

Hyperthermia effect of surface-modified magnetite nanoparticles in a microfluidic system

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ABSTRACT

Surface-modified magnetite nanoparticles (SMMNPs) were synthesized and characterized. Fe₃O₄ nanoparticles (NPs) was first synthesized via coprecipitation, and then poly(acrylic acid) (PAA) was coated on these NPs. The coating process is not possible at pH higher than 5.5 due to the electrostatic repulsion between Fe₃O₄ NPs and ionized PAA. It was found that both PAA-coated Fe₃O₄ and pure Fe₃O₄ are superparamagnetic. The dispersibility of the SMMNPs was greatly influenced by pH; as pH value becomes larger, the size distribution of the NPs becomes narrower. After applying a magnetic field (88.24 kA/m and 69.44 kHz), the SMMNPs can increase the temperature of the solution in the range of 4.7~6.5 °C in 15 min, which is suitable for hyperthermia applications.

Keywords: hyperthermia, magnetite nanoparticles, magnetite, nanoparticles, poly(acrylic acid), surface modification

1 INTRODUCTION

Magnetic NPs have been investigated for biomedical application, such as hyperthermia [1], cell therapy [2], magnetic resonance image (MRI) [3], drug delivery [4], and magnetofection [5]. For these applications, however, some issues, such as short blood circulation time, and nonspecific targeting, should be overcome. Besides, these magnetic NPs tend to aggregate, thus surface modifications on these NPs are necessary. In this report, SMMNPs were synthesized and analyzed. Besides, an *in vitro* approach was also proposed to investigate the hyperthermia effect of the SMMNPs on the cancer cells.

2 EXPERIMENTAL

2.1 Materials

Fe(III) chloride hexahydrate (99%) was purchased from Acros and used as received. Sodium hydroxide (99%) and sodium sulfite (99%) were purchased from Riedel-de Haën. Poly(acrylic acid) (MW~5,000) was purchased from Aldrich. Other chemicals were of analytical grade and used without further purification. Distilled and deionized water was used throughout the work.

2.2 Synthesis of surface-modified Fe₃O₄ NPs

Fe₃O₄ NPs were first synthesized via the modified coprecipitation according to literature [6]. In this process, 50 ml of 1.0 M FeCl₃ in 2.0 M HCl aqueous solution and 25 ml of 2.0 M Na₂SO₃ aqueous solution were added in 100 ml of 10.0 M NaOH aqueous solution with vigorous stirring at 70 °C under N₂. After precipitation, Fe₃O₄ NPs were purified by centrifugation, and the supernatant was discarded with the assistance of a magnet; this procedure was repeated several times. After purification, the precipitate was added into the PAA solution with vigorous stirring at 80 °C under N₂. The weight ratio of Fe₃O₄/PAA was maintained at 4/3. The procedure for purification of the PAA-coated NPs was the same as that for the uncoated Fe₃O₄ NPs, except with the addition of 0.1 M HCl, which is used to enhance the precipitation of the PAA-coated NPs. Up to this step, the precipitate included both PAA-coated and uncoated magnetite NPs. After lyophilization, the dried particles were added into a 0.1 M NaOH solution. The PAA-coated magnetite NPs precipitated much slower than un-coated ones under magnetic attraction due to electrostatic repulsion, and thus the uncoated ones can be excluded. A solution of 0.1 M HCl was then added to the supernatant obtained to precipitate the PAA-coated magnetite NPs, and the PAA-coated ones are purified several times according to the same procedure established for Fe₃O₄ purification. Finally, the PAA-coated magnetite NPs were dried by lyophilization.

2.3 X-ray diffraction analysis

X-ray diffraction measurements with monochromatic Cu K α radiation were done to investigate the crystal structure of the PAA-coated and pure magnetite NPs.

2.4 Particle size distribution and zeta potential of magnetite NPs

The zeta potential and the size distribution of the PAA-coated magnetite particles at different pHs were measured at 25 °C with a dynamic laser scattering analyzer (Malvern Zeta Sizer 3000H). Before the measurements, Fe₃O₄ particles were dispersed in water and pretreated by ultrasonication for 5 min.

2.5 SQUID analysis

The magnetization curves of the PAA-coated Fe_3O_4 particles and pure ones were measured at 25 °C with a SQUID magnetometer (superconducting quantum interface device, Quantum Design MPMS7). The applied magnetic field was ranged between $\pm 10,000$ gauss.

