

Preparation and characterization of novel spin labeled magnetic nanoparticles

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Received: 11 Jan 2011, Revised: 16 Feb 2011 and Accepted: 19 Feb 2011

ABSTRACT

Novel spin-labeled magnetic nanoparticles (MNPs) were prepared through the reaction between carboxylic acids modified Fe₃O₄ particles and 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (4-NH₂-TEMPO). And X-ray diffractometer (XRD), FT-IR spectra, thermogravimetric analysis (TGA), transmission electronic microscopy (TEM) and room temperature electron paramagnetic resonance (EPR) were employed to characterize the prepared materials. The results of EPR measurements indicated that the spin labeled MNPs exhibited both the paramagnetism of nitroxide free radicals and superparamagnetism of ferrimagnetic nanoparticles could act as a bio-probe or potential drug delivery vehicles tracking by EPR technique. Copyright © 2011 VBRI press.

Keywords: Multifunctional magnetic nanoparticles (MNPs); spin labeled; electron paramagnetic resonance (EPR); bio-probes; drug delivery.



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Ronghua Yu, a Ph.D candidate in Pharmaceutical Engineering and Technology of the East China University of Science and Technology. His major area is multifunctional nanosized drug delivery systems.

Introduction

Magnetic nanoparticles (MNPs) with interesting structure advantages have been developed a number of obvious biomedical applications. [1, 2] Recently, the researchers are highly interested in developing multifunctional magnetic nanoparticles probes in order to offer synergetic multi-modality biomedical applications. [3, 4] For example, biocompatible iron oxide nanoparticles with long-circulation time could be used as a drug delivery vehicle for magnetic resonance imaging (MRI) monitored magnetic targeting of tumors. [5, 6] And fluorescent magnetic nanoparticles, combining quantum dots (QDs) or near-infrared fluorescence (NIRF) dyes, and even radioisotope labeling with magnetic nanoparticles, have been reported, which bearing optical, radioactive and magnetic properties could be developed as dual-modality MR/optical or MR/positron emission tomography (PET) imaging probes, and even MR/PET/NIRF tri-modality imaging probes. [7-12]

The multifunctional nanosystems are playing a very important role in potential biomedical application. Employing appropriate paramagnetic probes, electron paramagnetic resonance (EPR) spectroscopy is a useful instrument which can provide the unique information of biological body *in vitro/ in vivo* sensitively and non-

invasively. [13-15] Spin-labeled MNPs bearing two attractive features, paramagnetism and superparamagnetism, could be developed as combined EPR and magnetic probes or potential drug delivery vehicles. [16] At present, we designed and synthesized novel functional MNPs with nitroxide free radicals spin labeling in order to investigate the new applications of those MNPs as EPR/MR bio-probes.

Experimental

Materials and methods

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) ammonium hydroxide ($\text{NH}_3 \cdot \text{H}_2\text{O}$), oxalic acid, propanedioic acid, hexanedioic acid and decanedioic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. (SCRC). 4-Amino-2,2,6,6-tetramethylpiperidin-1-oxyl (4-NH₂-TEMPO) used as building blocks in this study was prepared according to the reported method [21] in our laboratory. All solvents and reagents obtained from commercial source were of analytical reagent (AR) grade and used as received. And all the solutions were prepared with ultrapure water (High-Tech Instruments Co., Ltd., China).

Preparation of spin labeled MNPs: Preparation of carboxylic acids modified Fe_3O_4 nanoparticles

Fe_3O_4 magnetic nanoparticles were prepared and stabilized by the co-precipitation method described in the literature.[18] Then dicarboxylic acids or their sodium salts in 10 mL ultrapure water were added into the system to react for 1.5 h at 70 °C, and the introduction of -COOH groups onto the surface of Fe_3O_4 particles was achieved. After natural cooling, magnetic particles were separated with a magnet from the reaction mixture, and then they were dried in an oven at 40 °C for 24 h. For hexanedioic acid and decanedioic acid, their sodium salts solutions were employed, and the reaction products were dealt with 1 M HCl. At last the particles were washed with alcohol until most of the Fe^{3+} and Fe^{2+} were removed and dried at room temperature in air.

