Modified iron oxide nanoparticles with polyethylene glycol and citric acid for biomedical applications

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Abstract

Research on synthesis of superparamagnetic iron oxide nanoparticles and its surface modification for biomedical applications is of intense interest. The modified surface of NPs has resulted in magnetization softening. Also, this is very applicable to prepare biocompatible, surface-modified soft magnetic materials, which may offer enormous utility in the field of biomedical research. In this study, poly(ethyleneglycol) (PEG)-coated Fe_3O_4 magnetic and citric acid (CA)-coated Fe_3O_4 magnetic have been synthesized by the coprecipitation method. The nanoparticle size can be controlled by changing the concentration of PEG and CA. The nanoparticles were characterized by XRD, EDX, FESEM, FT-IR and VSM techniques. The size of nanoparticles from non-coating and coating of PEG, AC are 15-20 nm respectively, and the nanoparticles are relatively uniform which is evidenced by the results of FESEM. Measurement of VSM at room temperature showed that iron oxide nanoparticles had superparamagnetic properties.

Keywords. Iron oxide nanoparticles, PEG-coated Fe_3O_4 magnetic, CA-coated Fe_3O_4 magnetic.

1. INTRODUCTION

In nature, iron nanoparticles are classified into 3 main forms: hematit (α-Fe_2O_3), maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4).[1] All of these forms exhibit some important properties such as biocompatibility, relatively low toxicity to the human body, less affected by oxidation, stability in magnetic reaction. Iron nanoparticles are synthesized by coprecipitation, nucleation by simultaneous reaction of hydrolysis and dehydration of a mixture of iron (III) salt and iron (II) salt solutions. In the case of formation of magnetic particles, when increasing pH, $Fe(OH)_2$ combines with ferrihydrite and forms an intermediate compound of Fe^{2+}-ferrihydrite, then condenses into magnetic particles with inverse spinel structure. Meanwhile, the transfer of electrons between Fe^{2+} and Fe^{3+} plays a fundamental role in crystallization.[2]. According to the thermodynamics of the reaction to completely precipitate Fe_3O_4, the pH ranges from 8 to 14, with a chemical equilibrium ratio of 2:1 (Fe^{3+}/Fe^{2+}) in an oxygen-free environment.[3] LaMer[4] provides a diagram illustrating the formation of nanoparticles with crystallite creation and growth mechanisms.

Thus, to be able to apply Fe_3O_4 nanoparticles effectively in some biomedical applications such as drug delivery and contrast enhancer in magnetic resonance imaging (MRI), nanoparticles must have a maximum value of magnetization saturation (Ms) and a minimum value of coercivity (Hc), as well as residual magnetism (Mr).[5] To achieve this goal, ultrafine magnetic particles must be stably dispersed in a solution of water or organic liquid.[6,7] Therefore, the preparation of liquid magnetic substances is divided into two steps: The first step is the synthesis of magnetic nanoparticles and the second step is to stabilize the particles in the liquid by physical or chemical surface treatment.[8] Now, to stabilize liquid particles and higher biological compatibility on their surfaces, scientists often use electrostatic dispersants and create other coatings on the surface of Fe_3O_4 particles. In particular, they study the surface modification of Fe_3O_4 particles by adding organic anions including carboxylate, hydroxy carboxylate ions such as: citric acid,[9,11] gluconic,[12] oleic acid[13,14] ... or compounds which create polymer surfaces such as PEG,[15] dextran,[16] starch,[17] polyvinyl alcohol.[18] The presence of these organic ions on the surface of iron oxides can

prevent nucleation and then lead to larger particles or inhibit the growth of crystal nuclei, leading to small nanoparticles.

In this research, we have synthesized PEG-coated Fe_3O_4 magnetic and CA-coated Fe_3O_4 magnetic using the coprecipitation method. The effects of PEG concentration and CA concentration on the degree of crystallinity, average particle size and magnetic properties have been investigated.

