCUSSION

In this study, we present a simple polymerization method for preparing non-covalent molecularly imprinted polymer (MIP) with specific IgG recognition sites. We have employed the surface-imprinting technique that relies on electrostatic interactions between a functional monomer and the chosen template molecule L-lysine to produce MIP capable of selective recognition in aqueous media. For this purpose, first, we prepared N-methacryloyl-(L)-aspartic acid (MAAsp) as a functional monomer. Then, the L-lysine-imprinted, poly-(hydroxylethyl methacrylate-N-methacryloyl-(L)-aspartic acid methylester) [poly(HEMA-MAAsp)] nanoparticles were synthesized via mini-emulsion polymerization. Adsorption of IgG onto L-lysine-imprinted poly-(HEMA-MAAsp) nanoparticles were investigated in batch system under various medium conditions (i.e. pH, ionic strength, IgG concentration, temperature). The results show that the imprinted nanoparticle has high selectivity and sensitivity for IgG. IgG adsorption capacity and molecular recognition selectivity studies in a batch system versus other proteins such as human serum albumin (HSA) and hemoglobin (Hb) were investigated and characterized in detail. Characterization of nanoparticles was conducted using FTIR, TEM, zeta sizer and elemental analysis. The specific surface area of the L-lysine-imprinted particles was found to be 1872 m^2/g with a size range of 110 nm in diameter. Finally, the reusability of the L-lysine-imprinted poly(HEMA-MAAsp) were evaluated there is no significant loss in adsorption capacity after ten adsorption-desorption cycles.

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