

Synthesis of Novel Magnetic Water-Soluble Chitosan as Potential Magnetic Resonance Imaging Contrast Agents

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A magnetic water-soluble chitosan as potential contrast agents for MRI was synthesized by a simple new route. The morphology and structure of the magnetic water-soluble chitosan were characterized by FTIR, FESEM and XRD. The magnetic properties were characterized by *in vitro* and T₂-weighted FLASH image experiments. *In vitro* magnetic resonance imaging demonstrates that the magnetic water-soluble chitosan could greatly enhance the contrast for image under magnetic field and the contrast was enhanced with the concentration. The results of T₂-relaxivities indicates that the magnetic water-soluble chitosan provided high relaxivity. The paper presents a new, simple and economic route to preparing magnetic water-soluble chitosan using for potential magnetic resonance imaging contrast agents.

Key Words: Magnetic properties, Chitosan, Magnetic resonance imaging, Contrast agents.

INTRODUCTION

Since the early application of magnetic resonance imaging (MRI) in clinical diagnostics by Lauterbur¹, magnetic resonance imaging has been one of the most useful diagnostic techniques. Nowadays, more than 30 % of the examinations are performed after administration of a contrast agent, in order to enhance the contrast between normal and diseased tissue. The most commonly used contrast agents are paramagnetic materials like Gd-based compounds²⁻⁶, Mn-based compounds^{7,8} and superparamagnetic iron oxide (SPIO) nanoparticles^{9,10}. Recently, more and more attention has been drawn to SPIO nanoparticles because they may potentially provide higher contrast enhancement and efficiency in early detection of tumors, lymph node metastasis and liver sclerosis¹¹ than conventional paramagnetic Gd-based contrast agents due to their superparamagnetic property. Until now, SPIO nanoparticles (like Cliavist, Combidex, Feridex, Resovist, Sinerem) have been used successfully in some instances for liver tumor enhancement. However, the direct use of SPIO nanoparticles as *in vivo* MRI contrast agent results in biofouling of the particles in blood plasma and formation of aggregates that are quickly sequestered by cells of the reticular endothelial system such as macrophages¹². Therefore, many efforts have been devoted to modifying SPIO nanoparticles using coating agents with good biocompatible properties. Kim *et al.*¹³ successfully prepared biocompatible starch-coated SPIO nanoparticles and

investigated them *in vivo* in a rat brain by MRI. Lee *et al.*^{14,15} first synthesized ferrofluid made of SPIO and chitosan, then made the ferrofluid sprayed by a nozzle on the surface of the alkali solution to prepare embolic materials in the form of microspheres. The SPIO-chitosan microspheres which were about 100-150 μm showed a strong enhancement of MRI contrast similar to the ferrofluid *in vitro* when injected into the kidney of a New Zealand white rabbit *via* an angiographic catheter.

Chitosan, a nontoxic cationic polysaccharide derived by partial deacetylation of chitin, which exhibits good biocompatibility and biodegradability, diverse biological activities such as antifungal and antibacterial activity, has been widely used in biomedicine, food, cosmetic, agriculture and chemical industries. However, the molecular weight (Mw) of commercially produced chitosan is *ca.* 20000 and this high molecular weight makes the chitosan have very low solubility in neutral solution. Thus, most researchers usually have to add strong acid (such as HCl) to the solution in order to enhance the solubility, which will introduce new trouble to the system and limit many applications. Tsai *et al.*¹⁶ synthesized a potential MRI contrast agent (ferrofluid of chitosan-coated superparamagnetic iron oxide particles) by lowering the molecular weight of chitosan to *ca.* 13000 and adding HCl, mannitol and lactic acid in order to enhance the solubility.

In this paper, we use an easy, novel and effective method to prepare water-soluble chitosan which has excellent water

solubility in neutral environment and synthesize magnetic water-soluble chitosan by *in situ* reaction. First, the water-soluble chitosan with low molecular weight ($M_w = 1024$) and very narrow polydispersity index ($M_w/M_n = 1.06$) was synthesized by degradation from common chitosan under microwave irradiation. Then, magnetic Fe_3O_4 nanoparticles were obtained by co-precipitation and covered by the water-soluble chitosan by *in situ* reaction. The synthesized magnetic water-soluble chitosan which has high relaxivity, good water solubility, low toxicity, good biocompatibility and biodegradability can be used as a potential good contrast enhancement agent in magnetic resonance imaging. Moreover, the synthesis process suits large scale production. This interesting route might open a new, simple and economic route to preparing magnetic resonance imaging contrast agents with high relaxivity and low toxicity in bulk.

