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# Carboxylated magnetic polymer nanolatexes: Preparation, characterization and biomedical applications

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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.
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*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

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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

PBS and finally suspend them in PBS for further activity detection.

One hundred microliters of enzyme-labeled second antibody, RAM-HRP, dilution solution was added to 50 μg mouse IgG-bound magnetic polymer nanospheres and left for 1 h at 37 °C with continuous stirring. The latexes were washed 3 times with washing solution (PBS containing 0.05% Tween20). One hundred microliters of 3,3′5,5′-tetramethylbenzidine (TMB) and 100 μl $H_2O_2$ were added to the latexes and left to react for 30 min in the dark at 37 °C. A 50 μl of aliquot of $H_2SO_4$ was added to stop the reaction and the change of color of the solution was observed.

### 2.4. Characterization

The size and morphology of the latexes were determined using transmission electron microscopy (TEM, Model CM120, Philips). The magnetite content of the dried samples was measured by thermogravimetric analysis (TGA, Model 2050, TA Instruments). The magnetic properties of the latexes were measured by using a Vibrating Sample Magnetometer (VSM, Model 155, EG&G Princeton Applied Research). The electrokinetic characterization of the latexes was determined by measuring the electrophoretic mobilities and Zeta potentials at 25 °C under different ionic strengths (NaCl as the electrolyte) and pH conditions using a Zeta-Sizer (Model 2000, Malvern Instruments).

The presence of carboxyl groups on the surface of the latex was analyzed by Fourier Transform infrared spectrometer (FT-IR, Model 55, Bruker Equinox) and the amount of carboxyl groups on the surface was determined by potentiometric titration using a potentiometer (Model ZD-3, Shanghai Exact Science Instruments Ltd.). The amount of bound antibody was determined by measuring the unbound protein content in the supernatant after binding process by UV (Model UV 300, Unicam) at 280 nm.

## 3. Results and discussion

### 3.1. Synthesis process

Fig. 1 shows the TEM images of the $Fe_3O_4$-St dispersion. It was found that in styrene the oleic-acid-coated magnetite particles were dispersed independently with a size down to 8 nm.

For the encapsulation of magnetite particles into polymer matrix by a miniemulsion polymerization process, a stable dispersion of magnetite particles in monomer styrene is required. Fig. 1 showed that oleic acid was very efficient, which was coated on magnetite particles and made the particles well stabilized in styrene and related monomer mixtures.

The lipophilic dispersion could be miniemulsified in water by using SDS as a second emulsifier and hexadecane as osmotic agent along with sonication. Since hexadecane was added to monomer phase as an ultrahydrophobe to prevent Ostwald ripening, the miniemulsion was quite

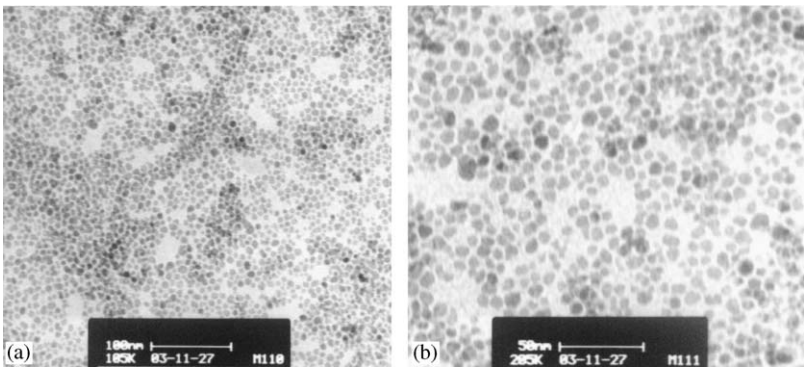


Fig. 1. TEM images of $Fe_3O_4$-St dispersion at different magnification (a) 100,000 ×; (b) 200,000 ×.

kinetically stable. Then the polymerization could be started by adding the hydrophilic initiator ACPA at 70 °C, which carried carboxyl groups and would also provide carboxyl end groups on the surface of polymer latexes. The resulting dispersion was stable and free of coagulation. A simple test with a magnet showed that the dispersion was a ferrofluid, i.e. was magnetic.

### 3.2. Size analysis

In the TEM images (Fig. 2), polystyrene latexes with encapsulated magnetite particles are shown. Latexes with an average diameter of about 250 nm were observed. A low-magnification image gives a feeling for the somewhat polydispersity of the nanospheres (Fig. 2a). At higher magnifications, a more detailed structure of the magnetite within the nanolatex is obtained (Fig. 2b). The distribution of magnetite particles in the polymer nanosphere seems to be rather homogeneous.

