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Dextran-coated Fe_3O_4 Nanoparticles for Hyperthermia Therapy Application

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ABSTRACT

In the present work, nanocrystals of magnetite were prepared by alkaline precipitation. The precursor used for synthesis was ferrous chloride only and the reaction was carried out in absence of any oxidant. Dextran is a material with excellent biocompatibility, water solubility and low cytotoxicity. Hence it was chosen as a potential coating material for the magnetic nanoparticles (MNPs). Both bare and coated MNPs (Fe_3O_4 and D- Fe_3O_4) showed particle size of diameter 20.8±4.1 nm and 16.3±3.2 nm, respectively. The magnetization values of both the MNPs were 53.62 emu/g and 47.86 emu/g at room temperature, respectively. The negligible coercivity and remanence values at room temperature implied superparamagnetic behaviour of the MNPs. Zeta potential of both bare and coated nanoparticles showed higher colloidal stability of coated nanoparticles than the bare one at a pH range of 2-10. The MNPs are studied for their heating induction abilities at 167.6 Oe, 251.4 Oe and 335.2 Oe (equivalent to 13.3, 20.0 and 26.7 kA m^{-1}, respectively), in order to use them in magnetic fluid hyperthermia therapy. At 335.2 Oe, D-Fe_3O_4 showed maximum Specific Absorption Rate (SAR) of 120.25 W/g, while bare MNPs showed SAR of 79.32 W/g. Both the particles had no cytotoxic effect on L929 cell line showing their strong possibility to be used for *in vivo* applications. The further work is in the process.

Key words: *Magnetic Nanoparticles, Alkaline precipitation, Fe_3O_4, Dextran, Hyperthermia*

Introduction

The γ-Fe_2O_3 or Fe_3O_4 particles with a core size of <20 nm are known as super paramagnetic iron oxide nanoparticles (SPIONs). They show a very impotent feature that they no longer show magnetic interaction after removal of the magnetic field. Super paramagnetic nature allows tracking of such SPIONs in a magnetic field gradient without losing the advantage of a stable colloidal suspension. The heating of the particles can be observed on application of an alternating magnetic field, based on the Néel relaxation mechanism Irfan *et al.* (2021). The surface modification of SPIONs by organic molecules has many advantages. It stabilizes the SPIONs in a biological suspension with a pH around 7.4. It provides functional groups on the surface for further derivatization. Additionally, it helps to avoid immediate uptake of SPIONs by the reticuloendothelial system (RES). Dextran is a biocompatible, biodegradable and water-soluble material extensively used in biomedical applications for coating nanoparticles. It helps to prevent agglomeration and toxicity of SPIONs. It also exhibits defined biological interactions with the complement system Li *et al.* (2021). *In vitro* effect of dextran-coated SPIONs was tested on human colon cancer cell lines and the results showed that the dextran-coated SPIONs had

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better biocompatibility compared with the uncoated nanoparticles Ayala *et al.* (2013).

In the present work, dextran-coated MNPs (D-Fe_3O_4) were prepared by chemical method and were studied for their possible use in cancer hyperthermia therapy application.

Materials and Methods

Fe_3O_4 NPs were synthesized via alkaline precipitation method reported earlier Shete *et al.* (2015). 2g of $FeCl_2.4H_2O$ was dissolved in 50 ml of 1M HCl by heating up to 70°C. 50 ml of 3M NaOH was added to it at 60°C drop by drop with constant stirring. Fe_3O_4 NPs were formed as black precipitate. The precipitate was allowed to settle down by applying external magnetic field. It was then separated and washed with distilled water till neutral pH is achieved. It was then dried at RT and used for coating procedure. The possible reaction is shown below:

$3FeCl_2.4H_2O + 6NaOH + 1/2\ O_2\ Fe_3O_4 + 6NaCl + 15H_2O$

The dextran solution was prepared by mixing 5g of dextran with 100 ml of deionized water. The solution was put in contact with 1g of Fe_3O_4 NPs at 100°C for 1h in order to achieve coating of the MNPs with dextran. After the solution was cooled at room temperature (28°C), the suspension was centrifuged at 800rpm for 15min in order to separate the newly synthesized materials. The final product was separated using a magnet and then washed with methanol, referred as D-Fe_3O_4 (Dextran-coated Fe_3O_4).

Both Fe_3O_4 and D-Fe_3O_4 MNPs were submitted for further studies - Transmission Electron Microscopy (TEM), Fourier Transmission InfraRed (FTIR) Spectroscopy, Vibrating Sample Magnetometry (VSM), Specific Absorption Rate (SAR) analysis and Cytotoxicity Assay.

