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PREPARATION AND MAGNETIC PROPERTIES INVESTIGATION OF Fe_3O_4 NANOPARTICLES ^{99m}Tc LABELED AND Fe_3O_4 NANOPARTICLES DMSA COATED

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DMSA-coated Fe_3O_4 nanostructure was prepared by adding 1:2:8 proportions of $FeCl_2$, $FeCl_3$ and NaOH to deionized water, separately, under atmosphere control condition (N_2). 0.01 M DMSA ($C_4S_2O_4H_6$) solution was prepared in deoxygenated deionized water. The solutions were added separately together slowly in a three spout balloon. Fe_3O_4 nanoparticles (5-20nm) were produced by co-precipitation method. Prepared Fe_3O_4 nanoparticles were labeled by ^{99m}Tc radioisotope directly at room temperature at the presence of $SnCl_2$ as a reducing agent that reduces surface charge of the ^{99m}Tc. The labeling efficiency was assessed by instant thin layer chromatography (ITLC) and was found above 99 percent. Magnetic and structure properties of DMSA-coated and ^{99m}Tc labeled nanoparticles were investigated by Alternating Gradient-Force Magnetometer (AGFM), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). Both of DMSA-coated Fe_3O_4 and ^{99m}Tc labeled Fe_3O_4 were super paramagnetic and their saturation magnetizations were determined 31 and 28 emu/g, respectively.

Key words: Fe_3O_4 nanoparticle, ^{99m}Tc radioisotope, radiolabeling, DMSA, coating.

1. Introduction

Nanoparticles have specific physical characteristics in size and shape. They have high proportion of surface to volume. These characteristics have made them appropriate to be used in many medical and biological applications [1]. They are smaller than cellular structures and therefore when they inject to animals, quickly distribute in most organs and tissues. It means they have a very strong cellular uptake phenomenon [2].

Magnetic nanoparticles have some important applications such as ferro-fluids, microwave absorbing and magnetic drug delivery for the treatment of various types of cancers [3-5]. There is an increasing interest in inventing new magnetic nanoparticles because of their wide applications.

Nowadays, in order to achieve the most effectiveness in biological systems, nanoparticles are coated by different biocompatible materials such as albumin, dextran [6], polyethylene glycol, polyethylene oxide [7], aspartic acid and DMSA (dimercaptosuccinic acid $C_4S_2O_4H_6$, Aldrich Chemical) [8]. Presence of such coatings help the stability of nanoparticles in biological solutions, blood circulation and tissue distribution as well as entrance to cells and also decrease nanoparticles toxic effects [9].

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On the other hand, the magnetically delivery of radioisotopes by magnetic nanoparticles is an innovative field of interest. Magnetic radioactive nanoparticles have the profit of being able to transport high concentrations of radioactivity to the certain areas, with minimum damage to surrounding normal tissue. For instance, magnetic poly lactic acid microspheres, as carriers for yttrium, have shown great promise for radiotherapy [10].

One of the most important magnetic nanoparticles is Fe_3O_4 which has the super paramagnetic property and acts as a powerful magnet at the presence of external magnetic field without any remanent at the absence of external magnetic field. In this essay an attempt is to compare the super paramagnetic property of Fe_3O_4 nanoparticles labeled with ^{99m}Tc radioisotope and Fe_3O_4 coated with DMSA and also present a simple way to producing these nanostructures.

2. Experimental

2.1. Synthesis of Fe_3O_4 nanoparticles coated with DMSA

DMSA-coated Fe_3O_4 nanoparticles (Fe_3O_4@DMSA) were synthesized by wetted chemical method. For this purpose, three solutions of $FeCl_2$ (0.01 M), $FeCl_3$ (0.02 M) and NaOH (0.08 M) (all from Merck company) were prepared in the distilled deionized water, under vigorous stirring. At first, $FeCl_2$ solution was poured into a three spout balloon container. Meanwhile, $FeCl_3$ solution was added to the same balloon. After that, 0.01 M DMSA solution was prepared in deoxygenated deionized water. In the construction process, every three or four seconds, one droplet of DMSA solution was added via nitrogen bubbling and magnetic stirrer. Finally, $FeCl_3$ solution was added to the balloon by the same way and under atmosphere control condition (N_2). The resulting solution was washed by deionized water and then was centrifuged in order to remove any impurity aggregate. Then, the precipitated sample was dried at room temperature. All processes were done at room temperature [11].

