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Preparation of magnetic polymer microspheres with reactive epoxide functional groups for direct immobilization of antibody

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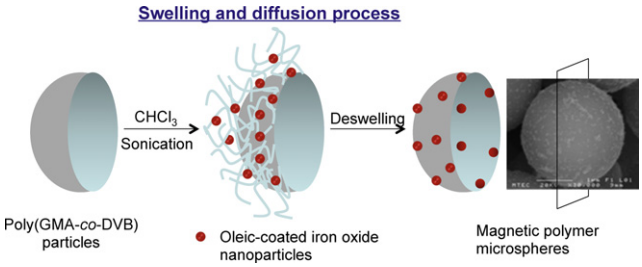
HIGHLIGHTS

- Monodisperse magnetic particles have been prepared by swelling and diffusion process.
- The particles exhibited superparamagnetism at room temperature.
- Epoxide groups on the surface allow for covalent attachment of biomolecules.
- Antibody was attached on particles without the signs of denaturation or degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

Monodisperse magnetic polymer microspheres with epoxide functional groups have been developed through *ex situ* swelling and diffusion process. Poly(glycidyl methacrylate-*co*-divinyl benzene) (poly(GMA-*co*-DVB)) microspheres were prepared by cross-linking precipitation polymerization of GMA and DVB monomers without the use of surfactants and stabilizers. The microspheres were subsequently swollen in chloroform in the presence of oleic acid-coated iron oxide nanoparticles (IOs). The size of poly(GMA-*co*-DVB) microspheres can be controlled by varying the concentration of DVB cross-linker. Based on FTIR, XRD, and vibrating sample magnetometer (VSM) analysis, IOs were successfully entrapped in poly(GMA-*co*-DVB) microspheres. For a constant IO feed, increase in DVB content resulted in the decrease in the IO loading. It was found that the reactive epoxide functional groups of the microsphere's surface can covalently conjugate with monoclonal antibody, specific to CD4 molecules expressed on $CD4^+$ lymphocytes, which was confirmed from flow cytometry. Thus, these microspheres are promising to be used in various biomedical applications including diagnosis, and monitoring of human diseases.

1. Introduction

The applications of magnetic polymer microspheres, consisting of one or more magnetic nanoparticles as a core and a polymer matrix or coating periphery as a shell, in biomedical fields, such as bio-separation [1–4], immunoassay [5–8], drug delivery [9–13], and imaging [14–16] have attracted increasing attention in recent decades. All these biomedical applications are usually based on the unique superparamagnetic property and other physicochemical properties, for examples, high saturation magnetization, monodispersity, stability under physiological conditions, and abundant surface functional groups, which correlate significantly with the methods of preparation [17].

In general, the preparation of magnetic nanoparticles is mostly based on coprecipitation of iron salts in an alkaline condition. These nanoparticles are, however, sensitive to oxidation and aggregation due to their magneto-dipole interparticle interaction. To improve their stability, surface-protective organic and/or inorganic layers have been introduced on the surface of magnetic nanoparticles in order to prevent the crystal growth, and self-assembly

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of primary particles to form clusters. Coating/encapsulating of magnetic nanoparticles with organic polymers to form magnetic polymer microspheres is now one of the most attractive methods. Additionally, specific functional groups and surface charges of polymer-based magnetic microspheres can be manipulated in order to facilitate their adherence with negatively charged phospholipid bilayer of cellular membranes and conjugation with drugs, proteins, and biomolecules [18].

Various approaches to the syntheses of magnetic polymer microspheres with reactive functional groups (*i.e.* anhydride, amine, hydroxyl, carboxyl, thiol, and epoxy) on the surface have been developed. Methacrylic acid (MAA) [19,20], 2-hydroxyethyl methacrylate (HEMA) [21], acrylic acid (AA) [22], and glycidyl methacrylate (GMA) [23–30] are often used as functional monomers. Among them, GMA is an excellent candidate. GMA possesses the reactive epoxide functional groups, which can be directly coupled with biomolecules *via* ring opening reactions or further modified to be various functional groups. In addition, this conjugation takes place easily at room temperature without an addition of activator and/or cross-linker. Several methods on preparation of magnetic poly(glycidyl methacrylate) microspheres have been proposed in the literature including dispersion [23–25], suspension [26,27], and emulsion [28–30] polymerization. However, these approaches involve the utilization of emulsifiers and/or stabilizers for preventing the aggregation of microspheres that may further interfere their conjugation with biomolecule or cause nonspecific binding, which could severely limit their applications in bio-related fields.

