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ORIGINAL RESEARCH PAPER

Synthesis and tissue distribution of $CoFe_2O_4$ Nanoparticles Coated with DMSA in rats liver

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ABSTRACT

***Objective(s)*:** According to the unique properties of magnetic nanoparticles, their usages in medicine and industry have increased in the last decade. Due to the vital role of liver in the body, the accumulation of $CoFe_2O_4$ and $CoFe_2O_4$@DMSA was studied.

***Materials and Methods*:** The nanoparticles were synthesized by co-precipitation method and were coated with DMSA. The techniques XRD, TEM, DLS, FTIR, AGFM and UV-Visible spectroscopy were used to characterize the nanoparticles. Nanoparticles were injected intraperitoneally in rat and blood samples were collected from the rats' heart, at 15 and 30 day post injection. The liver of each rat was removed and kept frozen at -70 °C.

***Results*:** The size of the pure cobalt ferrite and DMSA coated cobalt ferrite nanoparticles were about 10 nm and 12 nm, respectively. The saturation magnetization of $CoFe_2O_4$ and $CoFe_2O_4$@DMSA nanoparticles were 44.8 and 33 emu/g, respectively. Statistical analysis of the results indicated that cobalt ferrite nanoparticles were accumulated in liver in all groups.

***Conclusion*:** The accumulation of nanoparticles in liver was significantly higher than those of the control group and the level of liver iron accumulation was significantly lower after 30 days of injection in comparison with 15 days post injection in all groups.

Keywords: *$CoFe_2O_4$ nanoparticle, $CoFe_2O_4$@DMSA, DMSA coated, Liver, Tissue distribution*

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INTRODUCTION

Nanotechnology is almost related to all advanced technology and scientists believe that nano-technology related to all fields of engineering, biology, chemistry, medicine and physics [1]. The nanoparticle refers to the particles that are smaller than 100 nm. By reducing the particle size, new properties would appear [2]. Some nanoparticles because of their high potential for specific treatments, are frequently used in biology and medicine studies [3]. One method of improving the magnetic properties of nanoparticles is to use ferrite with the general formula MFe_2O_4 where M is bivalent cation such as Mg, Fe, Co or Ni. This non-toxic nanoparticles withhigher magnetic properties as ultra-sensitive probes are used in MRI imaging [4]. Researchers have shown that hydrophobic nanoparticles are surrounded faster than other nanoparticles by blood proteins leading to their rapid clearance from circulation. Since coated nanoparticles with hydrophilic surface have different properties, thus, by improving their stability, they could remain in the blood for a longer period of time [5-8]. DMSA (dimercaptosuccinic acid, $C_4S_2O_4H_6$) is a non-toxic substance which is used as chelating

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substance to for other elements injected into the patient's body. The coating creates an anionic cover to the surface of the nanoparticles and reduces the nanoparticles opsonization by blood proteins. Also it increase the cellular uptake and tissue distribution [9,10]. Due to the increasing interest in using cobalt ferrite nanoparticles (according to their characteristics compared to other nanoparticles), *in vivo* study of their accumulation is necessary. According to the studies conducted so far, the accumulation of DMSA coated cobalt ferrite nanoparticles in the rats liver has not been investigated. Therefore, in this study an attempt was made to assess the accumulation of DMSA coated cobalt ferrite nanoparticles in the rats liver following intraperitoneal injection.

MATERIALS AND METHODS

Synthesis of $CoFe_2O_4$ nanoparticles coated with DMSA

DMSA-coated $CoFe_2O_4$ nanoparticles ($CoFe_2O_4$@DMSA) were synthesized by co-precipitation method. For this purpose, three solutions of $CoCl_2$ (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, $CoCl_2$ solution was poured into a beaker container. Meanwhile, $FeCl_3$ solution was added to the same beakers. After reaching to the boiling temperature, the NaOH were added to the same beakers. Then, obtained nanoparticles solution was washed with deionized water. In order to coat the nanoparticles, 0.01 M DMSA solution was prepared in deoxygenated deionized water and added dropwise to the nanoparticle solution while stirring. The resulting particles were washed again by the deionized water and then was centrifuged in order to remove any impurity aggregate. The precipitated samples were dried at 100 ° C [11].

$$CoCl_2 + 2\ FeCl_3 + 8\ NaOH \ \text{---}> \ CoFe_2O_4 + 4\ H_2O + 8\ NaCl$$

Synthesis and coating efficiency was properly examined using XRD (Philips pw3040 λ=0.154 nm Cu Kα radiation, Netherlands), TEM (Philips 208 S 100 kV, Netherlands), DLS (ZS90 zeta potential analyzer), FTIR (FT/IR-6300, JASCO, Japan) and AGFM (AGFM, Meghnatis Daghigh Kavir Co, Iran).