2. EXPERIMENTAL

2.1. Materials

Ferric chloride hexahydrate ($FeCl_3.6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2.4H_2O$) was purchased form Merck. The PEG polymers (PEG-6000), AC and NH_3 solution were obtained from Macklin-China, India. Deionized water was used for the preparation of all aqueous solutions.

2.2. Synthesizing the PEG-coated magnetic and CA-coated magnetic (NPs)

The PEG-coated magnetic and CA-coated magnetic materials were synthesized in 3 steps as with the procedure for preparation of superparamagnetic iron oxide nanoparticles. In which PEG agent is added to step 1, CA agent is added to step 2 as shown in figure 1.

Step 1: Put 0.9950 g of $FeCl_2.4H_2O$ and 2.7050 g of $FeCl_3.6H_2O$ into a flask containing 100 ml of distilled water, heat the reaction system to a temperature of 80 °C and maintain that throughout the reaction. The reaction mixture is stirred for 30 minutes, while aerating nitrogen to obtain a yellow-orange solution.

Step 2: Slowly add 25-28 % NH_3 solution to the reaction flask so that the solution always maintains

pH=9-10. At this time, the solution turns from yellow-orange to brown then black. After adding sufficient NH_3 solution, continue the reaction for 1 h, maintaining the reaction conditions.

Step 3: The black precipitate product is obtained, filtered and washed with distilled water several times until the environment becomes neutral. Next, wash 2 more times with ethanol. The product is dried by oven at a temperature of 50 °C.

2.3. Characterizations

X-ray diffraction patterns of the solid powders were recorded with a P'Pret Pro - PANalytical X-ray diffractometer operating at 1.8 kW (40 mA/45 KV) using CuK_α ($\lambda = 1.5406$ Å) radiation. FTIR spectra of the solid samples were recorded by KBr pellet method using a Bruker FTIR spectrometer. FESEM – EDX measurements were carried out using a Hitachi S-4800, EMAX operating at 20 keV. The minimal amount of solid sample was dispersed in ethanol and small drops were placed on an aluminum grid. The grid was dried for 1-2 h in a vacuum over at 40 °C prior to the FESEM studies. Magnetic measurements of the solid samples were performed at room temperature (25 °C) using a Magnet B-10 Vibrating sample magnetometer (VSM).

3. RESULTS AND DISCUSSION

The PEG-coated magnetic and CA-coated magnetic are synthesized in the N_2 gas environment using PEG and CA dispersing agents. Surveying the effect of PEG concentration: 25 to 100 mg/l and CA concentration: 100 to 1200 mg/l to the formation of Fe_3O_4 crystals, the study samples are presented in table 1.

Table 1: Study on sample characteristics

No.	Sample	Concentration of PEG (mg/l)	Sample	Concentration of CA (mg/l)
1	M.80.25-P	25	M.80.1-A	100
2	M.80.50-P	50	M.80.2-A	200
3	M.80.75-P	75	M.80.6-A	600
4	M.80.100-P	100	M.80.12-A	1.200

The resulting black precipitate, which can be attracted by a permanent magnet proved the formation of Fe_3O_4 crystals and detailed characterization including: X-ray, VSM, EDX, FESEM and FTIR. Figure 2 depicts the powder X-

ray diffraction (XRD) patterns of the PEG-coated magnetic and CA-coated magnetic samples. The position and relative intensity of all diffraction peaks in both XRD pattern are fully matched. Also, all diffraction peaks were in agreement with the

standard card of Fe_3O_4 (00-072-2303). It is worth noting that the main peak (311) of both samples is clearly the characteristic peak of magnetite.[7,18] However, all the peak positions at 18.2 (111), 35.4 (311), 57.2 (511) and 62.6 (440) are consistent with