2.2. Synthesis of Fe_3O_4 nanoparticles labeled with ^{99m}Tc radioisotope

2.2.1. Synthesis of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were synthesized by co-precipitaion method. In this method, three solutions of $FeCl_2$ (0.01 M), $FeCl_3$ (0.02 M) and NaOH (0.08 M) (all from Merck company) were prepared in the distilled deionized water and were heated up to boiling point, separately. In order to get nanostructure, they were mixed together simultaneously. The resulting solution was washed by distilled deionized water and then was centrifuged in order to remove any impurity aggregate.

2.2.2. Fe_3O_4 labeling with ^{99m}Tc radioisotope

In order to radiolabeling Fe_3O_4 nanoparticles, the Technetium-99m (^{99m}Tc), with activity of 1.3 GBq was prepared from ^{99}Mo-^{99m}Tc generator. It was injected into a small sterile and vacuuming vial under the observance of radiation protection rules. Subsequently, 0.5 ml of stannous chloride (2.0 mg/ml) solution in 0.1N HCl, was injected into the same vial, and stirred for several seconds. The stennous chloride, which mainly contains $SnCl_2$, is a reducing agent that reduces surface charge of ^{99m}Tc to a suitable condition for labeling with other molecules. Then, 1 ml diluted Fe_3O_4 nanoparticles solution was added into the vial containing ^{99m}Tc and stannous chloride. The mixture was kept at room temperature for several minutes [12, 13].

3. Results and discussion

3.1. Coating test and magnetic properties of Fe_3O_4@DMSA

The chemical interaction between Fe_3O_4 and DMSA were investigated by FTIR (Fourier Transform Infrared Spectroscopy, JASCO FT/IR-680 PLUS). Fig.1 shows the FTIR curve of the Fe_3O_4, DMSA and Fe_3O_4@DMSA. As can be seen, in the Fe_3O_4 curve, 1628 and 3419 peaks are

related to OH junctions and it means that there is water in the material structure. The 581 peak shows that the spinel structure was formed and we will see it has a good agreement with XRD results. On the other hand, in the Fe3O4@DMSA curve, 1619 and 1376 peaks are related to the asymmetry and symmetry stresses of COO group, respectively. If we compare them with the 1699 and 1421 peaks in the DMSA curve, it can be concluded that the DMSA has coated the surface of the Fe_3O_4 nanoparticles. Also, decrease in the 581 peak is the other reason for this conjunction.

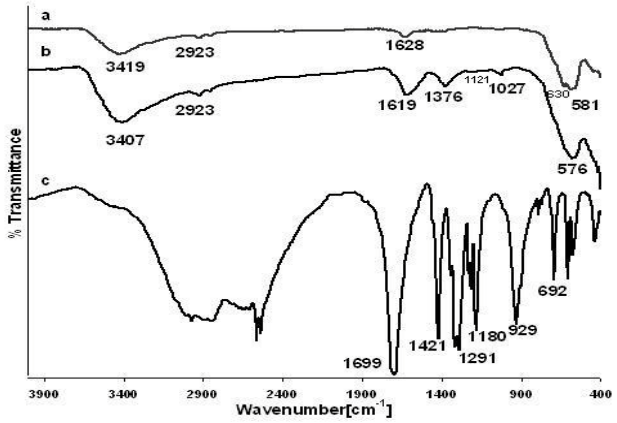

Fig1. ***a)*** *FTIR curve of* Fe_3O_4 ***b)*** *FTIR curve of* Fe_3O_4*@DMSA* ***c)*** *FTIR curve of DMSA*

Magnetic properties of the Fe_3O_4@DMSA nanoparticles were assessed by AGFM (Alternating Gradient-Force Magnetometer, Lake Shore). Fig.2 illustrates the AGFM curve of the Fe_3O_4 and Fe_3O_4@DMSA. As can be seen, both of them have the super paramagnetic property. The saturation magnetization was determined by extrapolation of magnetization curve on the basis of $1/H$ when$1/H \to 0$. It was measured 62 emu/g for Fe_3O_4 where as it was determined 27 emu/g for Fe_3O_4@DMSA.

