

Research Article

The Preparation of Glucan-Fe₃O₄ Magnetic Nanoparticles and Its In Vivo Distribution in Mice

Fengdan Jin

Key Laboratory of Applied Chemistry, Yanshan University, Qinhuangdao, Hebei 066004, China

Correspondence should be addressed to Fengdan Jin; fengdanjin@ysu.edu.cn

Received 30 October 2013; Revised 31 December 2013; Accepted 5 January 2014; Published 23 February 2014

Academic Editor: Lavinia Balan

Copyright © 2014 Fengdan Jin. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The glucan-Fe₃O₄ magnetic nanoparticles were prepared by hydrothermal method. The mixture of FeCl₂ and glucan was stirred vigorously for half an hour under low temperature (15°C). KOH of 1 mol/L was dropwise added, slowly, into the solution until the pH to 12. Immediately, KNO₃ was added and the temperature was raised to 75°C for an hour. All the processes of Fe₃O₄ crystal particles generation were under nitrogen. An atomic absorption spectrometry quantitative analysis method was built to determine the in vivo distribution of the glucan-Fe₃O₄ magnetic nanoparticles in mice. The diameter of glucan-Fe₃O₄ magnetic nanoparticles was about 25 nm and they were up taken by the liver primarily after intravenous administration via the tail.

1. Introduction

Magnetic induction heating treatment of cancer is a new development of heat treatment in recent years. Fe₃O₄ has a high specific surface area and a strong tendency to aggregate. It can reduce the surface energy of the particle by surface modification. It is the most widely used nanomagnetic materials in magnetic hyperthermia [1–3]. Wang et al. found that the Fe₃O₄ magnetic fluid demonstrates excellent stability and fast magnetotemperature response, which can be used both in magnetic resonance imaging and magnetic fluid hyperthermia [4]. Glucan can adjust the biocompatibility and response characteristics of the magnetic nanoparticles by modifying its surface, and therefore it could meet different aspects of the application requirements in biotechnology, medicine, and pharmaceuticals [5]. Research for the process of absorption, distribution, metabolism, and clearance in vivo of glucan-Fe₃O₄ magnetic nanoparticles can more fully investigate its effects and safety in vivo.

The biological effects and biological safety of nanomedicine are very important. In the development of nanotechnology, while simultaneously carrying out its safety studies, nanotechnology has the potential to become the first new technologies that benefit for humanity which were carefully evaluated before a negative effect even occurs [6, 7]. It is an important research direction for nanomaterials to

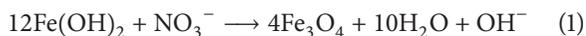
study the process of absorption, distribution, metabolism, and clearance in vivo and the mechanism of interaction between various nanomaterials and biomechanism of the target organ. Currently, due to the restriction of qualitative and quantitative detection to the nanoscale materials organism in vivo, these experimental data was little. This work requires long-term accumulation and development to establish and improve the research system.

Glucan-Fe₃O₄ magnetic nanoparticles were prepared by hydrothermal method and an atomic absorption spectrometry was built to investigate the distribution of glucan-Fe₃O₄ magnetic nanoparticles in mice tissue.

2. Materials and Methods

2.1. Preparation of Glucan-Fe₃O₄. 0.01 mol of FeCl₂·4H₂O was dissolved in 20 mL of deionized degassing water. It was poured into a solution containing 10 g of glucan. The mixture was stirred vigorously for half an hour under low temperature (15°C). Then, KOH (1 mol/L) was dropwise added into the solution slowly (0.003 mol/min) until the pH of the solution was increased to 10–12. Immediately, the temperature was raised to 75°C and 10 mL KNO₃ solution (250 mg) was added rapidly. The mixture was stirred for an hour. Finally, ferrous salt is oxidized to Fe₃O₄ crystal particles and a black colloid

was generated. All the processes of Fe_3O_4 crystal particles generation were under nitrogen. The chemical reaction equation is



The obtained black product was transferred to a beaker and adjusted to $\text{pH} = 7.0$ with 0.1 mol/L of hydrochloric acid. An NdFeB magnet was placed under the bottom of the beaker. After all of the particles sedimentation, the supernatant was decanted. The sediment was washed by deionized water 5 times repeatedly. The magnetic nanoparticles were purified with a $0.22 \mu\text{m}$ membrane filter (the purpose is to remove large particles unmodified) and were freeze-dried to powder.