EXPERIMENTAL

Sodium hydroxide, ferrous chloride tetrahydrate and ferric chloride hexahydrate, all of analytical grade, were purchased from Shanghai Chemical Reagents Corp. (Shanghai, China) and used without further purification. Deionized water was boiled for 0.5 h to remove oxygen before use. Chitosan was used as supplied by Hainan Provincial Key Lab of Fine Chem (Haikou, China). The chitosan was previously characterized by FTIR and gel permeation chromatography (GPC) for the determination of the degree of deacetylation and polymer weight, which were *ca.* 90 % and 20,000 g/mol, respectively.

General procedure: First, water-soluble chitosan was synthesized by degradation from common chitosan ($M_w = 20000$) in the dilute acetic acid solution under microwave irradiation. The GPC results show that the water-soluble chitosan has low polymer weight (*ca.* 1024 g/mol) and narrow polydispersity index ($M_w/M_n = 1.06$). Then magnetic water-soluble chitosan was prepared as follows: in a typical polymerization system, first, 0.1 g water-soluble chitosan was dissolved in 100 mL de-ionic water, then 0.1 g $FeCl_2 \cdot 4H_2O$ and 0.25 g $FeCl_3 \cdot 6H_2O$ were added in the solution and dispersed by ultrasonic irradiation at high power (150 W, 80 kHz) at room temperature under protection of nitrogen atmosphere. An aqueous solution of 0.2 g NaOH was added drop by drop. The reaction was completed in 3 h. The resultant product was obtained by magnetic separation and then washed by deionized water and ethanol three times respectively. Then the solid was dried under vacuum to yield magnetic water-soluble chitosan.

Detection method: The morphology of magnetic water-soluble chitosan was recorded on an S-4800II field emission scanning electron microscope (Hitachi, Japan) at an activation voltage of 15 kV. The FTIR was measured on a Nicolet Nexus 470 Fourier-transform spectrometer (Nicolet, America) at room temperature (25 °C). X-Ray diffraction patterns were recorded by using a D8 Super Speed (Bruker-AXS, Germany) equipped with CuK_{α} radiation performed at 40 kV and 40 mA. The polymer weight and polydispersity index of the water-soluble chitosan were determined on an IA001 LC-3000 GPC system (Beijing Chuangxintongheng science and technology, China) using the buffer of $CH_3COOH + CH_3COONH_4$ (pH = 4.5) as the mobile phase at a flow rate of 0.43 mL/min. MRI measu-

rements were performed at 0.5 T on an imaging and analyzing system (MicroMR, Niumag, China). Multi-slice Spin Echo sequence (TR = 30 ms, TE = 0.55 ms, NS = 32, slice thickness = 5.0 mm) was employed for the acquisition of T_2 -weighted FLASH images. MRI data were analyzed using the Image J software package (a public domain Java image processing program inspired by NIH Image that can be extended by plug-ins, <http://rsb.info.nih.gov/ij/>)^{17,18}.

RESULTS AND DISCUSSION

The crystalline structure of the products was characterized by XRD. The XRD patterns for magnetic water-soluble chitosan and pure Fe_3O_4 nanoparticles are shown in Fig. 1. The characteristic diffraction peaks for magnetic water-soluble chitosan are detected in the spectrum. Two characteristic diffraction peaks at 2θ value around 35.5 and 62.6° are observed. These two peaks correspond to the (311) and (440) phase of the Fe_3O_4 nanocrystal structure, which are consistent with the database in JCPDS 75-1609. This confirms that Fe_3O_4 nanoparticles are embedded in the magnetic water-soluble chitosan. The other characteristic peaks ($2\theta = 30.2, 43.0, 53.2$ and 57.3°) of Fe_3O_4 nanoparticles corresponding to (220), (400), (422) and (511) phase of Fe_3O_4 are not obvious in the XRD pattern of the magnetic water-soluble chitosan mainly because their intensities are relatively low and easily covered by the other wider diffraction peaks.

