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Preparation of polymethacrylic acid-grafted HEMA/PVP microspheres and preliminary study on basic protein adsorption

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ABSTRACT

The crosslinked copolymeric microspheres (HEMA/NVP) of N-vinylpyrrolidone (NVP) and 2-hydroxyethyl methacrylate (HEMA) were prepared using inverse suspension polymerization method. Subsequently, the reaction of methacryloyl chloride with the hydroxyl groups on the surfaces of HEMA/NVP microspheres was performed, leading to the introduction of polymerisable double bonds onto the surfaces of microspheres HEMA/NVP. Afterward, methacrylic acid was allowed to be graft-polymerized on microspheres HEMA/NVP in the manner of "grafting from", resulting in the grafted microspheres PMAA-HEMA/NVP. The grafted microspheres PMAA-HEMA/NVP were fully characterized with several means. The graft-polymerization of MAA on microspheres HEMA/NVP was studied in detail, and the optimal reaction conditions were determined. Thereafter, the adsorption property of the grafted microspheres PMAA-HEMA/NVP for lysozyme as a basic protein model was preliminarily examined to explore the feasibility of removing deleterious basic protein such as density lipoprotein from blood. The experimental results indicate that the PMAA grafting degree on microspheres HEMA/NVP is limited because an enwinding polymer layer as a kinetic barrier on the surfaces of HEMA/NVP microspheres will be formed during the graft-polymerization, and block the graft-polymerization. In order to enhance PMAA grafting degree, reaction temperature, monomer concentration and the used amount of initiator should be effectively controlled. The experimental results also reveal that the grafted microspheres PMAA-HEMA/NVP possess very strong adsorption ability for lysozyme by right of strong electrostatic interaction.

1. Introduction

With the development of life science, the polymeric materials with biocompatibility are playing an increasingly role in biology, medicine and pharmacy fields [1–6], and biocompatible polymeric materials are promising biomaterials. Polyvinylpyrrolidone (PVP) and poly(hydroxyethyl methacrylate) (PHEMA) are two kinds of polymeric materials with fine biocompatibility as well as with blood compatibility [7–10], and have important applications in various biotechnology areas such as tissue engineering, controlled drug release, separation of biomacromolecules as well as biosensor [11–14]. In the present work, the crosslinked copolymeric microspheres (HEMA/NVP) of 2-hydroxyethyl methacrylate (HEMA) and N-vinylpyrrolidone (NVP) were prepared via inverse suspension polymerization, and the microspheres HEMA/NVP are expected to be used in blood purification treatments in future.

In order to further improve performance, most of biomedical polymeric materials need to be surface-modified. Among various surface modification methods, graft-polymerization is received much attention [15–17]. Via graft-polymerization, functional macromolecules can be chemically introduced onto the surface of the matrix, and various new physicochemistry properties and biological properties can be imparted to the matrix polymers, such as hydrophilicity, affinity adsorption property, biocompatibility, blood compatibility as well as changed cell adhesion property. For example, Müller-Schulte et al. used radiation grafting technique to introduce poly(2-diethylaminoethyl methacrylate) into polyethylene and polyamide microcarriers for improving the DNA binding capacity of the microcarriers by utilizing the cationic character of the grafted polymer [18]; Nho et al. grafted acrylic acid, 2-hydroxyethyl methacrylate and three kinds of polyethylene glycol methacrylates onto the cellulose film surface by radiation grafting technique to improve surface blood compatibility of cellulose film for hemodialysis [10]; Mirzadeh et al. surface-grafted acrylamide and 2-hydroxyethyl methacrylate onto ethylene–propylene rubber using CO_2-pulsed laser as excitation source to improved tissue compatibility [19]; Fu et al. grafted acrylic acid (AA) onto the crosslinked chitosan beads by heterogeneous graft copolymerization method to improve both the low-density lipoprotein binding capacity and selectivity of porous chitosan beads [20]; Khorasani et al. grafted 2-hydroxyethyl methacrylate onto polydimethylsilox-

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ane to reduction of the platelet adhesion and aggregation on the surfaces of polydimethylsiloxane [21].

By referring to the above idea and method of preparing and constituting biomedical materials, in this investigation, the crosslinked microspheres HEMA/NVP were surface-modified by the graft-polymerization of methacrylic acid (MAA). HEMA/NVP microspheres were first acylated with methacryloyl chloride, and the polymerisable double bonds were introduced onto the surfaces of the crosslinked microspheres HEMA/NVP. Subsequently, the graft-polymerization of MAA was allowed to be conducted with the "grafting from" manner, and the grafted microspheres PMAA-HEMA/NVP were obtained. The grafted microspheres PMAA-HEMA/NVP not only are probably biocompatible, but also there is a great deal of carboxyl groups on their surfaces which are functional groups and make the surfaces of the microspheres PMAA-HEMA/NVP to be negatively charged. So the microspheres PMAA-HEMA/NVP are probably potential in hemoperfusion treatment for the removal of low-density lipoprotein which is a basic protein. In this work, the adsorption property of the microspheres PMAA-HEMA/NVP for basic protein was preliminarily tested using lysozyme as a basic protein model, but further experiments about the adsorption behavior will be carried out in depth to fully evaluate the properties of microspheres PMAA-HEMA/NVP. This investigation serves as a basis for the development of biocompatible polymeric adsorbents of basic proteins.

