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Carboxylated magnetic polymer nanolatexes: Preparation, characterization and biomedical applications

Weiming Zheng, Feng Gao, Hongchen Gu*

Engineering Research Center for Nano Science and Technology, Shanghai JiaoTong University, 1954 Huashan Road, Shanghai 200030, PR China

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Abstract

Carboxylated magnetic polymer nanolatexes were prepared by miniemulsion polymerization using 4,4′-azobis (4-cyanopentanoic acid) (ACPA) as initiator, which provided carboxyl end groups on the latex surface directly. The colloidal stability and the magnetic properties showed that these resulting carboxylated magnetic polymer nanolatexes were applicable in biomedical separation, which was performed by covalent coupling of activated antibody.

Keywords: Carboxyl groups; Magnetic polymer latex; Miniemulsion polymerization; ACPA; Antibody; Covalent coupling

1. Introduction

Magnetic polymer latexes with surface functional groups have extensive applications in the field of biomedicine (clinical diagnosis [1], immunoassay [2], target drug [3]), molecular biology (cDNA libraries [4], gene sequencing [5], isolation of DNA, mRNA [6]), cytology (cell labeling [7], cell separation [8]), and bioengineering (immobilized enzyme [9]). These applications require these functional latexes to fulfill some properties, such as colloidal stability, uniform size or narrow size distribution, high and uniform magnetite content, superparamagnetic behavior and enough surface functional groups for coupling active biomolecules.

Among functional magnetic polymer latexes, carboxylated magnetic polymer latexes are an interestingly important class for the biomedical applications. Carboxylated magnetic polymer latexes are generally prepared by copolymerization of hydrophobic monomer (usually styrene) with carboxylic acid monomers (such as acrylic acid (AA), methacrylic acid (MAA)) in the presence of the magnetite particles [2,10–12]. The features of the carboxylated magnetic polymer latexes are often related to the distribution of acid groups in the latex products. Therefore, carboxylic acid groups are required to reside on the outer surface of the magnetic polymer latexes as much as

*Corresponding author. Tel.: +86 21 6293 3731; fax: +86 21 6280 4389.
E-mail address: hcgu@sjtu.edu.cn (H. Gu).

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possible for the colloid stability and the efficient coupling of biomolecules. However, it is difficult to control the distribution of the incorporated carboxylic groups in the final magnetic polymer latexes. The unfortunate results that often occur are that most of the functional monomers are buried in the interior of the latex or present in the aqueous phase. Numerous studies have been performed to find out how carboxylic monomers are incorporated into emulsion polymers and to understand the role these monomers play in emulsion [13–16].

Besides the sufficient surface functional groups for efficient coupling of biomolecules, a significant reduction in particle size from about 50 to 500 nm must be achieved to provide enough surface area required for biological applications. However, particles with too small diameters may not carry enough magnetite and would have weak magnetic response.

To avoid these problems and to minimize the particle size, we developed an alternative approach where the surface carboxyl groups were introduced directly by the carboxylated initiator (4,4′-azo-bis(4-cyanopentanoic acid), ACPA) during a miniemulsion polymerization process. This method has the advantages that carboxyl end groups on the particle surface come from the hydrophilic initiator molecules [17–19] and the miniemulsion polymerization is well known to generate nano-size particles and narrow size distribution [20–22]. This paper will describe this method. The functional groups on the surface, the morphology and size distribution, the colloid stability, the magnetite content and magnetic properties were analyzed. Finally, the capacity of the latexes for coupling protein was determined.

2. Experimental

2.1. Materials

Fe_3O_4-St dispersion (Fe_3O_4 in styrene of 8 nm mean particle size, concentration about 15 wt%) was provided by our lab. Sodium dodecyl sulfate (SDS) was purchased from Shanghai Chemical Reagents Company, China. Hexadecane (99%) was purchased from Acros. ACPA was purchased from Fluka. Mouse IgG, sheep anti-mouse IgG conjugated with horse radish peroxidase (SAM-HRP) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) were from Sigma.

2.2. Preparation of carboxylated magnetic polymer latexes

A total of 0.24 g SDS as surfactant was dissolved in 80 g water to constitute the aqueous phase, and sodium hydroxide was added to adjust the pH value to 10. Then 10 g Fe_3O_4-St dispersion and 0.4 g Hexadecane as osmotic agent were mixed and added to the aqueous phase when it was agitated in a glass flask. After stirring for 1 h, the miniemulsion was prepared by ultrasonicating the emulsion for 5 min in an ice-cooled bath. For polymerization, 0.6 g initiator (ACPA) was added and the temperature was increased to the reaction temperature of 70 °C. The polymerization was left for about 20 h. The polymer latexes were washed with water several times with external magnetic field.

2.3. Binding of antibody to magnetic polymer latexes

For the binding of antibody, 20 mg of magnetic polymer latexes as obtained above were dispersed in 2 ml MES (0.1 M, pH 5.0). Twenty-five milligrams of EDAC was added to activate the carboxyl groups on the latex surface for 10 min with continuous stirring. The latexes were separated on a magnet and washed 3 times with 5 ml PBS (0.01 M, pH 7.4). After the last washing step the latexes were resuspended in 1 ml PBS and 1 ml of mouse IgG solution (1 mg/ml in PBS) was added. The suspension was incubated overnight at 4 °C with continuous stirring. The antibody-bound latexes were recovered from the reaction mixture by magnetic sedimentation and the supernatant was used for protein assay. The latexes were washed 3 times with PBS, then 1 ml blocking agent (PBS containing 0.75% Gly and 3% PBS) was added and allowed to react for 1 h at 4 °C with continuous mixing. Wash the latexes 3 times with