Preparation of spin labeled Fe_3O_4 nanoparticles

Spin labeled Fe_3O_4 nanoparticles were obtained through the reaction between Fe_3O_4 particles with -COOH on the surface and 4-NH₂-TEMPO. Briefly, 10 mg carboxylic acids modified Fe_3O_4 particles prepared above, 31.8 mg (0.15mmol) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) and 19.2mg (0.15mmol) N-hydroxysuccinimide (NHS) were activated by stirring in 10 mL ultrapure water at 0 °C for 30 min. Then, 4-NH₂-TEMPO was added into the above system and warmed up naturally to room temperature. After 24 h, the mixture was extracted by ether and dichloromethane for twice, respectively, to separate residual 4-NH₂-TEMPO. And then, the obtained product was separated with a magnet and dried under vacuum at 40°C.

Characterization of spin labeled MNPs

The crystalline structure and particle sizes of the particles were investigated with powder X-ray diffractometer (XRD, Rigaku RU-200, Japan). FT-IR spectra of the nanoparticles were obtained by a FT-IR spectrophotometer (Nicolet AVATAR360 FT-IR spectrophotometer) with KBr-nanoparticles mixture in the powder form. The thermogravimetric analysis (TGA) thermograms were also obtained by a TGA equipment (Q5000IR TGA Perkin Elmer, Inc., USA). The morphology of the nanoparticles was characterized by a JEM-2100 transmission electronic microscopy (TEM) (JEOL Ltd., Japan). Room temperature EPR measurements were performed at X-band, 9.85 GHz and at 298K, using a Bruker EMX EPR spectrometer.

Results and discussion

In this paper, four carboxylic acids modified MNPs (**1**, **2**, **3** and **4**) and further, four spin labeled MNPs (**5**, **6**, **7** and **8**) shown in **Table 1** were prepared and characterized.

Table 1. The structures of the studied materials

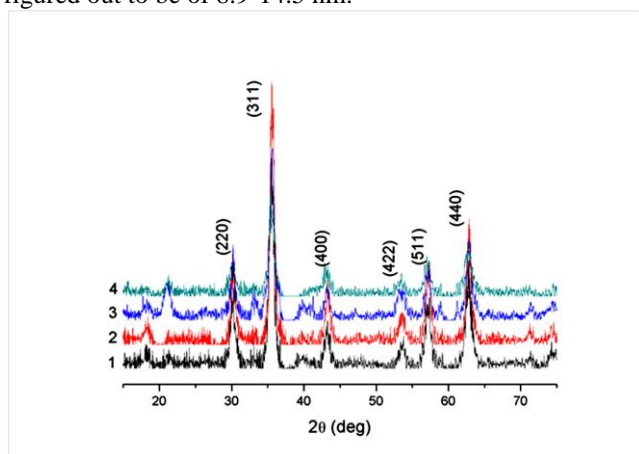
No.	Structural formula	No.	Structural formula
1		5	
2		6	
3		7	
4		8	

According to the differences of the dicarboxylic acids, the processes of the synthesis were slightly different. For short-chain dicarboxylic acids, such as oxalic acid and propanedioic acid, they were employed directly to prepare carboxylic acids modified Fe_3O_4 nanoparticles (**1** and **2**). While, for relatively long-chain dicarboxylic acids, hexanedioic acid and decanedioic acid, their sodium salts must be employed to obtain **3** and **4**, respectively. It suggested that short-chain dicarboxylic acids are favorable to the modified reaction, and it also can be confirmed by the thermogravimetric analysis (TGA) parameters shown in **Table 2**. There were two weight-loss steps of the magnetic particles. The first step observed at 30-100°C was due to the residual water, and the second step observed at 100-600°C was attributed to the burning of the organic ingredients on the surface of the Fe_3O_4 . [17] Therefore, the content of -COOH on the surface of the Fe_3O_4 could be calculated as shown in Table 2. According to the results of the TGA (**Table 2**), we could come to a conclusion that the surface of magnetic particles modified by propanedioic acid loaded more -COOH groups than those of modified by other acids, which is consistent with the reaction processes. And we take the propanedioic acid modified Fe_3O_4 nanoparticles as an example to illustrate the characterization of the materials in next parts of this paper.

