Reactive & Functional Polymers 77 (2014) 11–17

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Monodisperse macroporous poly(glycidyl methacrylate) microspheres coated with silica: Design, preparation and characterization

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ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 29 January 2014
Accepted 30 January 2014
Available online 8 February 2014

Keywords:
Multistep swelling polymerization
Poly(glycidyl methacrylate)
Poly(2,3-dihydroxypropyl methacrylate)
Monodisperse
Macroporous
Microspheres
Silanization

ABSTRACT

Monosized macroporous poly(glycidyl methacrylate) (PGMA) microspheres that were 9.3 μm in size were synthesized by multistep swelling polymerization using a modified Ugelstad technique. The PGMA microspheres and their hydrolyzed analogs derived from poly(2,3-dihydroxypropyl methacrylate) (PDHPMA) were coated by silanization with tetraethoxysilane (TEOS) and (3-aminopropyl)triethoxysilane (APTES), respectively. The particles were characterized by elemental and thermogravimetric (TGA) analysis, scanning and transmission electron microscopy (SEM and TEM) coupled with an energy dispersive X-ray analysis (EDAX) and FT-IR spectroscopy to determine the SiO_2 content, morphology, particle size, polydispersity and structure. These types of particles are expected to have improved biocompatibility relative to their starting polymers.

1. Introduction

Many techniques can be used for the preparation of microspheres. The first polymeric particles were produced at the beginning of the 20th century by suspension polymerization [1]. This technique yields large beads possessing diameters that span the low micrometer to millimeter range and that have a broad particle size distribution. Suspension polymerization is used for the commercial manufacture of many important polymers, including poly(vinyl chloride), poly(methyl methacrylate), expandable polystyrene and styrene-acrylonitrile copolymers, which are used as ion-exchangers, sorbents or adsorbents for water purification [2–4]. Precipitation and dispersion polymerization produces particles with diameters from 1 to 8 μm. The precipitation process gives larger and less regular particles than the dispersion process [5–8]. Current efforts have focused on the synthesis of monodisperse polymer particles. This synthesis along with the ability to control particle surface chemistry has led to important developments in clinical diagnostics [9,10], drug delivery [11], cell separation [12,13] and chemotherapy [14,15]. The most common production method for small-sized particles (100–600 nm) is emulsion polymerization. The technique is frequently used for production of polystyrene colloids [16,17]. Through the multistep swelling of seeds and polymerization, monodisperse microspheres are obtained [18,19].

The key issue in the design of particles for biomedical applications involves coating them with a proper shell. The importance of an appropriate shell is exemplified by the silanization of metal oxide or polymer particles to produce organic/inorganic hybrid materials that feature both biocompatibility and functionality, e.g., amino groups [20–25]. Silica can also coat metal nanoparticles, such as gold [26,27], glass and natural or synthetic polymeric particles [28–31]. Typically, silica has been applied to polystyrene [32,33] or silanization has been performed on cellulose microspheres [34] or fibers [35]. Only one report addresses silica microspheres that were obtained by the calcination of ethylenediamine-functionalized PGMA microspheres. The silica had, however, a porous or hollow structure [36]. Silanization eliminates undesirable nonspecific interactions and enables the functionalization required for biological applications. The introduced functional groups are then available for the attachment of proteins, peptides and antibodies. Silica particles are commonly synthesized by the sol–gel technique that was introduced by Stöber and Fink [37]. Primarily, this method is used for the preparation of silica nanoparticles that are < 100 nm in size. The process involves the hydrolysis of silica alkoxide precursors, such as tetraethoxysilane (TEOS), in a mixture of ethanol and aqueous ammonium hydroxide. Silicic acid is produced during hydrolysis, and when its concentration exceeds its solubility in ethanol, it nucleates homogeneously to form

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http://dx.doi.org/10.1016/j.reactfunctpolym.2014.01.010
1381-5148/

submicrometer silica particles [38]. These particles can then be functionalized with different silanization agents, usually (3-aminopropyl)triethoxysilane (APTES) [39]. This functionalization can be performed in the same step with the silanization or in a two-step process [40–42]. The silica shell on the particles facilitates a wide variety of surface reactions and allows for conjugation with biomolecules, such as proteins and DNA [13].

Silica nanoparticles are easy to prepare, nontoxic and amenable to simple surface modifications; thus, they have been widely studied and used in various applications, such as column chromatography solid support materials [43], insulating layers and silica–polymer composites in engineering and bio-imaging and drug/gene delivery systems in nano-biotechnology [44].

Herein, we report the preparation and characterization of monodisperse macroporous poly(glycidyl methacrylate) (PGMA) and poly(2,3-dihydroxypropyl methacrylate) (PDHPMA) microspheres and their subsequent coating with a silica shell. The PGMA microspheres were synthesized using a modified Ugelstad technique [45] based on the multistep swelling of polystyrene (PS) seeds. The PS particles were obtained by emulsifier-free emulsion polymerization. Compared to particles with broad size distributions that are subject to size classification and low product yields, monodisperse microspheres offer enhanced performance due to their uniform physical, chemical and biological properties. As far as we know, this is the first report of the direct silanization of monodisperse PGMA or PDHPMA microspheres. The well-known hydrophobicity of PGMA and polystyrene microspheres makes them highly bioincompatible; thus, the hydrophilicity imparted through silanization will improve their *in vivo* performance and enhance the wettability of PGMA.