2.6 FTIR measurement

To prepare the samples for the FTIR measurements (Bio-Rad FTS-3000 spectrometer), the PAA oligomers, used as received, the magnetite particles synthesized and the PAA-coated magnetite particles were grind-blended with KBr and then compressed to form a pellet. The transmission spectra were obtained for 64 scans at a resolution of 4 cm^{-1} .

2.7 Hyperthermia test

The heating effect of the PAA-coated magnetite particles under AC magnetic field produced by a medium-high frequency induction heater (New HP-Cube 15kw) was examined. The strength and frequency of the applied magnetic field were 88.24 kA/m and 69.44 kHz, respectively. Different weights of magnetite particles were added in water of 2 ml in the test tube situated in the middle of the heating coil of the induction heater. The time courses of temperature increase (ΔT) were recorded by a Pt thermocouple (Thermo Recorder TR-81).

3 RESULTS AND DISCUSSIONS

Magnetite NPs were first synthesized via the modified coprecipitation^[6], and then were surface-modified with PAA oligomer. This coating process is impossible at pH higher than 5.5 because of the electrostatic repulsion between the negatively charged magnetite NPs and the ionized PAA (see Fig. 1). According to the X-ray diffraction results (Fig. 2), the crystal structure of the PAA-coated magnetite particles is the same as that of the uncoated ones and belongs to the standard Fe_3O_4 crystal structure. The success of PAA coating process was confirmed by FTIR. FTIR spectra of the PAA-coated NPs, as shown in Fig. 3, indicate that the characteristic IR absorption peaks of PAA at 1710 and 1405 cm^{-1} are also noticed in the spectrum of PAA-modified NPs. The absorption peak appeared at 1710 cm^{-1} was due to the binding of the carboxylic acid groups to the surface of the NPs to form carboxylate groups [7]. The size or size distribution of the PAA-coated magnetite particles was influenced greatly by the pH of the solution. Fig. 4 indicates that as the pH value becomes larger, the size distribution of the NPs becomes narrower and nanosized. At higher pH, more carboxyl groups get ionized, thus providing more electrostatic repulsion among NPs (see Fig. 5), which prevents NPs from aggregation.

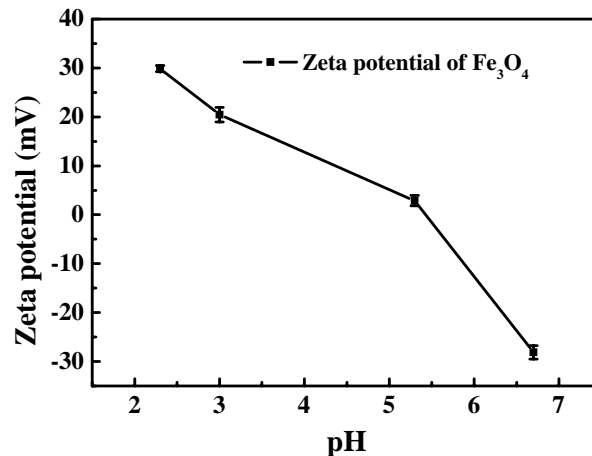


Fig. 1. Zeta potential of Fe_3O_4 particles at different pHs.

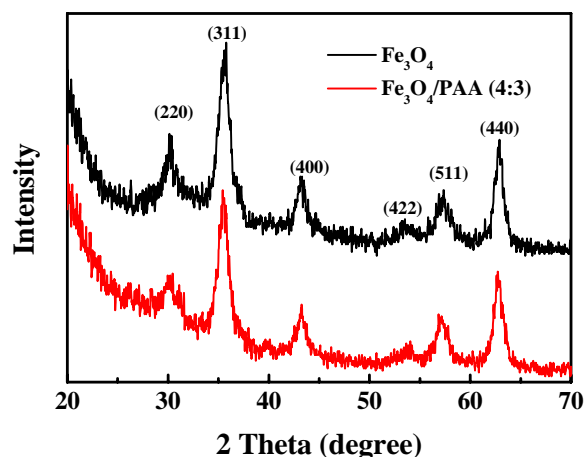


Fig. 2. XRD analysis of PAA-coated and pure Fe_3O_4 particles.