2. Experimental

2.1. Materials and instruments

N-Vinylpyrrolidone (NPV, Dexiang Medicine Inc., Shanghai, China) was of analytical grade, and was purified by distillation under vacuum prior to use. 2-Hydroxyethyl methacrylate (HEMA, Tianjin Chemical Reagent Institute, Tianjin City, China) was of analytical grade, and was purified by distillation under vacuum prior to use. N,N′-Methylene bisacrylamide (MBA, Xiangzhong Fine Chemical Plant, Province Hunan, China) was of chemical grade. Liquid paraffin (Tianjing BASF Chemical Company, Tianjing City, China) was of chemical grade. Ammonium persulphate (APS, Fushu Chemical Engineering Inc., Shanghai, China) was of analytical grade. Span-60 (Tianda Chemical Reagent Plant, Tianjin City, China) was of chemical grade. Methacrylic acid (MAA, Ruijinte Chemical Company, Tianjin City, China) was of analytical grade and was purified by distillation under vacuum before use. Ammonium persulphate (APS, Fushu Chemical Engineering Inc., Shanghai) was of analytical grade. Lysozyme (Lyz, Hangzhou Chunlei Feed Science and Technology Inc., Hangzhou City, China) was of chemical grade. Other reagents were all commercial chemicals with analytical grade and purchased from Chinese companies.

The instruments used in this study were as follows: Perkin-Elmer 1700 infrared spectrometer (FTIR, Perkin-Elmer Company, USA), Unic-2602 UV spectrophotometer (Unic Company, Shanghai, China), LEO-438VP scanning electronic microscope (STM, LEO Company, UK), Zetasizer Nano-Zeta potential analyzer (Malvern Instrument Company, UK), and THZ-92C constant temperature shaker equipped with gas bath (Boxun Medical Treatment Equipment Factory, Shanghai, China).

2.2. Preparation and characterization of crosslinked microspheres HEMA/NVP

According to the procedure described in Ref. [22], inverse suspension polymerization method was used to prepare crosslinked microspheres HEMA/NVP, and the typical process was as follows. The continuous phase (oil phase) was consisted of 60 mL of liquid paraffin containing 1.30 g of oil-soluble surfactant Span-60 which was used as suspension stabilizer. The oil phase was poured into a four-necked flask equipped with a mechanical stirrer, a reflux condenser and a N_2 inlet. HEMA (2 mL), NVP (2 mL) and 2.3 mL of redistilled water were mixed and dissolved each other, 0.27 g of MBA was dissolved in this solution, and the dispersed phase (water phase) was constituted. After the oil phase was heated to 35 °C, the water phase was added, and the mixture was stirred and sufficiently dispersed for 30 min, while N_2 was bobbled to exclude air. Then the mixture was heated to 65 °C with stirring, and 0.0213 g of APS was added. Under nitrogen atmosphere and with stirring at a rate of 450 r/min, the crosslinking copolymerization was carried out at the constant temperature of 65 °C for 8 h. Transparent microspheres were obtained. The resultant microspheres were washed with petroleum ether and acetone several times, dried under vacuum at 50 °C for 24 h, resulting in the transparent crosslinked microspheres HEMA/NVP with a mean diameter of 90 μm.

The chemical structure of the microsphere HEMA/NVP was characterized with FTIR, their morphology and size were examined with SEM.

2.3. Preparation and characterization of grafted microspheres PMAA-HEMA/NVP

2.3.1. Introducing polymerisable double bonds onto microspheres HEMA/NVP

Methacryloyl chloride was first synthesized by reaction of methacrylic acid with thionyl chloride. The crosslinked microspheres HEMA/NVP (2 g) were added into a reactor, followed by adding 40 mL of acetone, and the microspheres were soaked and swelled fully for 12 h. After adding 2 mL of methacryloyl chloride, the temperature of the content in the reactor was slowly heated to 40 °C, a little of Na_2CO_3 as capturing acid reagent was added, and the surface modification reaction of HEMA/NVP microspheres was allowed to be carried out at the constant temperature of 40 °C for 12 h. After finishing the reaction, the resultant microspheres were washed with distilled water repeatedly, dried under vacuum, and the surface-modified microspheres MAO-HEMA/NVP, on which methacryloyl (MAO) groups, were bond and the polymerisable double bonds were introduced, were obtained.

The infrared spectrum of MAO-HEMA/NVP microspheres was determined with KBr pellet method. The content of the polymerisable double bonds on the surfaces of MAO-HEMA/NVP microspheres were determined with KBr–$KBrO_3$ method. The MAO-HEMA/NVP microspheres prepared and used in this study had a double bond content of 1.12 mmol/g.

2.3.2. Graft-polymerizing of MAA on HEMA/NVP microspheres

The modified microspheres MAO-HEMA/NVP (1 g) were added into a four-necked flask equipped with a mechanical agitator, a reflux condenser and a N_2 inlet, followed by adding 70 mL of distilled water, and the microspheres were allowed to be soaked and swelled for 12 h. Methacrylic acid (3.5 mL) was added, N_2 was bobbled for 30 min to exclude air, the temperature was raised to 70 °C, and 0.011 g of initiator APS was added. The graft-polymerization was conducted under N_2 atmosphere with stirring for 12 h. After ending the reaction, the product microspheres were collected, and then extracted with ethanol in a Soxhlet extractor to remove the polymer physically attaching on the microspheres. Finally, by drying in vacuum, the grafted microspheres PMAA-HEMA/NVP were obtained.

The infrared spectrum of the grafted microspheres PMAA-HEMA/NVP was determined with KBr pellet method, and their morphology and size were examined with SEM. The grafting degree (g/100 g) of PMAA on the grafted microspheres PMAA-HEMA/NVP