2.2. Animal Experiments. The mice were injected with glucan- Fe_3O_4 nanoparticles solution (after high temperature sterilization) according to the dose of 5.68 mg/kg . The control group was injected with normal saline. After administration of 0, 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h, the mice were killed by cervical dislocation, dissected out heart, liver, spleen, lung, and kidney of each mouse. Each organization was weighed accurately and placed in digestion tanks, added 4 mL of HNO_3 (65%) and 0.5 mL of H_2O_2 (30%) and microwave digestion. The residue was cooling for a while and was diluted to 10 mL . The samples were determined in AA-7000 atomic absorption spectrophotometer (Shimadzu, Japan) at the atomic absorption wavelength of 258.9 nm , the spectral passband of 0.7 nm , and the operating current of 10 mA . The increased quantity of Fe in various organs after administration was obtained by the Fe content in various organs at each time minus the Fe content in corresponding organs of the control group. It was converted into the increased quantity of Fe_3O_4 in various organs after administration. The targeted rate in various organs was calculated by

$$\frac{\text{the increased } \text{Fe}_3\text{O}_4 \text{ in an organ at } t \text{ time}}{\text{the increased } \text{Fe}_3\text{O}_4 \text{ in the sum organs at } t \text{ time}} \times 100\%. \quad (2)$$

3. Results and Discussion

3.1. Preparation of Glucan- Fe_3O_4 . Preparation methods of magnetic nanoparticles were precipitation, oxidation, and microemulsion [8, 9]. Hydrothermal method was chosen because of the products narrow size distribution and low degree of aggregation.

To improve the experimental conditions, the dosage of glucan, KOH and KNO_3 and the experimental temperature were investigated. Dispersant effect of the surfactant was mainly in its adjustment of particle surface wettability. The concentration of surfactant is critical to this adjustment. When the quantity of glucan was less, glucan coated thin, and the soft agglomeration phenomenon occurred. The repulsive force (V_R) generated between the surfactants is insufficient to overcome the Van der Waal attractive force (V_A) between the magnetic particles and the magnetic attraction force (V_N). The macromolecule glucan was used as surfactant, and the electrostatic repulsion force between the particles was

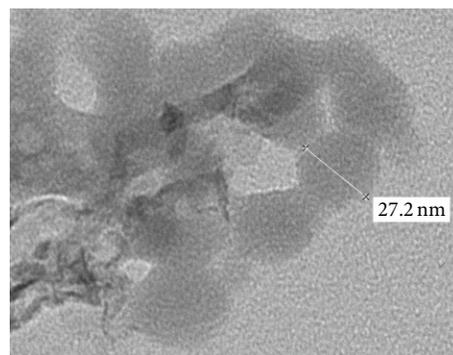


FIGURE 1: TEM of glucan- Fe_3O_4 nanoparticles.

composed of the electrostatic double layer repulsion force and steric effect. With the increase of the quantity of glucan, the glucan layer was thicker. The two repulsions force increased, and a barrier was formed which the V_A and V_N were difficult to penetrate. So, the particles were scattered well and covered well, and the number of nucleations increased, resulting in the fact that the average particle size was smaller. Excess glucan caused the coating layer to be thicker. The interaction between the polymer chains could not be ignored. Not only the particle sizes increased, but also the subsequent washing process became more difficult. When the glucan was less than 10 g , the products were layered. When the glucan was from 10 g to 15 g , there was little difference in the nanoparticle size and the morphology.

The magnetic particles were obtained by Fe^{2+} oxidized with weak oxidant KNO_3 under alkaline conditions. Slightly higher temperature and excess KOH were the suitable conditions for production of Fe_3O_4 magnetic nanoparticles. To temperature, the results showed that the oxidation reaction was difficult when the temperature was below 60°C , and a large number of black precipitates were produced when the temperature was too high (80°C). So, the temperature was set to 75°C in the oxidized process. The pH of the reaction system was changed to 8, a layered brown suspension was obtained, to 10, a black uniformly dispersed suspension was obtained, and, to 12, a black uniformly dispersed suspension was obtained. So, pH value was set to 10. Visible, in this condition (temperature was 75°C , pH value was 10), the smallest particle size was obtained.