To address these problems, the encapsulation of iron oxide nanoparticles by *in situ* precipitation polymerization without the use of surfactants and stabilizers has been reported. However, monodispersed magnetic microspheres with good spherical shape obtained from this polymerization technique were not practical [31]. Therefore, it is necessary to develop convenient, economic, and efficient methods for the preparation of monodisperse magnetic polymer microspheres containing high fraction of reactive surface functional groups without the use of stabilizers and surfactants.

In this study, monodisperse magnetic polymer microspheres with epoxide functional groups on their surface have been developed through *ex situ* swelling and diffusion process. Poly(glycidyl methacrylate-*co*-divinyl benzene) (poly(GMA-*co*-DVB)) microspheres were prepared by cross-linking precipitation polymerization of GMA and DVB monomers. The microspheres were subsequently swollen in chloroform in the presence of iron oxide nanoparticles. The effect of monomer ratio on particle size and iron oxide content was studied. The magnetic polymer microspheres prepared in this process are of interest from two perspectives. First, they exhibited superparamagnetism at room temperature, indicating that there was almost no remaining magnetization when the external magnetic field was removed. Later, the availability of epoxide groups located on the particle surface allows the surface modification and conjugation with biomolecules. Therefore, these well-defined magnetic polymer microspheres could be potentially useful in various applications, including magnetic separation, medical diagnostics, or drug delivery.

2. Experimental

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, Fluka), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, Fluka), oleic acid (Merck), acetonitrile (Carlo Erba), and ammonium hydroxide solution (40%, w/w, Merck) were used as received. Glycidyl methacrylate (GMA) and divinyl benzene (DVB, 55% mixture of isomers), obtained from Merck, were purified to remove inhibitors by using a column packed with alumina adsorbents. Benzoyl peroxide (BPO) was recrytallized twice from methanol. All other chemicals were commercially available and of analytical grade.

Table 1
The average particle size and morphology of poly(GMA-*co*-DVB) microspheres at various monomer concentrations.

mol% of monomer		Morphology	Average particle size (μm)
GMA	DVB		
20	80	Spherical shape	2.63
30	70	Spherical shape	1.89
40	60	Spherical shape	1.97
50	50	Spherical shape	1.82
60	40	Agglomeration of microspheres	–
70	30	Irregular shape	–
80	20	–	–

2.2. Preparation of oleic acid-coated iron oxide nanoparticles (IOs)

Iron oxide nanoparticles coated with oleic acid (IOs) were synthesized by a coprecipitation method. Briefly, $FeCl_2{\cdot}4H_2O$ (1.6 g, 2 M) and $FeCl_3{\cdot}6H_2O$ (4.32 g, 1 M) were dissolved in deionized water (20 mL), then ammonium hydroxide (80 mL, 1.4 M) was added immediately to the mixture under nitrogen atmosphere at 0–5 °C. After 30 min, the resulting black dispersion was washed repeatedly with deionized water until the pH was 7. Thereafter, the collected nanoparticles were dispersed in 0.01 M HCl and further refluxed in the presence of oleic acid (10 mL) for an hour. The black iron oxide nanoparticles were then purified by decantation and redispersed in cyclohexane. Finally, the nanoparticles were prepared at concentration of 20 mg/mL for the subsequent experiments.

2.3. Preparation of poly(GMA-co-DVB) microspheres

Poly(GMA-*co*-DVB) microspheres were synthesized based on a reported method with some minor modifications [32,33]. The microspheres were prepared by cross-linking precipitation polymerization of GMA and DVB monomers in acetonitrile medium without any stabilizer. The influence of the concentration of DVB, varied from 20 to 80 mol% with respect to the amount of GMA at 2% total monomer loading, on particle morphology and size of the product was studied. BPO was employed at 2 wt% with respect to the total amount of the monomers used. The reaction recipe is listed in Table 1. The polymerizing mixtures were purged with N_2 for 30 min and then placed into the shaking incubator with an agitation speed of 250 rpm at 70 °C to start the polymerization reaction. The reaction was continued for 24 h. The formed microspheres were purified by repeated centrifugation and washed with methanol and water.

2.4. Preparation of IO-entrapped poly(GMA-co-DVB) microspheres

A process of swelling and diffusion was adopted for the preparation of magnetic polymer microspheres. Prior to the experiment, 1 g of poly(GMA-*co*-DVB) microspheres was swollen in chloroform (50 mL) at room temperature overnight. Then, 7.2 mg of IOs was impregnated into the swollen poly(GMA-*co*-DVB) microspheres with the aid of sonication (Crest 275D, Crest Ultrasonics) for 15 min. The resulting microspheres were purified by decantation and concentrated at 1×10^7 beads/mL for subsequent experiments.