Study of nanoparticle accumulation in the liver

25, 50 and 100 mg/Kg (mg nanoparticle per kg weight of rat) of either coated or non-coated nanoparticles were injected intraperitoneally to separate groups of rats (each group contains 12 rats). Laboratory animals were randomly dissected 15 and 30 days post injection (each time 6 rat). The liver tissue was removed for analysis by AAS (VARIAN AA240FS USA). After separation of connective tissue and fat, samples were weighed (0.3 g) and were crushed into tiny pieces. They were poured into the test tubes and 3 ml of acid (perchloric acid and nitric acid 10% at 3:7 ratio) was added to the tubes. By mixing and combining, uniform mixture of samples was obtained. Then, the obtained mixture remained for 8-10 h in water bath at 100 °C in order to remove the residual acid. The specimens were then removed from the water bath and the impurities were removed from the solution by filtration. To compensate for the reduction in volume, 3 ml of twice distilled water was added to the final solution and centrifuged for 15 min. Finally, 50 µl of hydrogen peroxide (Sigma) solution was added to the samples. The obtained sample was used for testing by atomic absorption spectrometry [12, 13].

RESULTS AND DISCUSSION

Characterization of nanoparticles

Fig. 1 showed the transmission electron microscope (TEM) photograph of the $CoFe_2O_4$ and $CoFe_2O_4$@DMSA

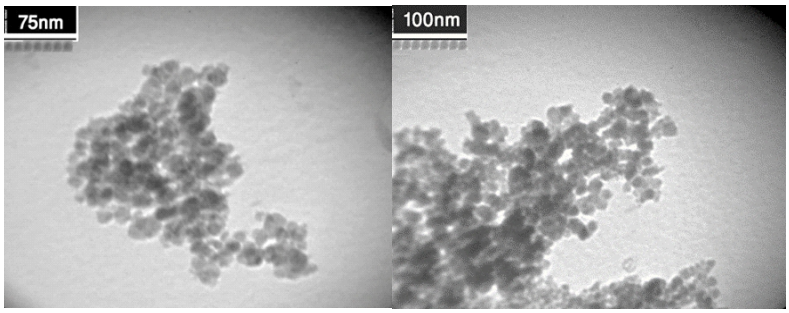

Fig. 1: TEM photograph of a) $CoFe_2O_4$ nanoparticles b) $CoFe_2O_4$@DMSA nanoparticles