3.2. Characterization of Glucan- Fe_3O_4

3.2.1. Transmission Electron Microscopy (TEM) and Atomic Force Microscope (AFM). The freeze-dried powder of magnetic nanoparticles was diluted into corresponding concentration solutions. They were pipetted a drop of liquid drops in the microgrid holes (for TEM) and mica sheet (for AFM), respectively, and dried by airing. The size and morphology of the products were examined by HT7650 transmission electron microscopy (Hitachi; Japan) and Multimode 8 atomic force microscope (Veeco; USA). The nanoparticles were in spherical shape with no agglomeration of particles. The dispersion was very uniform. The size of glucan- Fe_3O_4

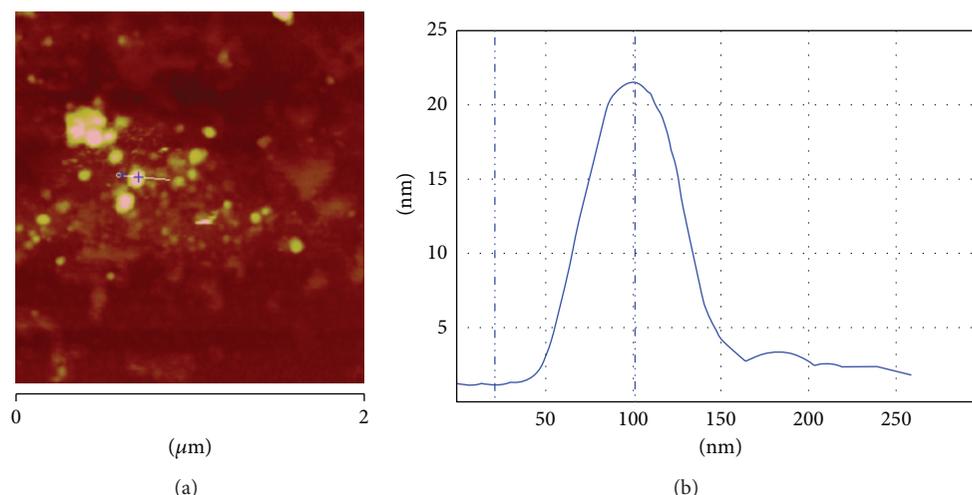


FIGURE 2: AFM of the glucan- Fe_3O_4 nanoparticles: the height of the selected particle in (a) was shown in (b).

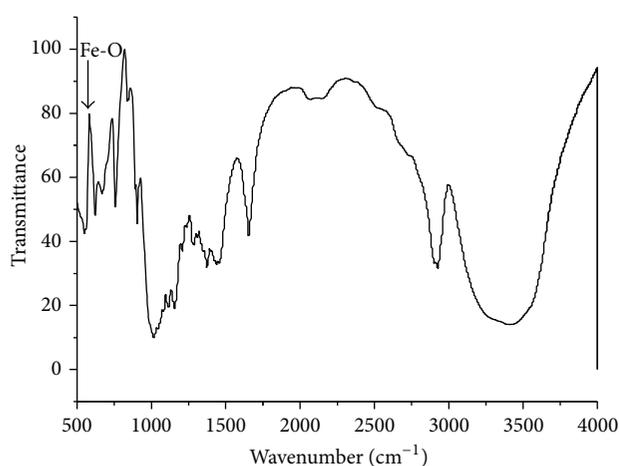


FIGURE 3: Infrared absorption spectrum of the glucan- Fe_3O_4 nanoparticles.

magnetic nanoparticles was calculated by Nano Measurer 1.2.5. The particle size was about 25 nm (Figures 1 and 2).

