

Supplementary Information

***In-vitro* Evaluation of Layered Double Hydroxide-Fe₃O₄ Magnetic Nanohybrids for Thermo-Chemo Therapy**

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1. Materials

Magnesium nitrate hexahydrate, aluminum nitrate nonahydrate, ferric chloride hexahydrate, ferrous chloride tetrahydrate, doxorubicin hydrochloride, and sulforhodamine B were procured from Sigma Aldrich. Sodium hydroxide, ammonia solution, citric acid, phenanthroline, hydrochloric acid, trichloroacetic acid, paraformaldehyde, and glycerol were purchased from Merck, India. Minimum essential medium, fetal bovine serum, penicillin/streptomycin antibiotics, trypan blue, PBS tablets, and tris buffer were procured from HiMedia, India. MilliQ water (18.2MΩ) was used from the Millipore water purification unit. All the chemicals and solvents were of analytical grade and were used without further purification.

2. Materials' Characterization

The powder X-ray diffraction (XRD) patterns were recorded on the Philips powder diffractometer PW3040/60 with Cu_{Kα} (1.5406 Å) radiation with step size of 0.017°. High-resolution transmission electron microscopy images and selected area diffraction patterns were obtained using field emission gun transmission electron microscope (FEG-TEM), JEOL JEM 2100F, which operated

at an accelerating voltage of 200 kV. Fourier transform infrared (FTIR) spectra were obtained on the JASCO spectrometer (6100 type-A) instrument in the range of 400–4000 cm^{-1} . The surface charge of the nanohybrids was measured by ZetaPALS zeta potential analyzer (BI-200 Brookhaven Instruments Corporation). The magnetization measurements were carried out using Magnetic Properties Measurement System (MPMS-XL, Quantum Design). In order to calculate the time-dependent calorimetric measurements, that is, the specific absorption rate, the amount of Fe in the suspension of the MNHs was obtained by using the phenanthroline spectrophotometric method (Cecil, Model No. CE3021) [1]. The fluorescence spectra of Dox were recorded using a Hitachi F2500 fluorescence spectrophotometer in the range of 500 to 800 nm at an excitation wavelength of 490 nm. The calorimetric studies of the Fe_3O_4 and MNHs under ACMF were carried out using an EASY HEAT, EZLI5060 RF generator that operated at a fixed frequency of 425 kHz. The confocal laser scanning images were captured by an inverted confocal microscope by Olympus, model IX81.

3. Magnetic separation of MNHs

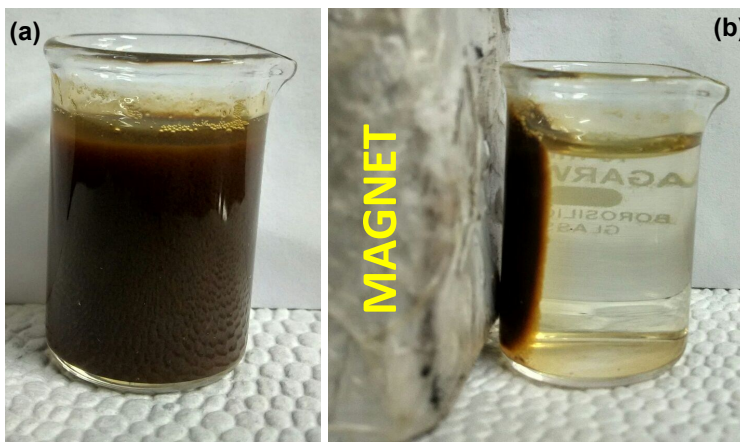


Figure S1. (a) Before and (b) After magnetic separation of magnetic nanohybrids from its aqueous suspension