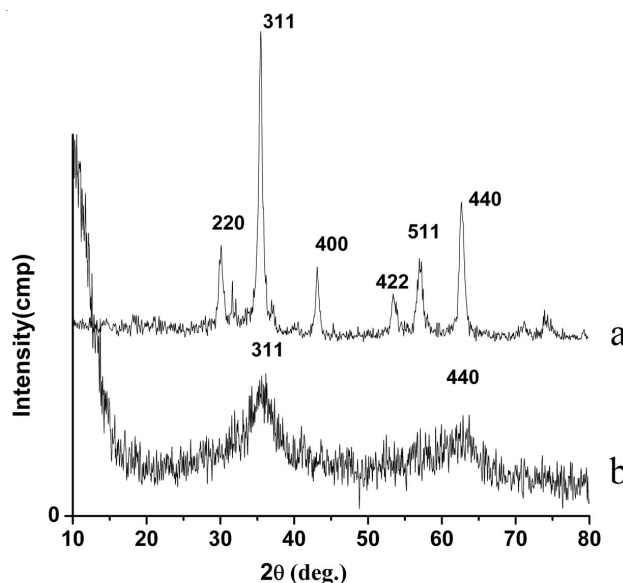


Fig. 1. XRD of (a) Fe_3O_4 nanoparticles and (b) the magnetic water-soluble chitosan

The chemical structure of the magnetic water-soluble chitosan was determined by FTIR (Fig. 2). FTIR of Fe_3O_4 and water-soluble chitosan were used for comparison. The spectra indicate the presence of water-soluble chitosan and Fe_3O_4 in the magnetic water-soluble chitosan. Thus, the broad peak at 3360 cm^{-1} arises from the combined O-H stretching and intermolecular hydrogen bonding vibrations. The vibrations of the N-H stretching from primary amines are masked in the same region. The C-H stretching vibrations are clearly seen at 2925 and 2876 cm^{-1} for the magnetic water-soluble chitosan. The

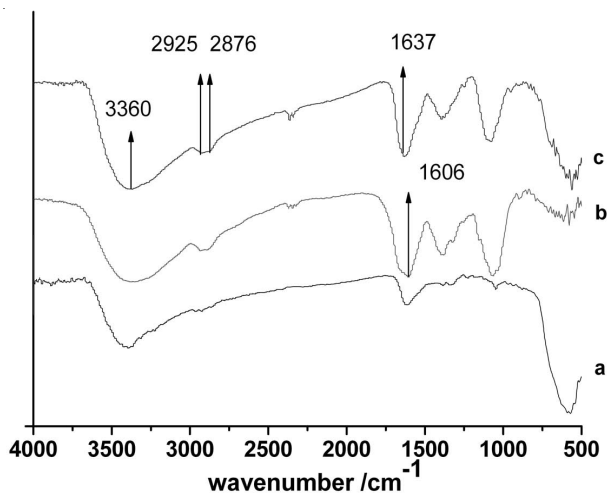


Fig. 2. FTIR of (a) Fe₃O₄, (b) water-soluble chitosan and (c) the magnetic water-soluble chitosan

result that the 1606 cm⁻¹ peak of the N-H bending vibrations from primary amines red-shifted to 1637 cm⁻¹ indicates that Fe₃O₄ nanoparticles are embedded in the water-soluble chitosan.

FESEM of the magnetic water-soluble chitosan are shown in Fig. 3. It is clear that the magnetic water-soluble chitosan basically resembles sphere morphology of a homogeneous structure. The nanoparticles exhibit well defined particles size smaller than 20 nm, embedded in the chitosan matrix.

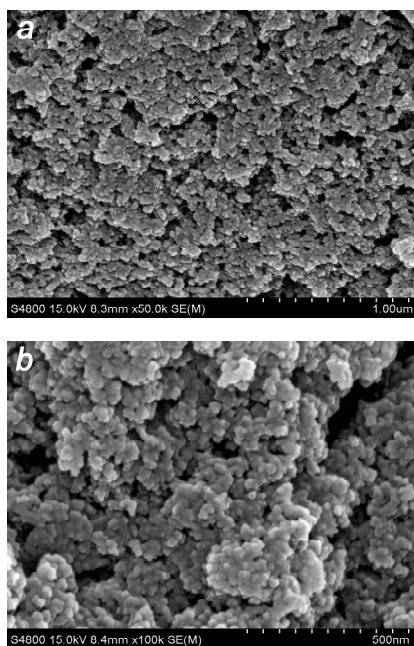


Fig. 3. FESEM of the magnetic water-soluble chitosan with different magnification: (a) 50000 and (b) 100000

To understand how the magnetic water-soluble chitosan perform as contrast agents for MRI, we studied their relaxivities under a 0.5 T imaging and analyzing system. Superparamagnetic iron oxide-based MR contrast agents shorten the T₂ (spin-spin) relaxation times and their net effectiveness is expressed as T₂ relaxivity (r₂), which represents the reciprocal of the relaxation time per unit concentration of metal ions, with units (Fe) mM⁻¹ s⁻¹. Fig. 4 displays the T₂ relaxivity of magnetic water-soluble chitosan. At the magnetic field of 0.5 T, magnetic water-soluble

