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Cholesterol removal onto the different hydrophobic nanospheres: A comparison study

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ABSTRACT

The aim of this study is to prepare two different hydrophobic polymeric nanospheres for cholesterol removal. Poly(HEMA–MAT) nanospheres with an average size of 100 nm and poly(HEMA–MAP) nanospheres with an average size of 158 nm were produced by surfactant free emulsion polymerization of HEMA and MAT and MAP monomers. These hydrophobic nanospheres were characterized by FTIR, SEM, elemental analysis, particle size and surface area measurements. Cholesterol removal experiments were performed in a batch experimental set-up and removal medium was methanol. Cholesterol adsorption capacity of poly(HEMA–MAP) nanospheres was approximately three times higher than that of poly(HEMA–MAT) nanospheres.

1. Introduction

Nanotechnology that deals with nanometer sized objects is the seat of a broad variety of interdisciplinary activity with applications as diverse as optoelectronics, microfluidics, and medicine [1]. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications [2]. Many published works focused on the synthesis of micrometer-sized polymer matrix and its application in the separation of biological molecules with the aid of a specific ligand coating the surface of the particles [3]. Only limited work has been published on the application of nanosized particles in the separation of biomolecules. Because of their nanoscopic size, nanoparticles can produce larger specific surface area and, therefore, may result in high binding capacity [4]. The properties like adhesion, wettability, biocompatibility and binding affinity of the surface can be altered and tuned to the specific requirements by chemical or physical modification of the surfaces [5]. Modified materials are nowadays well-known and have been investigated intensively due to their potential applications in many areas, such as biology, medicine and environment. Therefore, it may be useful to synthesize nanosized particles and utilize them for the separation of biomolecules.

A wide variety of separation techniques are available today, however, different types of affinity chromatography have become dominant because of their high resolving power. In dye-affinity chromatography, ion-exchange chromatography, immobilized metal-ion affinity chromatography and hydrophobic interaction chromatography (HIC), separation is dependent on the biological and physico-chemical properties of molecules; net charge, biospecific characteristics and hydrophobicity, respectively [6–8]. HIC takes advantage of the hydrophobicity of biomolecules promoting its separation on the basis of hydrophobic interactions between immobilized hydrophobic ligands and nonpolar regions on the surface of the biomolecules [9]. In fact, HIC has been successfully used for separation purposes as it displays binding characteristics complementary to other chromatographic techniques. A lot of different type of hydrophobic molecules, which have side chains of nonpolar amino acids such as alanine, methionine, tryptophan, and phenylalanine on their surface can be used as a ligand in HIC [10].

Cholesterol, chosen as a model biomolecule, commonly found as the component in cell membrane. Within the cell membrane, cholesterol functions in intracellular transport, cell signaling and nerve conduction. Biologically it is an important precursor for bile acid, provitamin D3 and several steroidal hormones [11]. On the other hand, it is known that cholesterol is a major ingredient of the plaque that collects in the coronary arteries and causes coronary heart diseases. If too much cholesterol circulates in the blood it can build up in the lining of the arteries and form atheromas or fatty acid deposits [12]. These can cause the arteries to narrow in a process called atherosclerosis and its complications, coronary heart disease,

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peripheral vascular disease, stroke and death [13]. A number of methods for the extracorporeal elimination of cholesterol from intravasal volume can be used. These techniques are plasmapheresis, cascade filtration, heparin induced extracorporeal LDL precipitation (HELP), thermofiltration and dextran induced LDL precipitation differing in their specificity and selectivity [14–18].

In this work, we have synthesized polymerizable derivative of tryptophan (*N*-methacryloyl-(L)-tryptophanmethylester – MAT) and phenylalanine (*N*-methacryloyl-(L)-phenylalaninemethylester – MAP) amino acids as a HIC ligand. Poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres were prepared by surfactant free emulsion polymerization by using 2-hydroxyethyl methacrylate (HEMA), *N*-methacryloyl-(L)-tryptophanmethylester (MAT) and *N*-methacryloyl-(L)-phenylalaninemethylester (MAP). These hydrophobic nanospheres were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), elemental analysis, particle size and surface area measurements. Then, cholesterol was adsorbed onto the poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres. Desorption of cholesterol and reusability of these hydrophobic affinity nanospheres were tested. Cholesterol removal capacities of these two different nanospheres were also compared.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Sigma (St. Louis, USA) and distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. Cholesterol was supplied by Sigma (St. Louis, USA). Methanol and acetonitrile used in HPLC analysis were HPLC grade and supplied from Merck A.G. (Darmstadt, Germany). All other chemicals were of the highest purity commercially available and were used without further purification. Ultra-pure water filtered by Millipore S.A.S 67120 Molsheim-France was used for all experiments unless otherwise stated. Before use, the laboratory glassware was rinsed with water and dried in a dust-free environment.