Results and Discussion

The size and shape of the D-Fe_3O_4 MNPs was observed using TEM (Fig. 1). The D-Fe_3O_4 MNPs show the particle size of 15.8±5.3 nm with nearly spherical shape. The bare MNPs show larger aggregates due to magnetic dipole-dipole interactions (Khmara *et al.*, 2020). While coating of dextran reduces particle size as non-magnetic surface inhibiting the magnetic interactions.

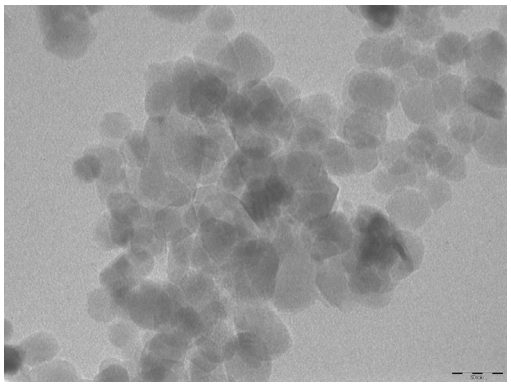

Fig. 1. TEM of D-Fe_3O_4 MNPs

To demonstrate the successful attachment of dextran onto the surface of Fe_3O_4 MNPs, both, bare coated MNPs were investigated using FTIR spectroscopy in the range of 450 to 4000 cm^{-1} (Fig. 2). The dips around 3376 cm^{-1}, 3400 cm^{-1}, 3434 cm^{-1} are assigned to stretching vibrations and the dip at 1620 cm-1 is assigned to bending vibrations of the –OH groups due to adsorbed water on surface of MNPs. The dip at 560 cm^{-1} corresponds to M–O vibrations, which is due to the Fe–O bond in the case of bare Fe_3O_4 Patil *et al.* (2014). The dips at 906 cm^{-1} and 1020 cm^{-1} correspond to α-glycosidic and α-1,6 glycosidic bonds, respectively (Rohiwal *et al.*, 2021). Hence, FTIR results confirm coating of dextran on MNPs.

Magnetic properties of the D-Fe_3O_4 MNPs were studied using their M–H curves. M–H curves of bare Fe_3O_4 and coated Fe_3O_4 MNPs at 100K and 300 K are shown in Fig.3. The graphs clearly show the superparamagnetic nature of D-Fe_3O_4 MNPs as the coercivity (Ce) and remanence (Mr) values are very negligible. Superparamagnetic behavior of MNPs at room temperature (28°C) is very useful in *in vivo*

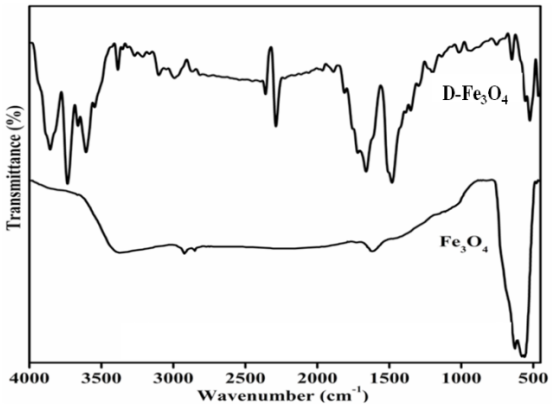

Fig. 2. FTIR of D-Fe_3O_4 MNPs

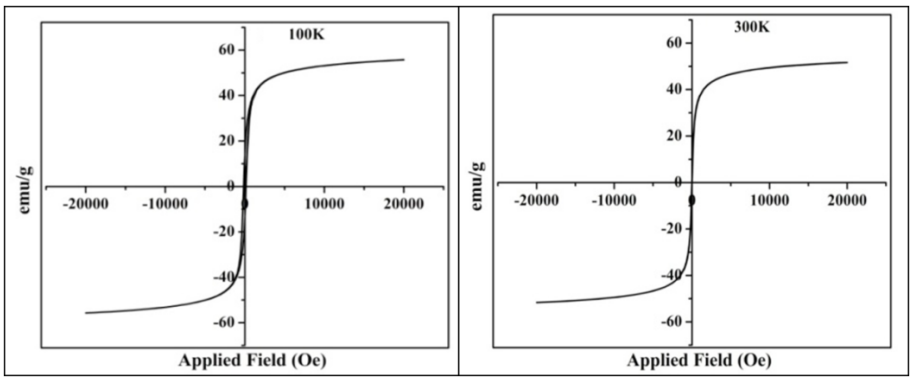

Fig. 3. M-H curves of D-Fe_3O_4 at 300K and 100K

applications as they do not retain magnetization before and after exposure to an external magnetic field, reducing the probability of particle aggregation due to magnetic dipole attraction.