4. Hydrodynamic diameter

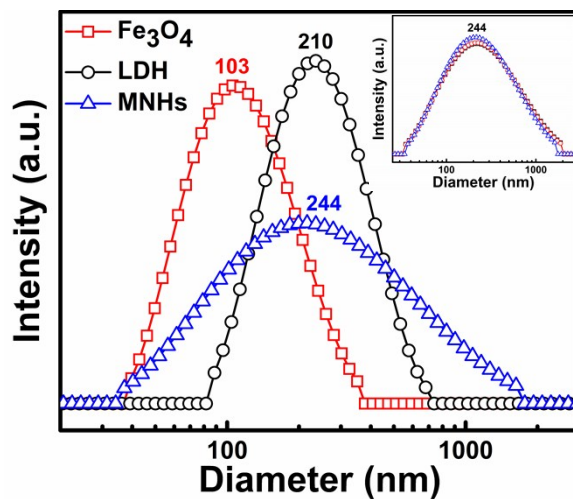


Figure S2. Hydrodynamic diameter of Fe₃O₄, LDH nanoparticles and MNHs. Inset represents the hydrodynamic diameter of MNHs in triplicate measurements ($\sigma \leq 5\%$)

5. Thermo Gravimetric Analysis (TGA)

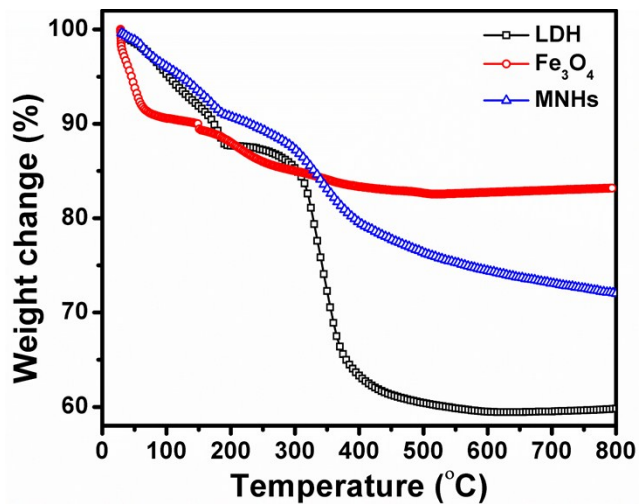


Figure S3. TGA plot of Fe₃O₄, LDH nanoparticles and MNHs

6. ZFC-FC Measurement

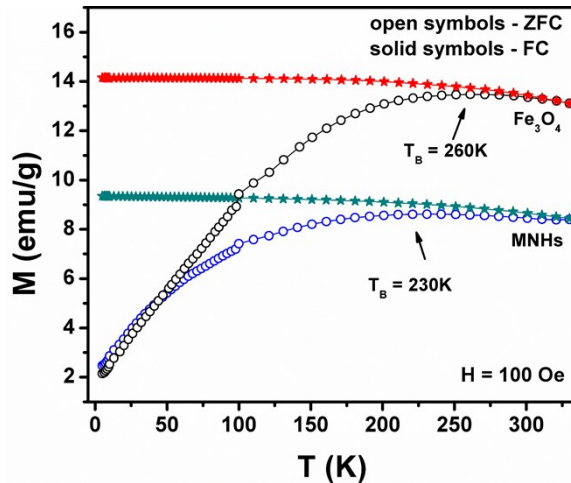


Figure S4. ZFC-FC curves of Fe_3O_4 and MNHs at 100 Oe field

7. Optimization of Calorimetric measurements of MNHs

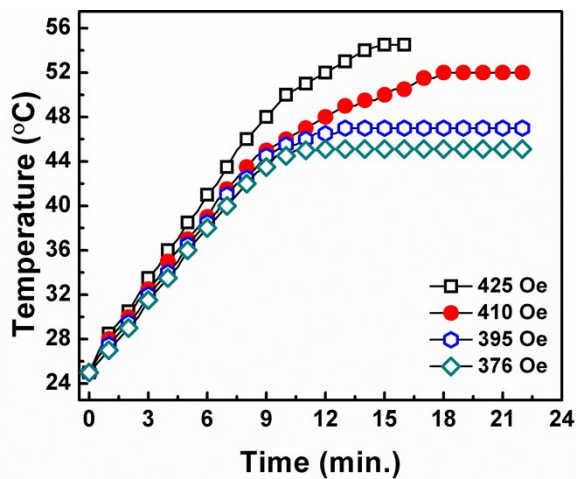


Figure S5. Heating profiles of MNHs at different field strengths

6. XPS Spectra

Figure S6 shows the XPS spectra for C1s, O1s, and Fe2p of citric acid-coated Fe₃O₄ nanoparticles. The C1s core levels were deconvoluted into three peaks at 284, 284.7, and 285.8 eV, which corresponded to C-H, C-C and C-O, respectively. These peaks arise due to the citric acid groups on the surface of Fe₃O₄. The O1s levels show two deconvoluted peaks at 532.2 and 532.6 eV, which correspond to C-O and C=O, respectively. The Fe2p levels reveal the presence of Fe2p_{3/2} and Fe2p_{1/2} oxidation states of iron oxide. The peak that occurs at 709.5 eV corresponds to the Fe-O bonds with oxygen of citric acid, while the peaks of Fe2p_{3/2} and Fe2p_{1/2} occur at 711.6 and 723.1 eV, respectively. The broad peak at 718 eV corresponds to the Fe³⁺ oxidation state of iron.