the standard X-ray data for the magnetite phase of the CA-coated magnetic samples,[12] and the intensity of these peaks is larger than the PEG-coated magnetic samples.
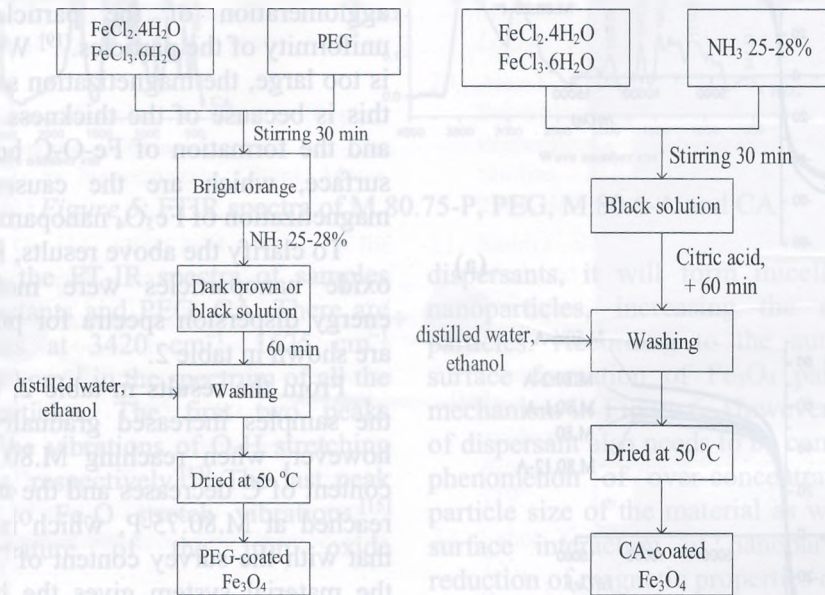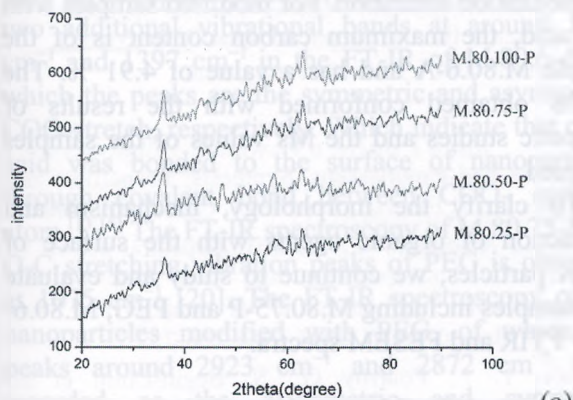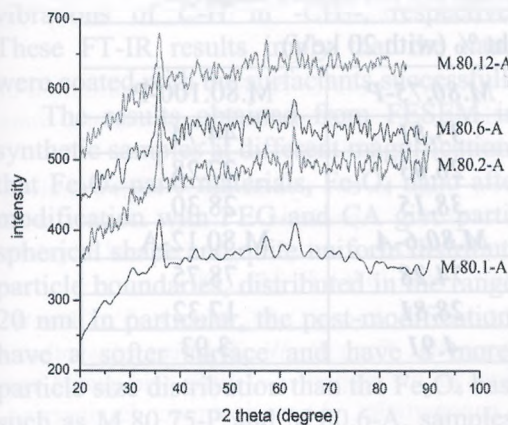

Figure 1: The procedure for preparation of PEG-coated magnetic and CA-coated magnetic

Figure 2: X-ray patterns of (a) PEG-coated magnetic and (b) CA-coated magnetic

Next, magnetic measurements were made using a vibrating sample magnetometer. Magnetization curves were measured at room temperature and parameters such as coercive field (Hc) and initial susceptibility (χi) were obtained. The saturation magnetization (Ms) was obtained by extrapolation to infinite field of the experimental data obtained in the high field range where the magnetization varies linearly with the inverse of the applied field. Saturation magnetization values were normalized taking into account the percentage of PEG and AC calculated from the samples.