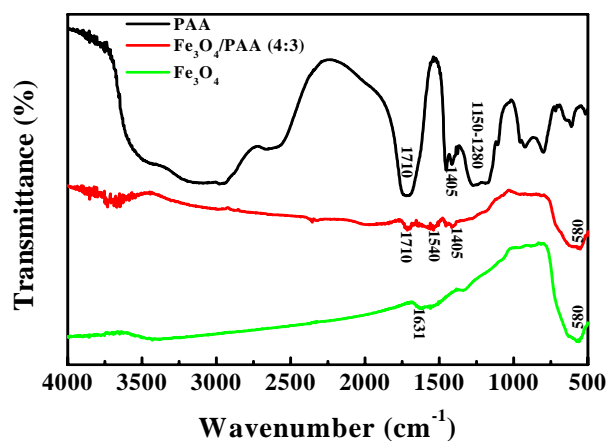


Fig. 3 FTIR analysis of PAA oligomers, pure and PAA-coated magnetite particles.

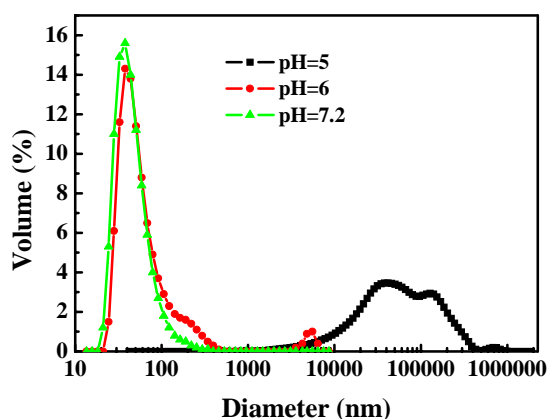


Fig. 4 Particle size distribution of PAA-coated particles at different pHs.

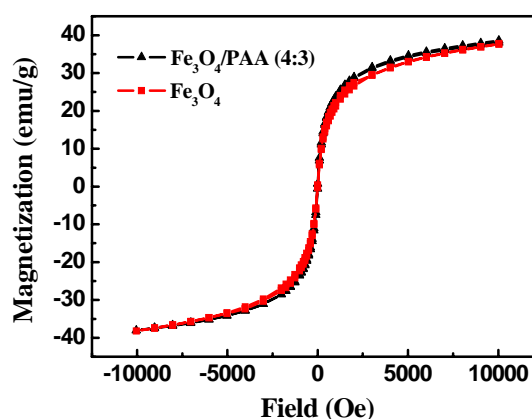


Fig. 6 SQUID magnetization curves of PAA-coated and pure magnetite particles at 298 K.

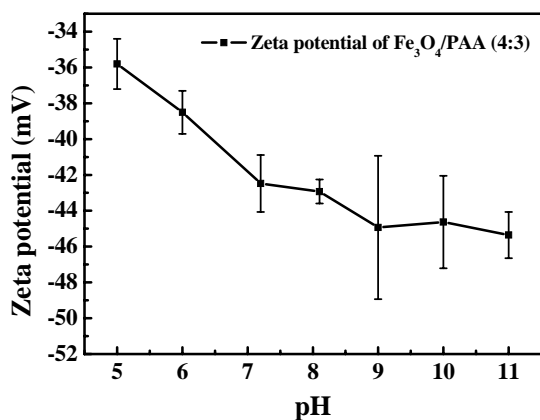


Fig. 5 Zeta potential of PAA-coated magnetite particles at different pHs.

The magnetic property of the PAA-coated and pure Fe_3O_4 particles were examined by SQUID. The results, as shown in Fig. 6, indicated that both of these particles were superparamagnetic and their saturated magnetization and susceptibility were almost the same. To test the feasibility of these PAA-coated particles for hyperthermia application, the predetermined amounts of these NPs were put into a test tube situated within the magnetic heating coil. After applying an AC magnetic field (88.24 kA/m and 69.44 kHz), these PAA-coated Fe_3O_4 particles can increase the temperature of the solution to 4.7~ 6.5 °C in 15 min (see Fig. 7), which is considered to be practical for hyperthermia. However, the amount of PAA coated on the magnetite particles should be lowered to enhance the heating effect of the PAA-coated magnetite particles.

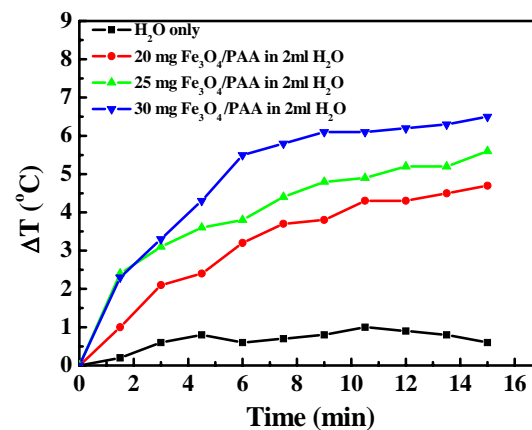


Fig. 7 The heating effect of the PAA-coated magnetite particles under AC magnetic field (88.24 kA/m, 66.94 kHz).

