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Atom transfer radical polymerization to fabricate monodisperse poly[glycidyl methacrylate-*co*-poly (ethylene glycol) methacrylate] microspheres and its application for protein affinity purification

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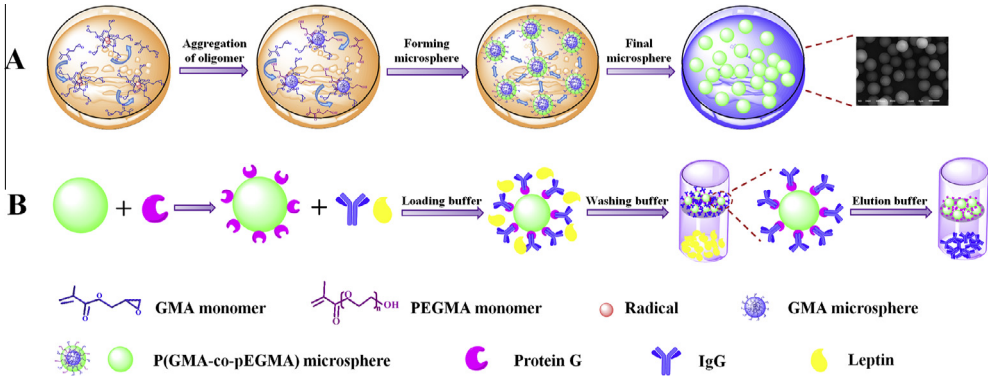
GRAPHICAL ABSTRACT



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ABSTRACT

Poly[glycidyl methacrylate-*co*-poly (ethylene glycol) methacrylate] microspheres for the first time were successfully synthesized by atom transfer radical polymerization (ATRP) method at room temperature. The co-polymerization approach was investigated to delicately control the microsphere morphology and size-distribution by reaction conditions including solvent percentage, monomer loading and rotation speed. The results show that the average size of the microspheres is ~5.7 μm with coexistence of epoxy, hydroxyl and ether groups, which provide plentiful functional sites for protein anchoring. The mechanism of the microsphere formation is proposed. The microsphere successfully demonstrates its unique application for affinity purification of proteins, in which the functional epoxy group facilitates a simple and efficient protein covalent immobilization to purify immunoglobulin G on the microspheres, while the hydrophilic poly (ethylene glycol) motif can repulse nonspecific protein adsorption for good specificity. This microspheres can be used in broad protein biosensors due to their abundant functional groups and high surface to volume ratio.

1. Introduction

Functional polymer microspheres have become one of the attractive materials because of their potential applications in

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biomedicines, catalysis, coatings, carriers, adhesives and electronics [1–8]. Some size-controlled polymer microspheres have been synthesized by various techniques such as emulsion polymerization, dispersion polymerization and precipitation [2–5]. In particular, narrowly dispersed and cross-linked polymer microspheres have been synthesized. Polyethylene (PE), polystyrene (PS) and poly(methyl methacrylate) (PMMA) microspheres are popular types of polymer microspheres because of their capability to expedite procedures such as cell sorting and immune-precipitation [9–12]. These polymer beads are generally hydrophobic and often require additional surfactant to ensure ease of handling. Importantly, PS, PMMA and silica microspheres are lack of active functional groups as covalent binding sites for immobilization of capture molecules such as antibodies, oligo-nucleotides and peptides for diagnosis or separation applications.

Glycidyl methacrylate (GMA) is a functional monomer with reactive epoxy groups allowing uses in polymer synthesis. Horák and Shapoval [2] have synthesized poly (glycidyl methacrylate) microspheres by dispersion polymerization. Nevertheless, the approach for GMA microspheres raise concerns of non-specific adsorption because of its hydrophobic property and side reactions by GMA incorporation with other monomers. Smigol and Švec [13] and Wang et al. [14] have synthesized monodispersed poly(GMA-*co*-ethylene dimethacrylate) microspheres by seed swelling and glass film emulsion polymerization. Poly(ethylene glycol) methacrylate (PEGMA) is a hydrophilic polymer containing a long chain of poly (ethylene glycol) (PEG) motif, which is one of the most used materials to modify hydrophobic biomedical surface for hydrophilicity improvement. PEGMA swellable spherical gel beads with controlled hydrophilicity have been synthesized by suspension polymerization [15]. Nevertheless, the seeds welling-polymerization process is relatively complex while the glass film emulsion polymerization requires special reaction equipment.