3.2.2. Infrared Absorption Spectrum (IR). The freeze-dried powder of magnetic nanoparticles and glucan-40 were pressed into tablets with KBr, respectively. The infrared absorption was examined by Nicolet iS10 FT-IR (Thermo Fisher Scientific, USA). The result was shown in Figure 3. The magnetic nanoparticle has a strong absorption peak in 580 cm^{-1} which was the Fe-O stretching vibration peak. The IR spectra characteristic absorption peaks of glucan were reflected at the magnetic nanoparticles, especially in $1,000\sim 1,200\text{ cm}^{-1}$ fingerprint region. Since magnetic nanoparticles have been cleaned several times after the synthesis, the not coated glucan-40 could not subside to bottom of the beaker by the magnet. However, the obvious characteristic peaks of glucan appeared in the IR spectra of the final products. This proved that the prepared magnetic nanoparticles have been covered with glucan.

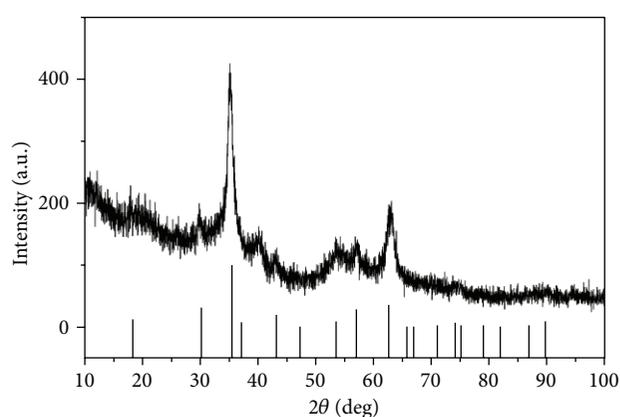


FIGURE 4: XRD of the glucan- Fe_3O_4 nanoparticles.

TABLE 1: The recovery of atomic absorption spectrometry method ($n = 5$).

Tissue	Content of Fe ($\mu\text{g}/\text{mg}$)	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
Heart	22.9	2.0	24.6	98.6	1.0
		10.0	10.0	99.8	1.1
Liver	41.6	2.0	43.3	99.3	1.6
		10.0	10.1	100.6	2.3
Spleen	39.7	2.0	41.4	99.4	1.5
		10.0	10.1	100.6	1.4
Lung	18.6	2.0	20.4	98.9	2.1
		10.0	10.0	100.3	2.7
Kidney	18.9	2.0	20.8	99.5	2.8
		10.0	9.9	98.7	1.8

3.2.3. X-Ray Diffraction (XRD). The information of composition of the materials and the structure or morphology of atoms or molecules can be obtained by a D/max-2500/PC X-ray diffractometer (Rigaku Corporation; Japan). Measurement result was shown in Figure 4. The characteristic

TABLE 2: The distribution of the glucan-Fe₃O₄ nanoparticles in mice at different sampling points (% ID/g, n = 6).

Tissue	0 min	0.25 h	0.5 h	1 h	3 h	6 h	12 h	24 h	48 h
Heart	3.21	0.94	0.75	1.65	0.68	7.98	8.45	6.42	5.42
Liver	25.46	20.75	14.52	47.25	10.45	14.16	24.87	12.65	7.64
Spleen	9.24	18.57	16.81	10.34	12.73	11.41	19.67	16.34	12.98
Lung	13.61	3.21	6.14	2.46	2.11	3.98	3.14	3.51	5.68
Kidney	1.72	0.43	0.34	0.51	0.65	1.99	1.81	2.31	3.57

peaks of various crystal faces in the map (2.968, 2.535, 2.103, 1.719, 1.614, 1.478, and 1.271) were consistent completely with the Fe₃O₄ characteristic peaks in XRD pattern of the standard PDF card (JCPDS card number 19-0629) provided by International Powder Federation. This indicates that the prepared magnetic nanoparticles were Fe₃O₄.

3.3. Method Validation of Atomic Absorption Spectrometry. The stock solution was formulated by spectral pure Fe liquid. Fe standard series solutions (1.00, 2.00, 3.00, and 4.00 μg/mL) were determined according to the working conditions of the instrument, establishing a standard curve automatically by the instrument. Regression equation was $Y = 5.9876X + 3.2654$. The correlation coefficient was 0.9992. Intraday precision was less than 4% interday precision was less than 6% of the various organs. The recovery data of the method was described in Table 1. The glucan-Fe₃O₄ magnetic liquid was made for immediate use. The 2 h stability of the liquid was 1.26%. From these results, the distribution of Fe in mice was reliable and accurate.