Application of these PAA-coated magnetite NPs for hyperthermia is not practicable without further modifying the surface with more biocompatible polymers and the home-device, such as folic acid. Conjugation of folic acid and poly(2-ethyl-2 oxazoline) (MW~5000) with the PAA-coated magnetite particles is under investigation. Poly(-2-ethyl-2-oxazoline) is a highly hydrophilic polymer and used to increase the blood circulation time of the PAA-coated magnetite particles [8]. On the other hand, folate receptors are often overexpressed on the tumor cells [9], and therefore the carriers conjugated with folic acid can bind more specifically to the tumor cells. Here, an *in vitro* approach was proposed to investigate the hyperthermia effect of the SMMNPs on the cancer cells. The fibroblast cells and human breast cancer cells (BT-20) are immobilized on the cavities of the interaction chamber (see Fig. 8), situated in the middle of the magnetic coil, and the survival rate of these two cells will be compared after circulation of these

SMMNPs under applying an AC magnetic field. These experiments are under investigation.

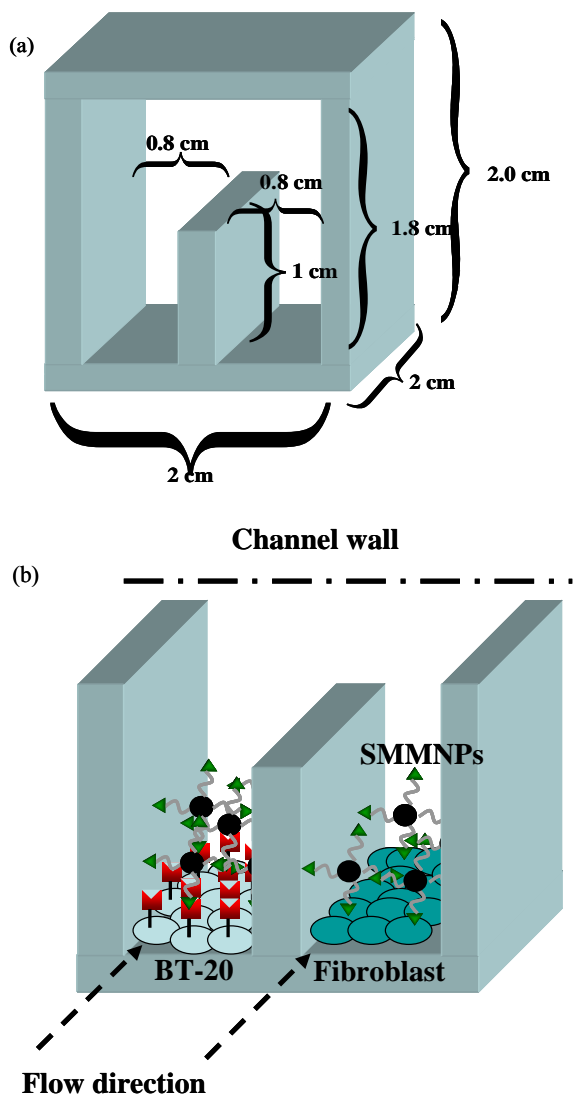


Fig. 8(a) The dimensions of the interaction chamber situated in the middle of the magnetic coil. (b) The schematic of the interaction between SMMNPs and cells.

4 CONCLUSION

Superparamagnetic PAA-coated magnetite NPs were synthesized and their physical properties are examined. The particle sizes and the size distribution of these NPs were greatly affected by the pH of the solution. Besides, these PAA-coated magnetic particles disperse well at pH 7.2, which is desired for use in physiological conditions. Furthermore, these particles are able to heat the water with an increment of at least 4.7 °C in 15 min and can be used for hyperthermia. To specifically investigate the interactions between cancer cells and normal cells, an *in vitro* approach is proposed. Although the latter approach is still under

investigation, we believe that by modifying the surface of the magnetite NPs, difference in interactions between NPs and cancer cells and normal cells could be amplified. Thus, the hyperthermia effect on cancer cells could be more obvious than that on normal cells.

5 ACKNOWLEDGEMENT

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