Growing poly(GMA-*co*-pEGMA) polymer brush on a flat glass and gold surfaces has been reported [16,17]. The polymer brush functionalization can offer a biocompatible surface to efficiently immobilize probe protein and prevent non-specific adsorption toward high sensitive and specific immunoassays [16,17]. Although growing a layer of poly(GMA-*co*-pEGMA) polymer brush on a solid surface is an effective approach to functionalize a defined subtract but it is restricted to immobilize target molecules along 1-dimensional direction on a planar substrate. Attractively, microspheres could capture probe molecules from 3-dimensional directions inside and outside of microspheres to fully use the microsphere merit of high surface area-to-volume for a high target biomolecule loading. More importantly, poly(GMA-*co*-pEGMA) microspheres also have highly potential for affinity-isolating protein and cells from mixtures through filtration that is not feasible based on polymer brush layer-modified planar surfaces.

Atom transfer radical polymerization (ATRP) is one of the investigated controlled/living radical polymerization (CRP) methods [18–22]. This strategy has been successfully used to mainly synthesize the polymer on a flat solid surface [23–25]. Recently, Zhang et al. have reported ATRP-synthesized poly(4-VP-*co*-EGDMA) and poly(HEMA-*co*-EGDMA) microspheres [26]. Herein we further investigate whether it is feasible in an aqueous solution at an ambient condition to ATRP-synthesize poly(GMA-*co*-pEGMA) polymer microspheres with incorporated active functional groups of glycidyl methacrylate and poly(ethylene glycol) methacrylate. The effects of solvent volume percentage, monomer loading concentration and rotating speed on the formation of microspheres were investigated. The mechanism to form this novel poly(GMA-*co*-pEGMA) microspheres was also discussed. The morphology, size distribution, surface chemical groups and its thermal stability of the microspheres were studied. Furthermore, protein affinity purification was demonstrated by epoxy-mediated immobilization of protein G on as-synthesized microspheres.

2. Materials and methods

2.1. Materials

Glycidyl methacrylate (GMA, 97%), poly(ethylene glycol) methacrylate (PEGMA, Mn = 360), 2,2′-bipyridyl (Bipy, ⩾99%), copper (II) bromide ($CuBr_2$, 99%), L-ascorbic acid, methanol(⩾99.9%), rabbit IgG (whole molecule), protein G and leptin were purchased from Sigma–Aldrich. Albumin form bovine serum was ordered from Aladdin, China. 30% Acr–Bis (29:1), ammonium persulfate, Tetramethylethylenediamine (TEMED), prestaining molecular weight maker, sampler buffer, SDS–PAGE electrophoresis buffer, Coomassie blue stain and de-stain buffer were purchased from Beyotime Biotechnology, China. All chemicals were used without further purification unless otherwise indicated. All solution was prepared with deionized (DI) water produced by PURELAB flex system, ELGA Corporation.

2.2. Preparation of Poly (GMA-co-pEGMA) microspheres

To synthesize co-polymerized polymer microspheres, a 5 mL reaction mixture of $CuBr_2$ (3.35 mg/mL), Bipy (4.6 mg/mL) and GMA monomer were prepared in methanol or ethanol. The reaction mixture was sealed in a glass Petri dish (d = 6 cm) by a parafilm membrane after adding 300 μL 0.1% (M/V) sodium citrate and 800 μL of 200 mg/mL ascorbic acid. The solution was shade from light and put on a shaker for 0.5 h at room temperature. Then PEGMA monomer was added into the reaction mixture and reacted for another 2–5 h. The resulted polymer microspheres were separated by centrifugation and washed with methanol for five times to remove free components. The concentrations of methanol or ethanol, GMA and PEGMA monomers as well as rotating speed to synthesize co-polymerized polymer microspheres were optimized.

2.3. Characterization of microspheres

The morphologies, particle size and size distribution of microspheres were characterized by scanning electron microscopy (SEM, Japan JSM-6510LV) operating at 25 kV. First, the microspheres were washed with methanol and DI water then put into a vacuum oven (DZF-6020) at 40 °C for 6 h. The microspheres were coated with a gold layer for improved SEM image. Fourier transform infrared (FTIR) spectra of the co-polymerized polymer microspheres were measured with a Thermo-Nicolet 6700 spectrometer. The clean samples were dried for two days in oven before FTIR test. X-ray Photoelectron Spectroscopy (XPS, Thermo) was used to determine the surface chemical composition of synthesized microspheres.

2.4. Surface tension measurements of reaction liquids

Bulk reaction solutions were injected from a 0.5 mL syringe to form a droplet at the tip using a contact angle measurement equipment (model JC2000D1, Powereach, China). Image capture software was used to take an image of the droplet when it equilibrated over timescales of 2 min at room temperature. The digitized images were analyzed and the surface tensions were obtained by fitting the experimental drop profile according to instruction of JC2000D1 software (Powereach, China).