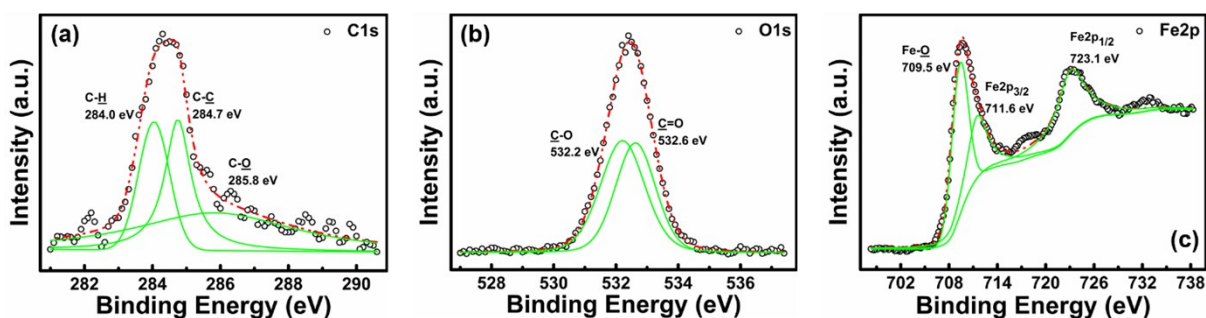


Figure S6. Deconvoluted XPS spectra of citric acid coated Fe₃O₄ nanoparticles showing (a) C1s, (b) O1s and (c) Fe2p core levels

Figure S7 shows the deconvoluted XPS spectra of LDH nanoparticles with C1s, O1s, Al2p, and Mg2p core levels. The C1s levels were seen to comprise three deconvoluted core levels arising at 284.8, 286.3, and 288.5 eV. These peaks could be attributed to the binding interactions of the carbon of the carbonate ion, the hydroxide of water that is present in the interlayer spacing, and, Mg²⁺ from the LDH layers and to oxygen within the carbonate anion, respectively. The O1s core levels give rise to two asymmetric deconvoluted peaks at 530.1 and 532.5 eV, which arise due to the carbonate anion and hydroxide anion, respectively. The spectra of Al2p consist mainly of a

symmetric peak at 75.2 eV, corresponds to interactions of Al^{3+} with interlayer hydroxide anions. A small shoulder peak is also seen at 74 eV, indicating the presence of Al^{3+} in the oxide form, but in minor proportions. The $\text{Mg}2p$ core levels are deconvoluted into two primary peaks at 55.3 and 57.2 eV, indicating the interactions of Mg^{2+} with oxide/hydroxide and the carbonate anions, respectively. Thus, these results confirm the successful synthesis of Fe_3O_4 and LDH nanoparticles.