### 3.3. Magnetite content measurement

Thermogravimetric analysis quantified the amount of magnetite within the polymer latex. Fig. 3 shows a 78.5 wt% loss of organic material up to 350 °C, which reveals that the magnetite loading in the final polymer latex is 21.5 wt%. It is found that the magnetic polymer latexes moved quickly under external magnetic field and could be separated completely from water in a very short time. Therefore the magnetic force is strong enough for magnetic separation in various biological applications.

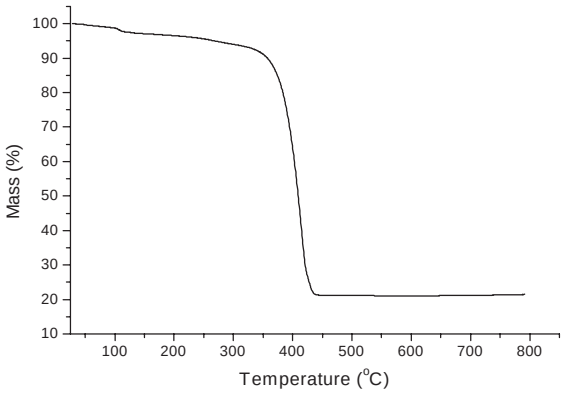


Fig. 3. TGA curve of magnetic polymer latexes.

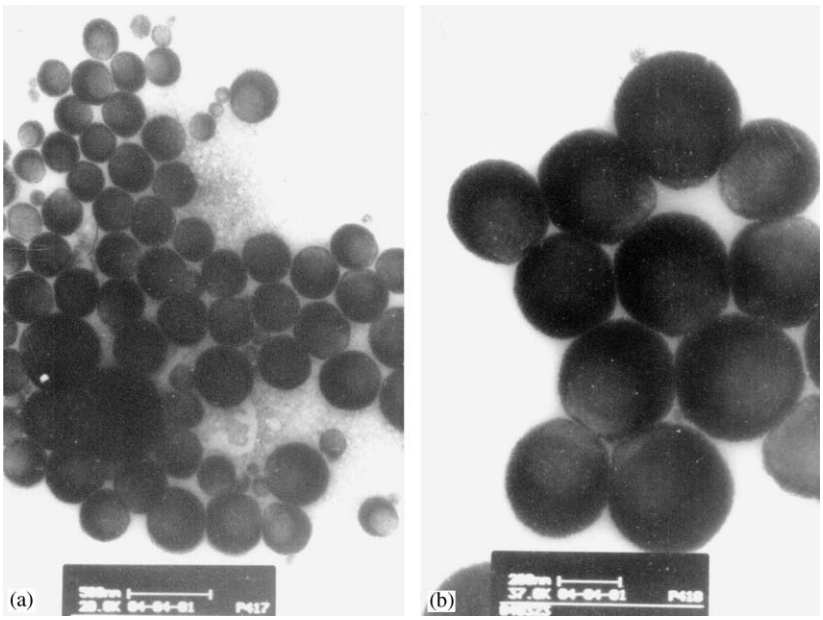


Fig. 2. TEM images of magnetic polymer latex (a) 20,000 × ; (b) 37,000 × .

### 3.4. Magnetic properties

The magnetic properties of the synthesis polymer latex encapsulated magnetite particles are of high interest for further applications. Fig. 4 is a typical magnetization curve measured by VSM. The magnetic properties including saturated magnetization ($M_s$), the residual magnetizations ($M_r$) of 1 g of samples, the relative saturation remanence ($m_r = M_r/M_s$), and the coercivities ($H_c$) are also given in Fig. 4. The sample shows a typical superparamagnetic behavior at room temperature without any hysteresis loop, which is also reflected in the low $M_r/M_s$ ratio and in the small $H_c$ value.

### 3.5. Electrokinetic characterization

The electrophoretic mobility values ($\mu_e$) and Zeta potentials ($\zeta$) versus pH are shown in Fig. 5. The pH was controlled using different buffers (acetate at pH 4 and 5, phosphate at pH 6 and 7, borate at pH 8 and 9, and carbonate at pH 10), and the ionic strength was kept at a constant value of 0.01 M. From Fig. 5, the $\mu_e$ and $\zeta$ values were positive at lower pH. With the increase of pH, the values became negative and almost constant when pH increased from 7 to 10. It is possible to recognize the weak acid character of the surface end groups of the latexes, which confirmed the presence of carboxyl acid on the surface. The isoelectric point (pI) of the carboxylated latexes is approximately at 4.32. The low values of $\mu_e$ and $\zeta$ at acid pH explained the low colloid stability of the carboxylated latexes, especially at pH around pI. Therefore the latexes should be performed at basic pH, where a good colloid stability can be achieved.