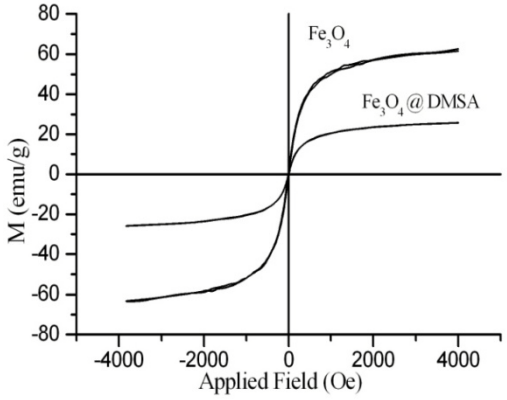

Fig2. AGFM curves of Fe_3O_4 *and* Fe_3O_4*@DMSA*

The structure of the Fe_3O_4@DMSA nanoparticles was investigated by XRD (X-Ray Diffraction, Bruker D8 ADVANCE λ=0.154nm Cu Kα radiation). Fig.3 demonstrates the XRD pattern of the Fe_3O_4 and Fe_3O_4@DMSA. It can be seen that, both of the samples have single phase and also have the ferrite spinel structure. The intensity of XRD background toward peak has increased after coating which is for the reason of DMSA structure. The mean size of the particles was determined by Debye-Scherer formula. It was calculated 7.5 nm for Fe_3O_4 and 8.3 nm for Fe_3O_4@DMSA.

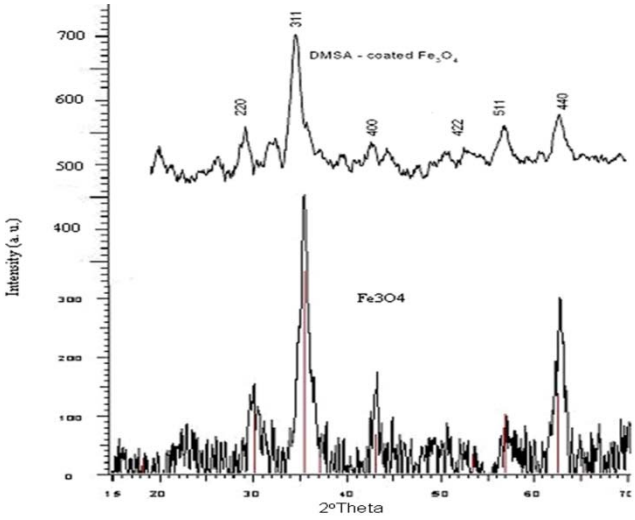

Fig3. XRD pattern of Fe_3O_4 and Fe_3O_4@DMSA

3.2. Labeling efficiency and magnetic properties of Fe_3O_4 labeled ^{99m}Tc

The labeling efficiency of Fe_3O_4 nanoparticles labeled ^{99m}Tc, was vrified by using ITLC (Instant Thin Layer Chromatography) method, since it is an easy way and well accepted process to assess the radiolabeling quality in nuclear medicine. firstly, a droplet of solution, containing labeled Fe_3O_4 nanoparticles, was applied onto the end of the strip of silica gel (1×10 cm). After that, the strip was placed into a chromatography development tank containing acetone solvent. Thus, the solvent was moved slowly from one end to the other end of the strip, passing through the droplet spot. Then, the strip was cut into two or three parts which the proximal part contenting the labeled Fe_3O_4 and the distal part contenting the unbounded ^{99m}Tc pertechnetate. The radioactivity of each part was determined by HPGe spectroscopy (High Purity Germanium spectroscopy, CANBERRA). The labeling efficiency was obtained more than 98%. The same ITLC tests were done after 2, 4 and 6 hours and the labeling efficiency of 90% was determined.