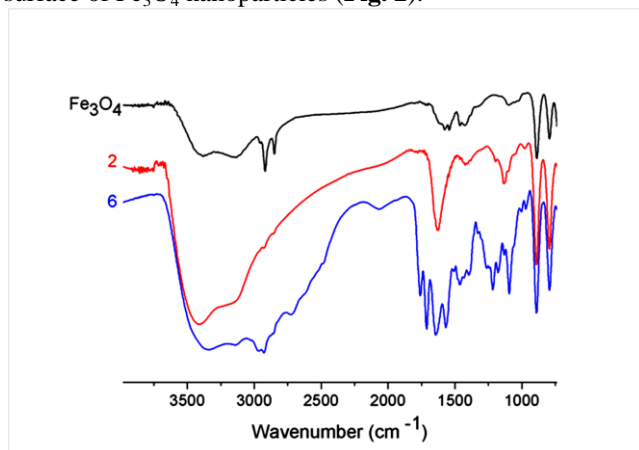
Table 2. TGA parameters of the magnetic nanoparticles.

No.	Weight loss %			Content of acid mmol/g Fe ₃ O ₄
	Total (30)	30	100	
1	8.13	3.95	4.18	0.506
2	12.07	5.30	6.77	0.740
3	10.52	1.74	8.78	0.601
4	8.40	2.16	6.24	0.309

The crystal phases of the nanoparticles were confirmed by X-ray diffraction (XRD). The XRD power patterns of the carboxylic acids modified Fe₃O₄ nanoparticles are shown in **Fig. 1**. And all the different peaks can be matched to the Fe₃O₄ of cubic structures. [18-20] According to the Scherrer equation [19], the crystallite sizes could be further figured out to be of 8.9-14.5 nm.

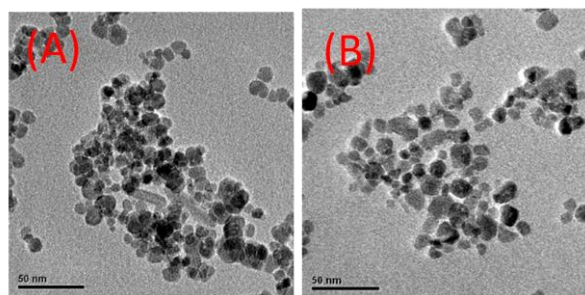
**Fig. 1.** The XRD patterns of the carboxylic acids modified Fe₃O₄ nanoparticles.

FT-IR spectroscopic shows the evidence of -COOH groups and even nitroxide radicals' formations on the surface of Fe₃O₄ nanoparticles (**Fig. 2**).

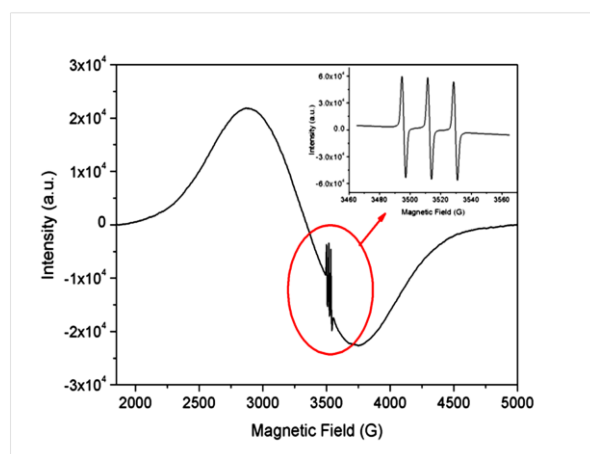
**Fig. 2.** FT-IR spectra of the studied nanoparticles. Curve A: Fe₃O₄ nanoparticles, Curve B: propanedioic acid modified Fe₃O₄ nanoparticles, and Curve C: spin labeled propanedioic acid modified Fe₃O₄ nanoparticles.