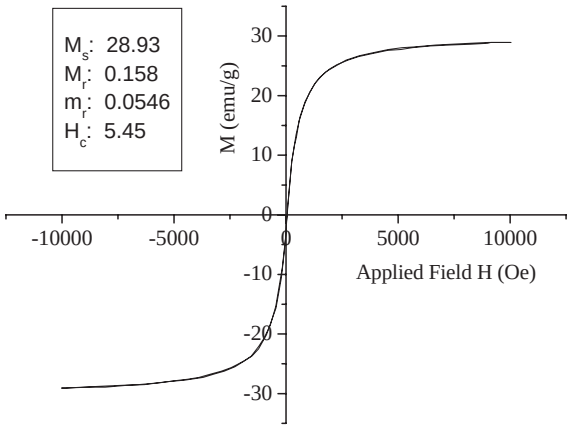


Fig. 4. Magnetization curves of magnetic polymer latex by VSM.

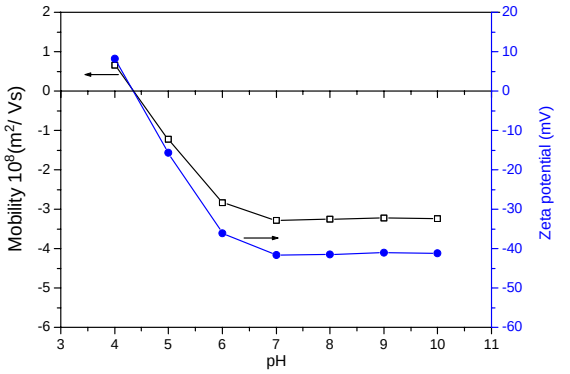


Fig. 5. Dependence of electrophoretic mobility and Zeta potential on pH for latexes.

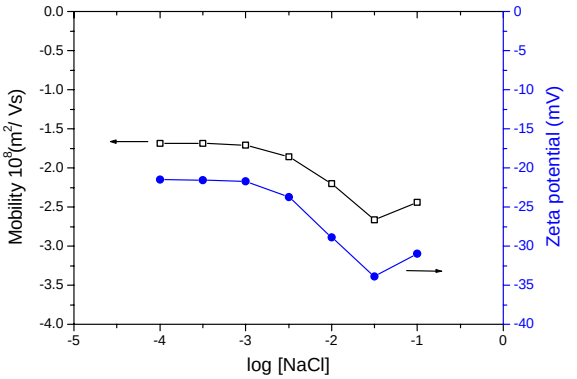


Fig. 6. Dependence of electrophoretic mobility and Zeta potential on electrolyte concentration (NaCl) for latexes.

Fig. 6 shows the $\mu_e$ and $\zeta$ of the latexes measured at different electrolyte concentrations (NaCl). At lower ionic strength, the $\mu_e$ and $\zeta$ values almost kept constant. However, there is a pronounced increase at high ionic strength and a little decrease when the ionic strength is higher. The optimum ionic strength for the good colloid stability is about 0.0316 M.

### 3.6. Surface functional group analysis

As mentioned above, the dependence of electrophoretic mobility and Zeta potential on pH for the

magnetic polymer latexes (Fig. 5) shows the weak acid character of the surface end groups on the latexes. To further confirm the presence of these carboxyl groups on the surface of latexes, a FTIR spectrum was obtained as shown in Fig. 7. The carboxyl band is observed at 1725.31 $cm^{-1}$ (C═O), 1448.67 $cm^{-1}$ (C–O), and 3459.49 $cm^{-1}$ (OH), which indicated the presence of COOH on the latex surface.

Potentiometric titration was carried out to quantify the amount of surface carboxyl groups. According to the electrokinetic characterization of the carboxylated latexes (as shown in Fig. 5), the latexes had lower colloid stability at lower acid pH. So it is difficult to determine the total carboxyl groups from direct titration in the latexes with weak acid groups on their surface, as it is necessary to protonate all the carboxyl groups, which occurs at pH around 3 and under these conditions the latexes would be aggregated. Therefore, a back titration was carried out to obtain the curve, which is shown in Fig. 8.