The survey results showed that, from the saturation of Fe_3O_4 nanoparticles when not using dispersant reached 56.77 emu/g, when using dispersant PEG gives saturation from 64.94 emu/g for samples using PEG concentration of 75 mg/l.

The research results showed that the sample M80.75-P had the greatest magnetization saturation, the remaining samples had lower magnetization, with two samples used at concentrations of 25 and 50 mg/l giving lower corresponding magnetizations of 54.45 emu/g; 57.26 emu/g and equivalent when not using dispersant. This may be explained as follows, if using surfactants less than 75 mg/l PEG, it is not enough to surround nanoparticles to form micelles, otherwise if greater than 75 mg/l micelles

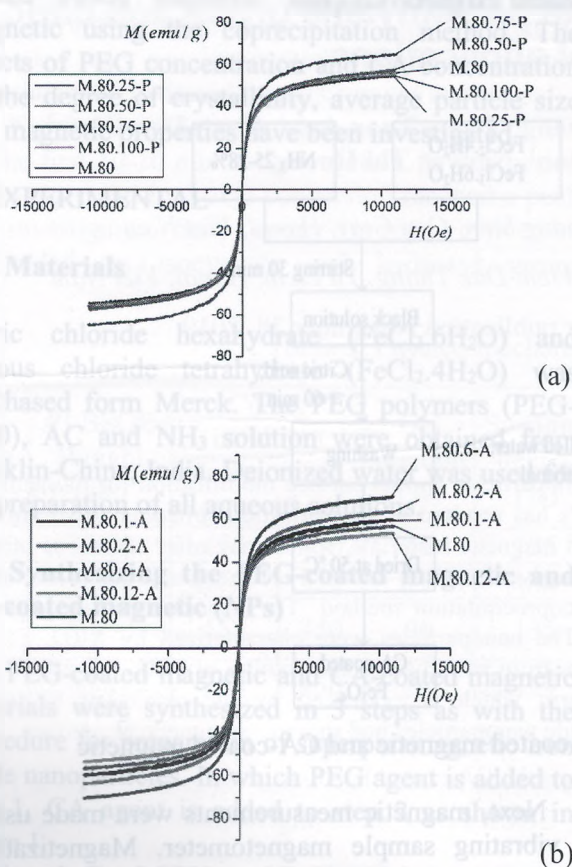are broken so the magnetization value has not reached the optimal value.

(a)

(b)

Figure 3: M-H curves for (a) PEG-coated magnetic and (b) CA-coated magnetic

When using citric acid as dispersant, the results are 59.64; 63.64; 70.52 and 53.47 emu/g equivalent to M.80.1-A; M.80.2-A; M.80.6-A and M.80.12-A samples. So when the acid content increases to 600 mg, the magnetization increases, if the acid content

continues to increase, the magnetization decreases gradually and when the acid content increases to 1200 mg, the magnetization decreases to near the value when not using dispersant. The results shown above are due to, when adding acid agents with appropriate content, hydrophilic micelles are created, covering the nanoparticles, reducing the agglomeration of the particles, increasing the uniformity of the particles.[19] When the acid content is too large, the magnetization saturation is reduced, this is because of the thickness of the acidic cover and the formation of Fe-O-C bonding layer on the surface, which are the causes of reduction of magnetization of Fe_3O_4 nanoparticles.

To clarify the above results, Fe_3O_4 magnetic iron oxide nanoparticles were measured with EDX energy dispersion spectra for proof and the results are shown in table 2.

From the results in table 2, the content of C in the samples increased gradually from M.80.25-P. however, when reaching M.80.100-P sample, the content of C decreases and the maximum content is reached at M.80.75-P, which is 38.15 %, proving that with the survey content of 75 mg/l of PEG for the material system gives the best dispersion, the results of this study are consistent with the results of FESEM images as well as the results of determining magnetization saturation. For modified samples with CA acid, the maximum carbon content is of the sample M.80.6-A and has a value of 4.91 %. The results obtained conformed with the results of magnetic studies and the Ms values of the samples obtained.