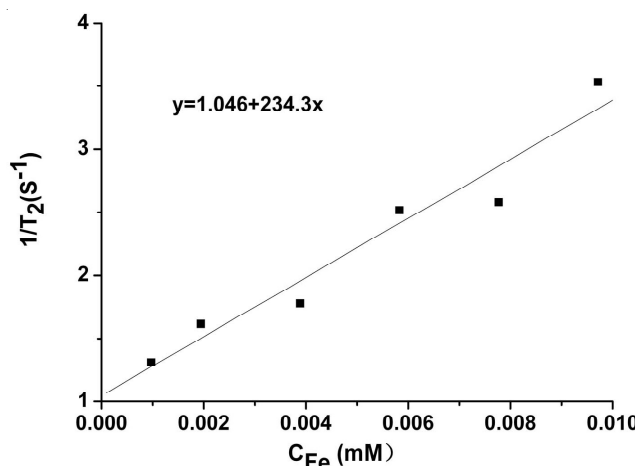


Fig. 4. Relaxivity (r₂) of the magnetic water-soluble chitosan

chitosan have a T₂ relaxivity of 234 (Fe) mM⁻¹ s⁻¹. Fig. 5(a) shows image of phantoms prepared by magnetic water-soluble chitosan. The intensity in the center tube, which included only the water, was used as baseline intensity. It was seen that proton signal intensity increased with contrast agent concentration. Fig. 5(b) shows the plots of signal enhancement levels (ΔS %) with the concentration of magnetic water-soluble chitosan. ΔS % was defined as follows: ΔS % = (pixel signal intensity/averaged signal intensity in the center tube - 1) × 100. The results show that good linear relation was obtained between the concentration of magnetic water-soluble chitosan and ΔS %.

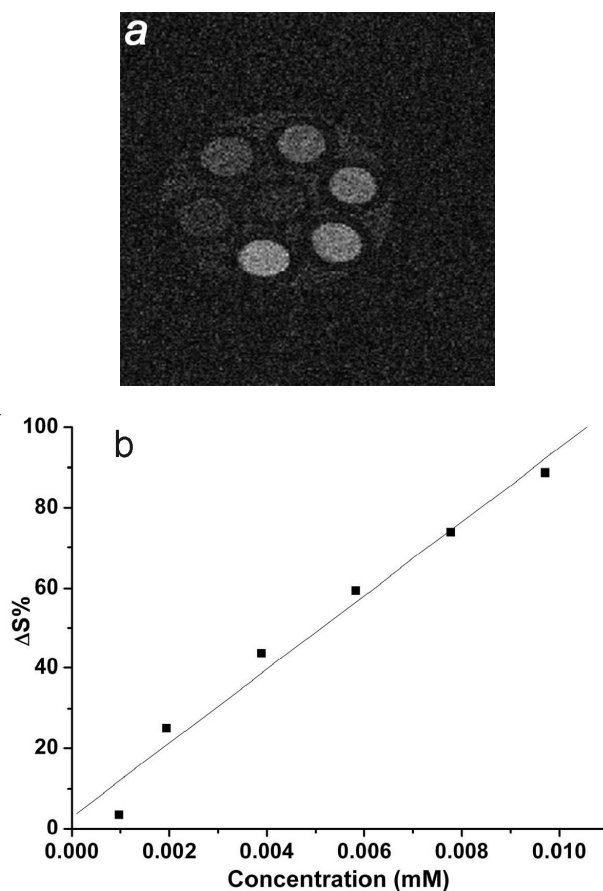


Fig. 5. (a) T₂-Weighted signal enhancement effect by the magnetic water-soluble chitosan with different concentration; (b) plots of ΔS % versus the concentration of the magnetic water-soluble chitosan

Conclusion

In summary, magnetic water-soluble chitosan as potential contrast agents for MRI were successfully prepared *via* a new, simple and effective route. The structure of the magnetic water-soluble chitosan was identified by FTIR and XRD. FESEM shows that the magnetic water-soluble chitosan basically resembles sphere morphology of a homogeneous structure. *In vitro* magnetic resonance imaging demonstrates that the magnetic water-soluble chitosan could greatly enhance the contrast for image under magnetic field and the contrast is enhanced with the concentration. The results of T₂-relaxivities indicate that the magnetic water-soluble chitosan has a high T₂ relaxivity of 234 (Fe) mM⁻¹ s⁻¹.

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