2.2. Synthesis of poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres

N-Methacryloyl-(L)-tryptophanmethylester (MAT) and *N*-methacryloyl-(L)-phenylalaninemethylester (MAP) were prepared as outlined in [19,20]. The poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres with an average particle size of 100 nm and 158 nm, respectively were produced by surfactant free emulsion polymerization. Briefly, the stabilizer, PVAL (0.5 g), was dissolved in 50 mL deionized water to prepare the continuous phase. Then, the comonomer mixture was added to this dispersion, which was mixed in an ultrasonic bath for about half an hour. Before polymerization, initiator was added to the solution and nitrogen gas blown through the medium for about 1–2 min to remove dissolved oxygen. Polymerization was carried out in a constant temperature (70 °C) with shaking under nitrogen atmosphere for 24 h. After the polymerization, the polymeric nanospheres were cleaned by washing with methanol and water several times to remove the unreacted monomers. For this purpose, the nanospheres were precipitated at the rate of 18,000 × *g* for 2 h in a centrifuge (Zentrifugen, Universal 32 R, Germany), the collected precipitate was resuspended in methanol and water several times. After that poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres were further washed with deionized water. Fig. 1 shows that the hypothetic structure of poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres.

2.3. Characterization of poly(HEMA–MAT) and poly(HEMA–MAP) nanospheres

FTIR spectra of the nanospheres were obtained by using a FTIR spectrophotometer (Varian FTS 7000, USA). The dried nanospheres

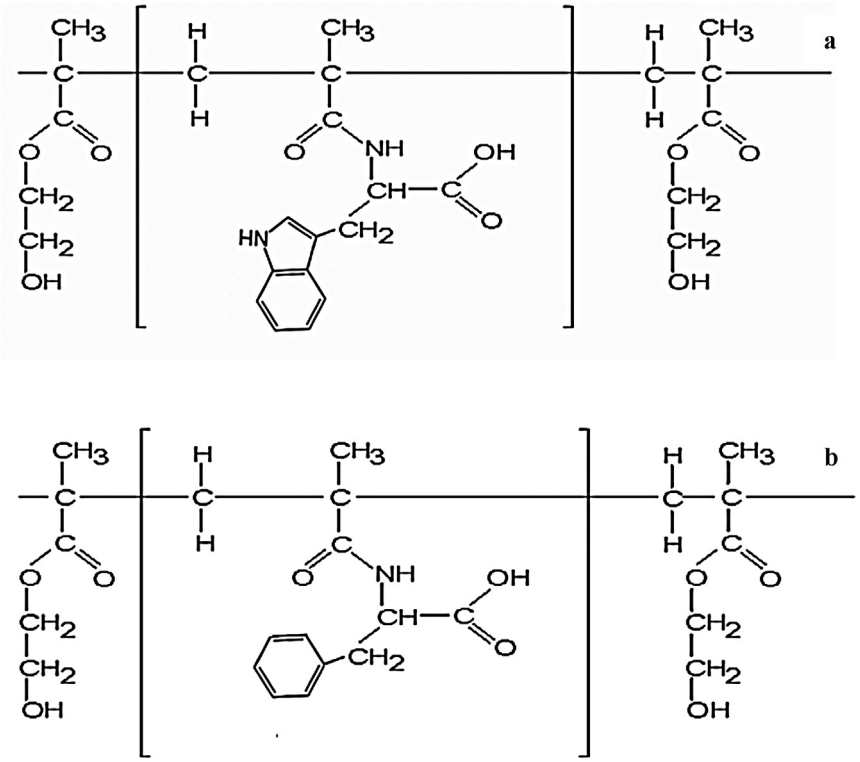

Fig. 1. The structure of (a) poly(HEMA–MAT) and (b) poly(HEMA–MAP) nanospheres.