3.4. The Distribution of Glucan-Fe₃O₄. The tissue distribution of glucan-Fe₃O₄ at different times was shown in Table 2. The distribution was different in Fe₃O₄ in tissues and organs. The concentration was more in liver and spleen. Liver and spleen might be its target organ in vivo. The targeted rate in liver at 1 h after administration was 47.25%. Furthermore, the time of the distribution peak of Fe₃O₄ in various tissues and organs was different. In the heart and liver, the concentration peak was at 7 h and 48 h. The distribution curve in the spleen changed wavy. The concentration was higher at 0.25 h and 12 h and then decreased gradually. In the lungs, the concentration was great at the moment of administration and decreased rapidly. Then it was at a certain level with no significant fluctuations.

4. Conclusions

The present study described the successful preparation method of glucan-Fe₃O₄ magnetic nanoparticles and the particle size is about 25 nm. Also, the development of simple, sensitive, selective, and rapid atomic absorption spectrometry quantitative analysis method for the accurate determination of Fe₃O₄ in mice's tissues was formulated. The tissue distribution data was conducive to investigate the actions and safety in vivo of nanoparticles.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Financial support from the National Natural Science Foundation of China (Grant 51202213), Excellent Young Scientist Funding of Hebei Province (Grant Y2012005), and Qinhuangdao city Science & Technology Research & Development Program (no. 201001A120) is acknowledged.

References

- [1] W. Pon-On, S. Meejoo, and I.-M. Tang, "Substitution of manganese and iron into hydroxyapatite: core/shell nanoparticles," *Materials Research Bulletin*, vol. 43, no. 8-9, pp. 2137-2144, 2008.
- [2] F.-Y. Cheng, C.-H. Su, Y.-S. Yang et al., "Characterization of aqueous dispersions of Fe₃O₄ nanoparticles and their biomedical applications," *Biomaterials*, vol. 26, no. 7, pp. 729-738, 2005.
- [3] A. A. Gouda and Z. Al. Mallah, "Development and validation of sensitive spectrophotometric method for determination of two antiepileptics in pharmaceutical formulations," *Spectrochimica Acta A*, vol. 105, pp. 488-496, 2013.
- [4] Y. M. Wang, X. Cao, G. H. Liu et al., "Synthesis of Fe₃O₄ magnetic fluid used for magnetic resonance imaging and hyperthermia," *Journal of Magnetism and Magnetic Materials*, vol. 323, no. 23, pp. 2953-2959, 2011.
- [5] X. L. Li, K. L. Yao, and Z. L. Liu, "CFD study on the magnetic fluid delivering in the vessel in high-gradient magnetic field," *Journal of Magnetism and Magnetic Materials*, vol. 320, no. 11, pp. 1753-1758, 2008.
- [6] Y. L. Zhao, *Nanotoxicology*, in *Encyclopedia of Nanoscience and Nanotechnology*, American Scientific, Stevenson Ranch, Calif, USA, 2005.
- [7] E. J. Furlani and E. P. Furlani, "A model for predicting magnetic targeting of multifunctional particles in the microvasculature," *Journal of Magnetism and Magnetic Materials*, vol. 312, no. 1, pp. 187-193, 2007.
- [8] R. Y. Hong, J. H. Li, H. Z. Li, J. Ding, Y. Zheng, and D. G. Wei, "Synthesis of Fe₃O₄ nanoparticles without inert gas protection used as precursors of magnetic fluids," *Journal of Magnetism and Magnetic Materials*, vol. 320, no. 9, pp. 1605-1614, 2008.
- [9] Y. Zhao, Z. Qiu, and J. Huang, "Preparation and analysis of Fe₃O₄ magnetic nanoparticles used as targeted drug carriers," *Chinese Journal of Chemical Engineering*, vol. 16, no. 3, pp. 451-455, 2008.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