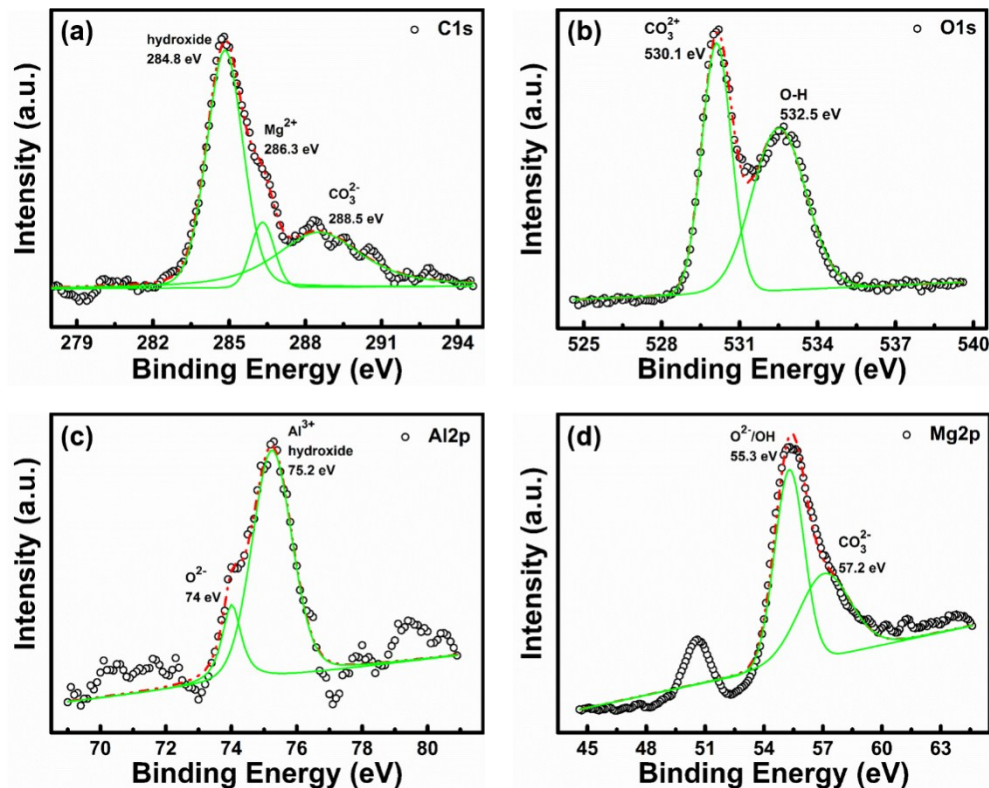


Figure S7. Deconvoluted XPS spectra of LDH nanoparticles showing (a) C1s, (b) O1s, (c) Al2p and (d) Mg2p core levels

Table S1. Elemental analyses of Fe_3O_4 , LDH and MNHs obtained from ICP-AES, TGA, CHN(O)S

Sample	Mg (wt%)	Al (wt%)	Fe (wt%)	C (wt%)	O (wt%)	H ₂ O (wt%)
LDH	13.72	4.03	--	1.48	26.47	12.31
Fe ₃ O ₄	--	--	40.53	2.05	35.66	12.01
MNHs	6.89	2.12	26.42	1.89	45.65	9.22

The quantification of Mg, Al and Fe were obtained from ICP-AES measurements, C and O from CHN(O)S measurements and H₂O is from the weight loss between 25 to 200 °C from TGA measurement of dry sample.

Table S2. Ratios of absolute area of the deconvoluted peaks of fluorescence spectra of Dox

	A1/A2	A3/A2
Dox	0.73	1.01
MNHs_25%	0.61	0.67
MNHs_50%	0.54	0.64
MNHs_75%	0.52	0.63
MNHs_99.8%	0.51	0.61

References

1. Ding, B., et al., *Preparation and pH Stability of Ferrous Glycinate Liposomes*. Journal of Agricultural and Food Chemistry, 2009. **57**(7): p. 2938-2944.