To clarify the morphology, mechanism and interaction of organic agents with the surface of Fe_3O_4 particles, we continue to study and evaluate two samples including M.80.75-P and PEG, M.80.6-A by FTIR and FESEM spectra.

Table 2: EDX analyses of (a) PEG-coated magnetic and (b) CA-coated magnetic

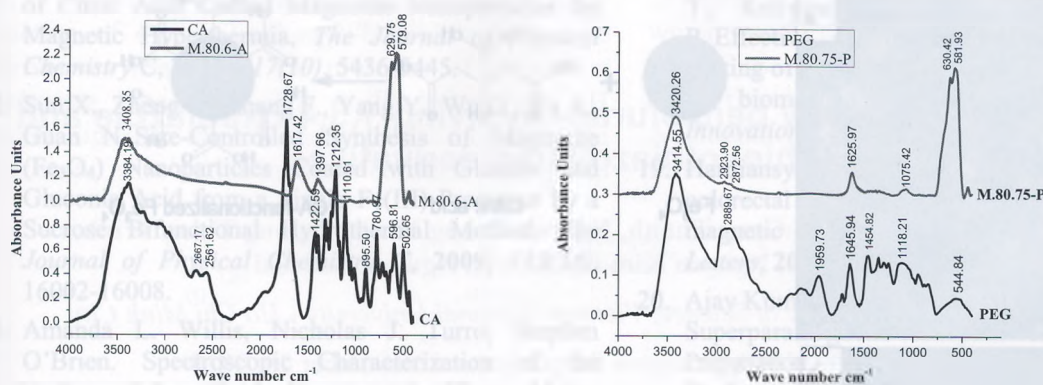| Element (a)	Samples/Atomic weight % (with 20 keV)			
	M.80.25-P	M.80.50-P	*M.80.75-P*	M.80.100-P
Fe	54.98	43.92	*35.81*	44.74
O	30.53	30.62	*26.03*	27.28
C	14.49	25.44	*38.15*	28.30
Element (b)	M.80.1-A	M.80.2-A	*M.80.6-A*	M.80.12-A
Fe	81.99	79.76	*74.28*	78.75
O	16.10	18.08	*28.81*	17.32
C	1.91	2.16	*4.91*	3.93

Figure 5: FTIR spectra of M.80.75-P, PEG, M.80.6-A and CA

Figure 5 shows the FT-IR spectra of samples modified with surfactants and PEG, CA. There are three obvious peaks at 3420 cm^{-1}, 1625 cm^{-1} (M.80.75-P), and 580 cm^{-1} in the spectrum of all the synthesized nanoparticles. The first two peaks basically represent the vibrations of O-H stretching and H-O-H bending, respectively.[13] The last peak can be attributed to Fe-O stretch vibrations,[15] confirming the nature of the iron oxide nanoparticles.

The most noticeable changes are the disappearance of the CO stretching band at 1728 cm^{-1} in the spectrum of CA and the appearance of two additional vibrational bands at around 1617 cm^{-1} and 1397 cm^{-1} in the FT-IR of M.80.6-A, of which the peaks are the symmetric and asymmetric COO-stretch, respectively. Which indicate that citric acid was bonded to the surface of nanoparticles through covalent bond between COO- and Fe atom.[13,15] The FT-IR spectroscopy of M.80.75-P, C-O-C stretching vibration peaks of PEG is observed at 1075 cm^{-1} [20]. The FT-IR spectroscopy of the nanoparticles modified with PEG, of which the peaks around 2923 cm^{-1} and 2872 cm^{-1} were regarded as the asymmetric and symmetric vibrations of C-H in -CH$_2$-, respectively.[15,16,20] These FT-IR results imply that the nanoparticles were coated with the surfactants successfully.