In **Fig. 2** curve A is the FT-IR spectrum of Fe₃O₄ nanoparticles. After propanedioic acid modification, the appearance of the peaks of carbonyl group (in -COOH) at 1619 cm⁻¹ is obvious to confirm the formation of -COOH

groups onto the surface of Fe₃O₄ (Curve B in **Fig. 1**). In addition, in curve C, two well-resolved peaks, carbonyl group (in -CONH) at 1631 cm⁻¹ and bending vibration of the N-H in -CONH at 1562 cm⁻¹, are found, and the stretching vibration of -N-O can be further found at 1390 cm⁻¹, which confirmed the formation of 4-NH₂-TEMPO spin labeled Fe₃O₄ nanoparticles. Transmission electronic microscopy (TEM) images of the nanoparticles are shown in **Fig. 3** A and B, which revealed that modified Fe₃O₄ nanoparticles were stable and mono-disperse in aqueous medium, with an average size of approximate 10 nm. The results were coincident with the consequences obtained from the XRD analysis.

**Fig. 3.** TEM images of the modified magnetic particles. A is propanedioic acid modified Fe₃O₄ nanoparticles, and B is spin-labeled propanedioic acid modified Fe₃O₄ nanoparticles.

Furthermore, EPR spectra of spin labeled Fe₃O₄ nanoparticles were measured at room temperature with parameters as follows, microwave power 0.2 mW, modulation frequency 100 kHz, modulation amplitude 1.00 G, time constant 2.560 ms, scan time 10.486 s and receiver gain 2.00. EPR spectra of the spin labeled propanedioic acid modified Fe₃O₄ nanoparticles at 298K are shown in **Fig. 4**.

**Fig. 4.** EPR spectra of the spin labeled propanedioic acid modified Fe₃O₄ nanoparticles suspension in water at 298K (Sweep width 3000G), and inset is hyperfine part of marking by red line scanned again at the same conditions (Sweep width 100G).

It can be seen that the EPR spectra show a broad line in the range of linewidth 1000 G from the superparamagnetic MNPs suspension in water in **Fig 4**. Meanwhile, the hyperfine triplet of the piperidine nitroxide radicals is obvious in the suspension. Furthermore, the hyperfine part

of the EPR spectra of the suspension was scanned again and a typical EPR signal of piperidine nitroxide radicals can be found (Fig. 4 inset) [21]. It confirmed the formation of the spin labeled MNPs. The results of the EPR measurement further indicate that the spin-labeled MNPs with both the superparamagnetism of ferrimagnetic nanoparticles and the paramagnetism of nitroxide free radicals could act as a potential vehicle to deliver drugs tracking by EPR technique.

Conclusion

In this paper, novel spin-labeled MNPs were prepared and characterized. The results of EPR measurements showed that the materials exhibited both broad line of ferrimagnetic nanoparticles and the hyperfine triplet of the piperidine nitroxide free radicals, which is favorable to investigate the MNPs behavior *in vitro* and *in vivo* tracking by EPR technique. Spin-labeled MNPs, combination of nitroxyl radicals and magnetic particles, could be further developed as dual-modality EPR/MR imaging probes or potentially targeted drug delivery vehicles.

Acknowledgements

This work was supported by Science and Technology Commission of Shanghai Municipality (STCSM, contract No. 10dz2220500 and No.09391911500), the Ministry of Science and Technology of the People's Republic of China (No. 2010DFA44300), and Natural Science Foundation of China (NSFC, No. 21074033).

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