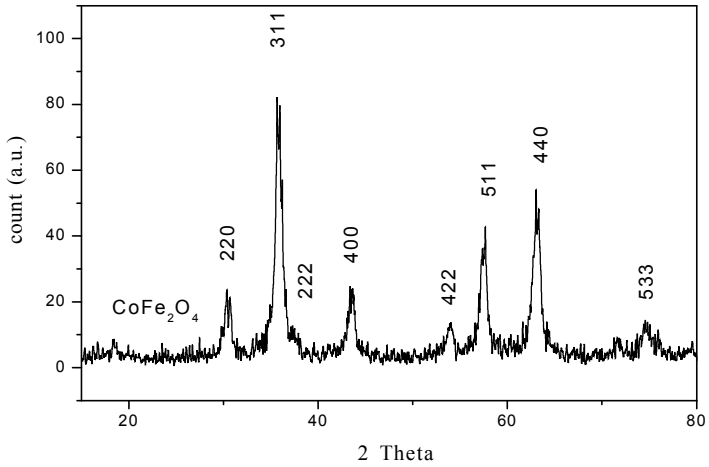

Fig. 2: X-ray diffraction curve of cobalt ferrite nanoparticles

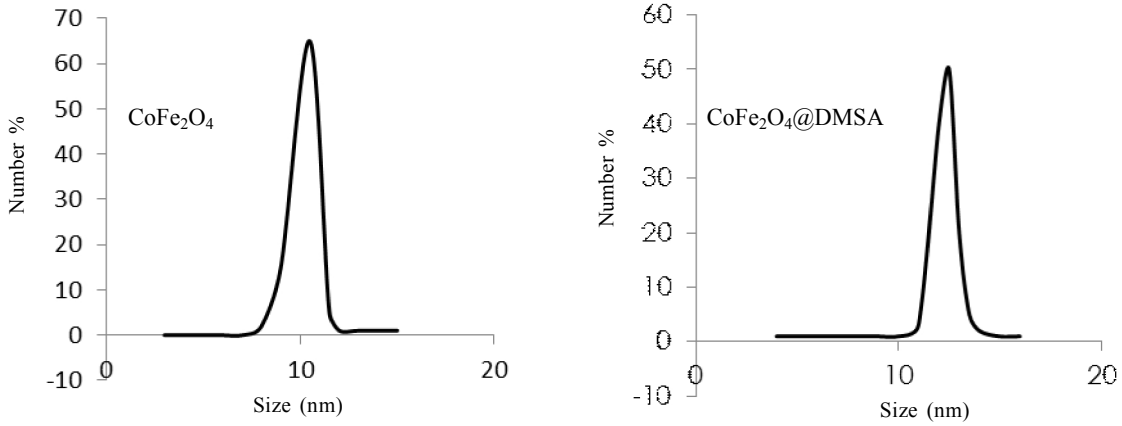

Fig. 3: Dynamic light scattering curves $CoFe_2O_4$ and $CoFe_2O_4$@DMSA

nanoparticles. The size of the pure cobalt ferrite nanoparticles and DMSA coated cobalt ferrite nanoparticles were around 10 nm and 12 nm, respectively with approximately uniform size distribution. The mean size of the nanoparticles was also determined from XRD pattern (Fig. 2) by Debye-Scherer formula which showed a good agreement with TEM results (approximately same sizes were obtained) [14,15].

Fig. 3 indicated the DLS graph obtained from both coated and non-coated nanoparticles. As can be seen, approximately 70% of cobalt ferrite nanoparticles have sizes about 10-11 nm and approximately 50% of DMSA-coated cobalt ferrite nanoparticles showed sizes around 12-13 nm and size distribution is uniform. These results confirmed the results obtained by XRD and TEM analysis.

Fig. 4 shows the FTIR spectra of $CoFe_2O_4$, DMSA, and $CoFe_2O_4$@DMSA. As can be seen, in the $CoFe_2O_4$ spectra, peaks at 1331.61, 1624.73 and 3409.53 cm^{-1} are related to hydroxyl groups suggesting that there was water in the material structure. On the other hand, in $CoFe_2O_4$@DMSA spectrum, peaks at 1604, 1387 and 1151 cm^{-1} corresponds to the asymmetric and symmetric stretch of carboxyl group, respectively. In comparison with different peaks at 1699.94, 1614.64 and 1118.19 cm^{-1} in DMSA represents a bond between cobalt ferrite and DMSA.

Fig. 5 indicated the AGFM spectra of $CoFe_2O_4$ and $CoFe_2O_4$@DMSA. As can be seen, both compounds

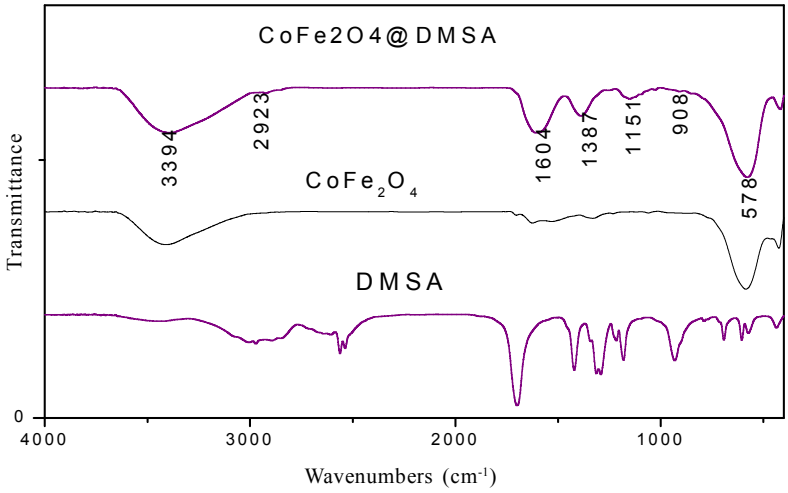

Fig. 4: Fourier transform infrared spectroscopy curve of $CoFe_2O_4$, $CoFe_2O_4$@DMSA and DMSA

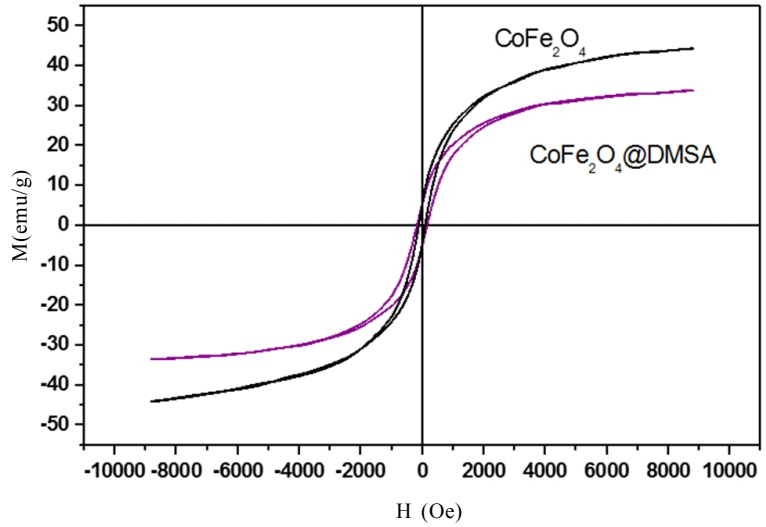

Fig. 5: AGFM curves of $CoFe_2O_4$ and $CoFe_2O_4$@DMSA

exhibited ferromagnetic properties. According to the image, pure cobalt ferrite sample has the saturation magnetization of 44.8 emu/g while that of coated samples is 33 emu/g. Magnetization obtained from these nanoparticles are less than that of bulk cobalt ferrite (about 80 emu/g) [16].