2. Experimental

2.1. Materials

Monomers, such as styrene (Synthos, Kralupy, Czech Republic), glycidyl methacrylate (GMA; Fluka; Buchs, Switzerland) and ethylene dimethacrylate (EDMA; Ugilor, France), were vacuum-distilled before use. 2,2′-Azobisisobutyronitrile (AIBN), dibutyl phthalate (DBP), sodium dodecyl sulfate (SDS) and (3-aminopropyl)triethoxysilane (APTES) were obtained from Sigma–Aldrich (St. Louis, USA). Tetraethoxysilane (TEOS) and 2-hydroxyethyl cellulose (HEC) were purchased from Fluka. Lithium persulfate, lithium hydrogen carbonate, ammonium hydroxide, sodium hydroxide and sulfuric acid were purchased from Lach-Ner (Neratovice, Czech Republic). Poly (vinyl alcohol) (PVA) was purchased from Wacker (Burghausen, Germany). Ultrapure Q-water ultra-filtered on a Milli-Q Gradient A 10 system (Millipore, Molsheim, France) was used throughout this work. All other solvents were from Lach-Ner (Neratovice, Czech Republic).

2.2. Preparation of monodisperse macroporous PGMA microspheres by multistep swelling polymerization

The method consists of four steps:

(i) Polystyrene (PS) latex (750 nm particles) was obtained by emulsifier-free emulsion polymerization. A 150-mL reaction vessel equipped with an anchor-type stirrer (300 rpm) was charged with styrene (10 g) and water (90 mL) containing 0.185 mM lithium persulfate (initiator) and 0.37 mM lithium hydrogen carbonate to adjust the pH to 7.5. Polymerization proceeded at 70 °C for 20 h under nitrogen atmosphere. The resulting PS latex was repeatedly washed with water.

(ii) A dispersion of PS latex (0.6 g corresponding to 0.3 g dry weight) in a 0.25 wt% SDS aqueous solution (5 mL) was swollen for 50 h with a dispersion of DBP (3 g) in a 0.25 wt% SDS solution (20 mL) prepared by 5-min sonication using a W-385 sonicator (Heat System – Ultrasonics; Farmingdale, NY, USA). Swelling with the DBP dispersion was performed three additional times with DBP, increasing to 4 and 5 g in the last two swellings. The final solvent-swollen PS dispersion contained 15 g of DBP.

(iii) DBP-swollen PS seeds were gradually swollen with a dispersion of monomers and an initiator (192 g of GMA, 128 g of EDMA and 1.6 g of AIBN) as well as a dispersion of porogens (8 g of dodecan-1-ol and 457 g of cyclohexanol) in a 0.1 wt% SDS solution (1.65 L) for 5 h.

(iv) The dispersion of monomer/porogen-swollen PS seeds in 0.1 wt% SDS solution was then transferred to a 4-L reaction vessel and a 3 wt% PVA aqueous solution (240 mL) and 6 wt% HEC aqueous solution (240 mL) were added. Polymerization proceeded at 70 °C for 16 h under a CO_2 atmosphere. The macroporous PGMA microspheres were washed successively with water, methanol, toluene and methanol (three times each with 1 L) and then air-dried.

Optionally, PGMA microspheres (1 g) were hydrolyzed with 0.1 M H_2SO_4 (30 mL) at 60 °C for 5 h under mechanical stirring. The resulting poly(2,3-dihydroxypropyl methacrylate) (PDHPMA) microspheres were separated by centrifugation and washed 10 times with water until the effluent was neutral and vacuum-dried.

2.3. Modification of PDHPMA and PGMA microspheres with TEOS and APTES

PDHPMA microspheres (0.2 g) were dispersed in 2 mL of ethanol/water (1/1 v/v) and 0.1 M NaOH aqueous solution (0.2 mL) was added under mechanical stirring until pH 11 was reached. A solution of TEOS (0.2 mL) in ethanol (1 mL) was then added, and the reaction proceeded at 70 °C for 5 h. The resulting PDHPMA/SiO_2 microspheres were separated by centrifugation (4000 rpm), three times washed with ethanol and water, separated by centrifugation and vacuum-dried at 50 °C.

Two approaches were employed for the coating of PGMA microspheres with APTES. In the one-step procedure, PGMA microspheres (0.2 g) were dispersed in methanol (2 mL), APTES (0.2 mL) in methanol (1 mL) was added under mechanical stirring and the reaction proceeded at 60 °C for 8 h. The resulting PGMA/SiO_2 microspheres were washed with methanol, separated by centrifugation and vacuum-dried at 50 °C.

In the two-step procedure, PGMA microspheres were first coated with APTES in toluene and then hydrolyzed in water. PGMA microspheres (0.2 g) were dispersed in toluene (1.5 mL), and APTES (0.2 mL) in toluene (0.5 mL) was added under mechanical stirring and the reaction proceeded at 50 °C for 5 h. The particles were washed with toluene, methanol and water to remove unreacted reagents and were separated by centrifugation. The second step included the hydrolysis and condensation of alkoxides in water (2 mL) to yield silica. An ammonium hydroxide solution was added until pH 11 was reached. The reaction proceeded under mechanical stirring at 50 °C for 3 h. PGMA/SiO_2 particles were separated by centrifugation, washed with water and vacuum-dried at 60 °C.

2.4. Characterization of microspheres

The microspheres were observed with an Opton III light microscope (Oberkochen, Germany), a 200S Quanta scanning electron microscope (SEM; FEI, Brno, Czech Republic) equipped with an