The results obtained from FESEM images of synthetic samples at different magnifications showed that Fe$_3$O$_4$ nano materials, Fe$_3$O$_4$ nano after surface modification with PEG and CA give particles with spherical shape and quite uniform distribution, clear particle boundaries, distributed in the range of 10 to 20 nm. In particular, the post-modification particles have a softer surface and have a more uniform particle size distribution than the Fe$_3$O$_4$ base sample, such as M.80.75-P and M.80.6-A, samples give the sizes of obtained nanoparticles in the range of 10-15 nm. This is because when using the PEG and CA

dispersants, it will form micelles covering some nanoparticles, increasing the uniformity of the particles. According to the authors [21, 22] the surface formation of Fe$_3$O$_4$ particles follows the mechanism in Figure 6. However, the concentration of dispersant also needs to be controlled to avoid the phenomenon of over-concentration affecting the particle size of the material as well as affecting the surface interaction of nanoparticles resulting in reduction of magnetic properties of the material.

Also, according to Hardiansyah [23], the covering citric acid surface does not change any crystalline structure or properties of Fe$_3$O$_4$ nanoparticles, citric acid agents create micelles with a hydrophilic tip on the outside and covering nanoparticles from the inside. Furthermore, when investigating the nano solution after creation, 10 to 120 days afterwards, it has not been observed to have deposited particles.

4. CONCLUSION

We have successfully prepared PEG-coated Fe$_3$O$_4$ magnetic and CA-coated Fe$_3$O$_4$ magnetic using the coprecipitation method. Particles obtained by this method are very uniform in size, with an average size from 15 nm to 20 nm. All the as-synthesized magnetite nanoparticles are superparamagnetic, and the saturation magnetization is greatly related to the particle size and concentration of surfactants (PEG and CA). The saturation magnetization of PEG-coated Fe$_3$O$_4$ magnetic, CA-coated Fe$_3$O$_4$ are 64.94 emu/g and 70.52 emu/g, respectively.

These study results imply that the nanoparticles were coated with the surfactants successfully. The modified surface of nanoparticles has resulted in softening the magnetization and its surface modification for applications in biomedicine such as magnetic resonance imaging contrast enhancement, drug delivery.
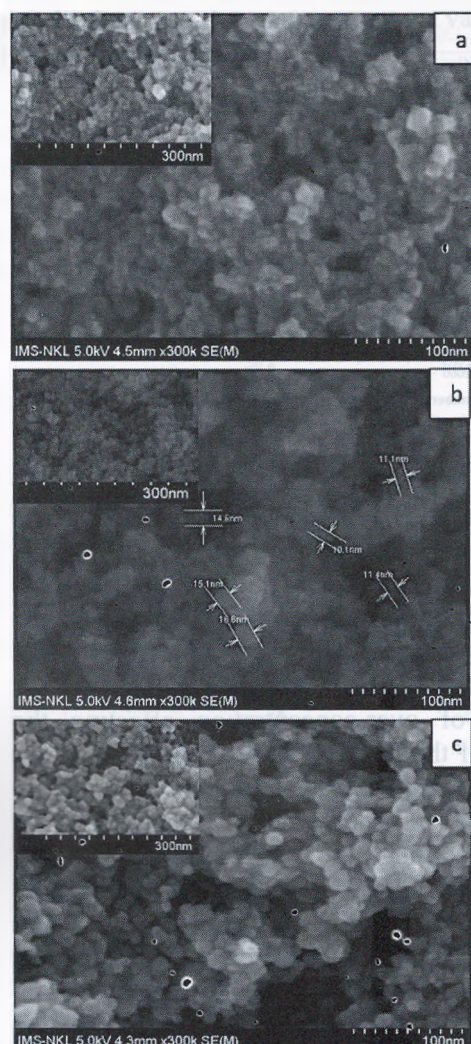
Figure 6: FESEM image of *a*-Fe₃O₄, *b*-M.80.75-P and
c-M.80.6-A; Scheme for the preparation of PEG-coated
magnetic and CA-coated magnetic

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