Nanoparticles accumulation in the liver

The average liver iron levels in different animal groups is showm in Table 1. As can be seen, the results of 15 days post injection, there is a significant increase in liver iron concentration in all groups compared to the control group ($P<0.001$). The level of liver iron accumulation significantly decreased 30 days post injection in comparison with 15 days post injection in all groups. Also, there were significant differences between control and all groups except the group received 25 mg/kg.

Fig. 6 indicated the mean iron accumulation in the rat liver 15 and 30 days post injection. It can be seen that the amounts of iron concentration 30 days post injection were significantly less than that of 15 days post injection in all groups tested ($P<0.001$). The results are completely in agreement with those published previously [17-19].

Table 1: Mean iron concentration in rat liver

Day	Dose mg/kg	Group	Mean µg/kg	SD	P-value
		Control	4.05300	0.332932	
	25	$CoFe_2O_4$	6.59300	0.596708	P<0.001
	50	$CoFe_2O_4$	8.46900	2.147126	P<0.001
15	100	$CoFe_2O_4$	7.80700	0.499158	P<0.001
	25	$CoFe_2O_4$@DMSA	7.74700	1.261515	P<0.001
	50	$CoFe_2O_4$@DMSA	8.67000	1.323993	P<0.001
	100	$CoFe_2O_4$@DMSA	7.33800	0.104312	P<0.001
		Control	3.91200	0.190990	
	25	$CoFe_2O_4$	4.10500	0.160614	P>0.05
	50	$CoFe_2O_4$	6.23500	0.681048	P<0.001
30	100	$CoFe_2O_4$	5.66600	0.170666	P<0.001
	25	$CoFe_2O_4$@DMSA	5.25600	0.493425	P<0.05
	50	$CoFe_2O_4$@DMSA	5.43900	0.767080	P<0.05
	100	$CoFe_2O_4$@DMSA	4.41500	0.050764	P<0.05

Results are the mean ± SD of six separate experiments

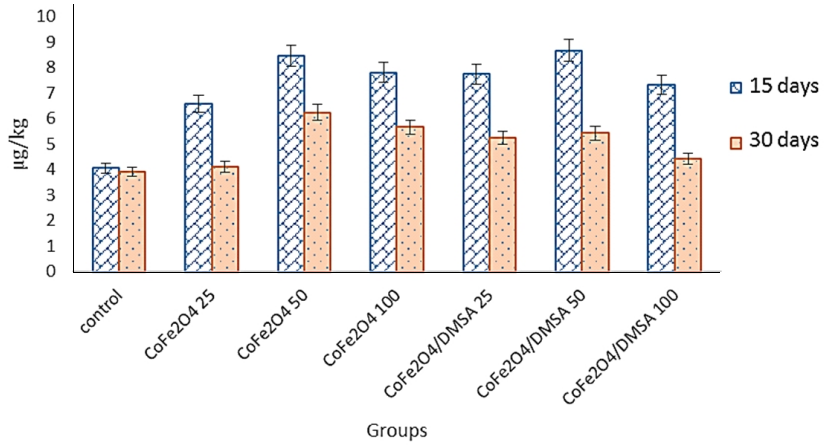

Fig. 6: Mean iron concentration in different tissues.

According to results, iron aggregation in the liver was relatively low even at high doses. Except the group received 25 mg/kg, the accumulation of iron in the groups received coated nanoparticles were less than the groups received non-coated nanoparticles. This result indicated that the coating of nanoparticles could decrease the uptake of nanoparticles by the reticuloendothelial system and therefore increased the persistence of nanoparticles in the blood circulation. which could be attributed to the effect of coating. The nanoparticles accumulation in liver significantly increased compared to control group and the level of iron accumulation in liver was significantly lower 30 days post injection in comparison with 15 days post injection in all groups. Therefore, it is concluded that there was no adverse effects on the liver function which was related to the use of coated and uncoated cobalt ferrite nanoparticles.

CONCLUSION

DMSA-coated nanoparticles were synthesized by a simple manner. The saturation magnetization of $CoFe_2O_4$@DMSA nanoparticle was 33 emu/g which was a little less than that of non-coated nanoparticles

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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