From the SAR graph (Fig.4), it is observed that the hyperthermia effect of Fe_3O_4 MNPs enhances dramatically after functionalization with dextran when the applied field is above 250 Oe. The first possible reason for the enhanced hyperthermic effect after coating is that the ability of the coating to retain the superparamagnetic fraction of the Fe_3O_4 is much better as compared to Fe_3O_4 alone. The second is that the coating layer prevents the formation of larger aggregates of Fe_3O_4 which possibly makes a better suspension of D-Fe_3O_4 MNPs enhance the hyperthermic effect through Brownian and Neel's spin relaxations. Thus the hyperthermia study also strongly supports the coating of Fe_3O_4 MNPs and prevention of particle agglomeration.

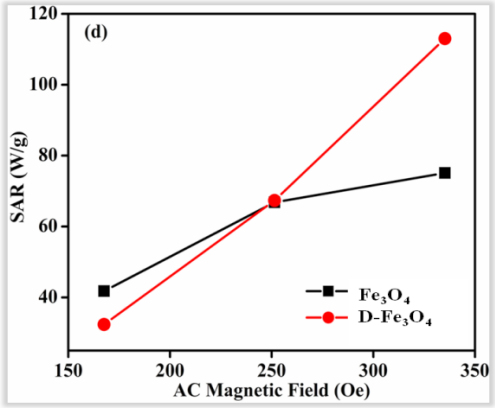

Fig. 4. SAR of Fe_3O_4 and D-Fe_3O_4 MNPs

Cytotoxicity assay was performed in order to check the cytotoxic effect of MNPs (Fig. 5). The cytotoxicity study of both, bare and coated MNPs was done on the L929 cell line with different concentrations of MNPs. The L929 cell line was incubated with MNPs for 48 h with the concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 mg ml^{-1} at 37 ^oC in a 5% CO_2 atmosphere. The relative cell viability (%) compared with the control well containing cells without MNPs are calculated by the equation: [A]tested/[A]control × 100. Fig. 5 shows the cell viability after incubation with different concentrations of both bare and coated Fe_3O_4 MNPs. It clearly reveals that after 48 h, bare MNPs started to exhibit their cytotoxicity while coated MNPs still showed almost 100% viability. Therefore D-Fe_3O_4 MNPs are more suitable for *in vivo* applications than the bare MNPs, owing to their lower cytotoxicity.

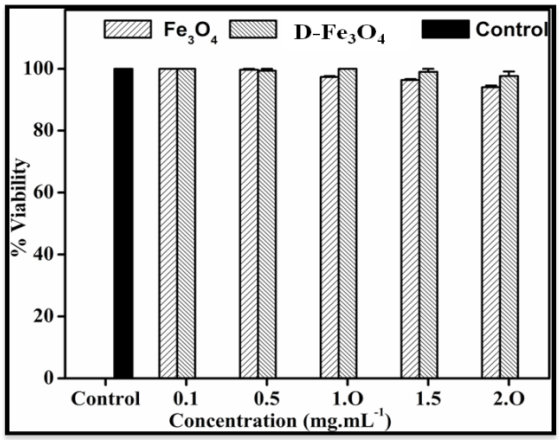

Fig. 5. Cytotoxicity assay of Fe_3O_4 and D-Fe_3O_4 MNPs

Conclusion

This study demonstrates the effect of capping of dextran on the surface behavior of Fe_3O_4 MNPs. TEM image of D-Fe_3O_4 MNPs showed that the particles are monodispersed, spherical shaped having

diameter of 15.8±5.3 nm. M-H curve D-Fe_3O_4 MNPs are superparamagnetic at room temperature with negligible Ce and Mr. Cell viability assay of both the MNPs showed very low cytotoxic effect on L929 cell line even after 48 h incubation period. Thus, synthesized MNPs are suitable for hyperthermia therapy applications owing to their smaller size, superparamagnetic behavior at room temperature (28°C), higher magnetization values and low cytotoxicity.

Conflict of interest

There is no any conflict of interest.

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