There are three different slopes in the potentiometric curve, each is caused by: (i) the titration of the excess of NaOH previously added (since this plot represents a back-titration), (ii) the titration of the carboxyl groups of the latexes, and (iii) the last slope is caused by the excess of titration agent (HCl). Knowing the total latexes employing ($W$), the HCl concentration ($N$), and the total volume ($V$) of this solution consumed in the titration, we can calculate the amount of surface carboxyl groups according to

$$-\mathrm{COOH}\ (\mathrm{mmol/g\ latex}) = NV/W. \quad (1)$$

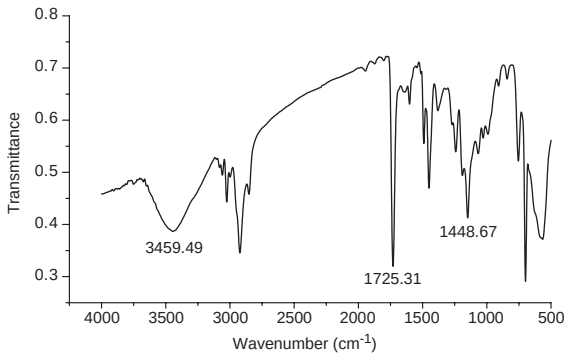


Fig. 7. FTIR spectrum of magnetic polymer latexes.

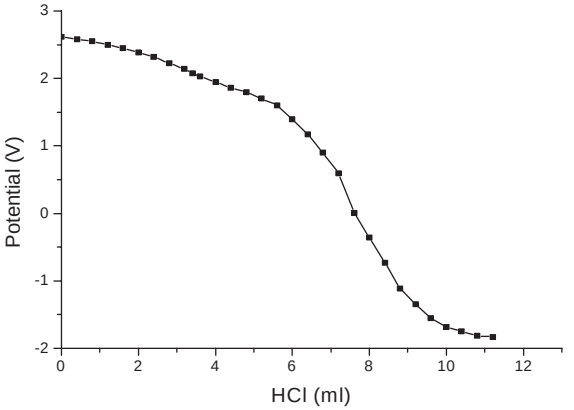


Fig. 8. Potentiometric titration curve of magnetic polymer latexes.

From the curve, about 3.8 ml HCl (0.01 M) was consumed in the titration of the carboxyl groups of the latexes (200 mg). Therefore, the amount of surface functional COOH groups is about 0.19 mmol/g, which is enough for the magnetic polymer latexes to couple protein in various biological applications such as immunoassay and cell separation.

### 3.7. Covalent coupling of protein

To investigate the feasibility of surface carboxyl groups of the magnetic polymer latexes for use in biological applications, we perform the experiment of covalent coupling protein. Mouse IgG was bound on the surface of the latex using the conventional carbodiimide chemistry as shown in Fig. 9.

By assaying the absorption value of the unbound protein in the supernatant after the binding process at 280 nm using UV and comparing the value before binding, it was found that mouse IgG was indeed bound on the surface of the latex and the bound amount was about 12.8 mg/g latex.

The activity of bound mouse IgG was determined by a conventional ELISA method as described. A strong color response was obtained in the ELISA with antibody-bound magnetic polymer nanolatexes compared to un-coated latexes. The absorbance values at 450 nm was $0.652 \pm 0.005$ versus $0.082 \pm 0.002$ for the un-

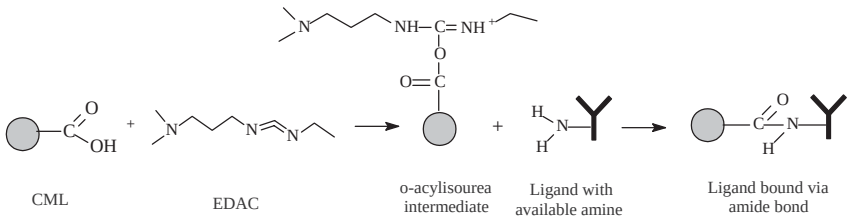


Fig. 9. Covalent coupling reaction of protein with carboxylated latex by carbodiimide activation.

coated latexes. It is verified that the antibody was still immunologically active by reacting with an enzyme-labeled second antibody (SAM-HRP) and by measuring the amount of enzyme bound.

## 4. Conclusion

We showed that carboxylated magnetic polymer nanolatexes can be efficiently achieved via a miniemulsion process using ACPA as initiator, which also provides the carboxyl groups on the surface of latexes. TEM, TGA and VSM analysis indicated that up to 20% of 8 nm magnetite particles were homogeneously encapsulated into the polymer latex resulting in the average diameter of 250 nm, and the latexes are superparamagnetic. Electrokinetic characterization showed that the functional magnetic polymer nanolatexes had good colloid stability in water at basic pH and high ionic strength. FTIR and potentiometric titration confirmed that there are abundant carboxyl function groups on the surface, which can be efficient in covalently coupling protein such as antibody for further biological applications.

## Acknowledgements

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