Fig.4 indicates the AGFM curve of Fe_3O_4 and Fe_3O_4 labeled ^{99m}Tc. It can be seen that both of them have the super paramagnetic property. The saturation magnetization was determined by extrapolation of magnetization curve on the basis of $1/H$ when$1/H \to 0$. It was measured 54 emu/g for Fe_3O_4 where as it was determined 28 emu/g for Fe_3O_4 labeled ^{99m}Tc.

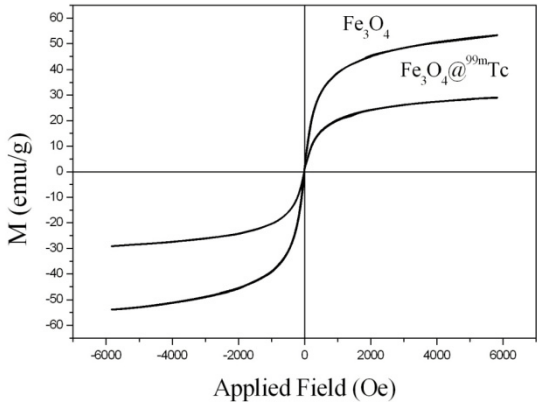

Fig4. AGFM curves of Fe_3O_4 and Fe_3O_4 labeled ^{99m}Tc.

4. Conclusions

It can be concluded that, Fe_3O_4 nanoparticles can be coated with biocompatible structures like DMSA and also can be labeled with different radioisotopes like ^{99m}Tc. The obtained compounds will also have the super paramagnetic property but with less saturation magnetization in comparison with Fe_3O_4. It is an interesting prospect to labeling and coating the Fe_3O_4 nanoparticles simultaneously and delivering the obtained compound by the magnetic field to the desire body organs.

References

[1] CC. Berry, ASG Curtis, *J. Phys. D Appl. Phys*, 36, 198-200 (2003).
[2] OV. Salata, *J. Nanobiotech*, 2(3), (2004).
[3] G. Nedelcu, *Digest Journal of Nanomaterials and Biostructures*, 3(2), 99 (2008).
[4] G. Nedelcu, *Digest Journal of Nanomaterials and Biostructures*, 4(3), 103 (2008).
[5] Gh. R. Amiri, M. H. Yousefi, M. R. Aboulhassani, M. H. Keshavarz, D. Shahbazi, S. Fatahian, M. Alahi, *Digest Journal of Nanomaterials and Biostructures*, 5(3), 1025(2010).
[6] CC. Berry, S. Wells, S. Charles, ASG. Curtis, *J. Biomaterials*, 24, 4551-4557 (2003).
[7] LM. Lacava, ZGM. Lacava, MF. Da. Silva, O. Silva, SB. Chaves, RB. Azevedo, et al *Biophys. J*, 80, 2483-2486 (2001).
[8] N. Sadeghiani, LS. Barbosa, LP. Silva, RB. Azevedo, PC. Morais, ZGM. Lacava, *J. Magn. Magn. Mater*. 289, 466-468 (2005).
[9] VI. Shubayev, TR. Pisanic, S. Jin S, *Adv. Drug. Delivery. Rev*, 61, 467-477 (2009).
[10] U. O. Häfeli, S. M. Sweeney, B. A. Beresford, B. A. Humm, and R. M. Macklis, *Nucl. Med. Biol.*, 22(1), 147 (1995).
[11] TR. Pisanic, JD. Blackwell, VI. Shubayev, RR. Finones, *Biomaterials* 28, 2572–2581 (2007).
[12] C. M. Fu, Y. F. Wang, Y. C. Chao, H. S. Huang, and M. D. Yang, *IEEE Trans. Magn*, 40(4), 3001–3003, Jul (2004).
[13] T. Banerjee, A. K. Singh, R. K. Sharma, A. N. Maitra, *International Journal of Pharmaceuties*, 289, 189-195 (2005).