Chapter 5
Assessment of $T_1$-$T_2$ dual MRI contrast enhancement by a system having both manganese oxide and iron oxide

5.1 Introduction

This chapter discusses development of manganese oxide ($\text{Mn}_2\text{O}_3$) and iron oxide ($\text{Fe}_2\text{O}_3$) nanoparticles encapsulated in mesoporous 3D carbon framework (CF) and assessment of their MRI contrast enhancement property.

Single mode contrast agents face a lot of challenges during imaging of small biological targets. This issue can be addressed by use of dual mode MRI, involving $T_1$ and $T_2$ simultaneously to acquire complementary diagnostic information from both the modes. The emergence of dual mode MRI has led to efforts by researchers to develop $T_1$-$T_2$ dual mode contrast agents [1-9].

We already discussed about the merit of manganese based systems as $T_1$ based MRI contrast agents in the sub-section 1.4.2 of Chapter 1. On the other hand, magnetic iron oxide nanoparticles have set a successful example for application as a $T_2$ MRI contrast agent [10]. Therefore, in this work, a nanosystem having both manganese oxide and iron oxide nanoparticles encapsulated together within a mesoporous 3D carbon framework (MOIO@CF nanosystem) was developed and its $T_1$ and $T_2$ based MRI contrast enhancement efficiency was assessed. The synthesis of $\text{Mn}_3\text{O}_4$ nanoparticles discussed in the Chapter 3 involves use of nitrogen gas which lessens its acceptance as a technology. Therefore, in this chapter, $\text{Mn}_2\text{O}_3$ nanoparticles have been studied.

The schematic presented in Figure 5.1 summarizes the design and the formation mechanism of the nanosystem. Various characterizations (microstructure, surface morphology, composition etc.) were performed to ensure high quality product. Cell viability studies were performed to confirm the applicability of
the nanosystem for biomedical purpose. This was followed by T₁-T₂ based relaxivity studies and *in vitro* MRI scans using the developed nanosystem.

![Figure 5.1 Schematic of formation of MOIO@CF nanosystem](image)

**Figure 5.1** Schematic of formation of MOIO@CF nanosystem

### 5.2 Material development

#### 5.2.1 Starting materials

Manganese (II) sulphate monohydrate (MnSO₄·H₂O), Iron sulphate (FeSO₄·7H₂O), Ammonium bicarbonate (NH₄HCO₃) and Ethanol (C₂H₅OH; as a solvent) used in the reaction for precipitation of Manganese carbonate (MnCO₃) and Iron carbonate (FeCO₃), precursor for manganese oxide nanoparticles, were procured from Merck. Polyvinylpyrrolidone (PVP), which was used as the carbon source was procured from Sigma-Aldrich. Throughout the synthesis processes and characterizations, deionized water was used.

#### 5.2.2 Synthesis procedure

6.7 mmol of MnSO₄·H₂O, 6.7 mmol FeSO₄·7H₂O and 4 g of PVP were dissolved into 400 mL of deionized water under stirring. Then, solution of 164 mmol of NH₄HCO₃ dissolved in 200 mL of deionized water, and 40 mL of ethanol were added to the above mixture with vigorous stirring. The final mixture is kept under stirring for 1 hour. After adding 6 g of PVP into the solution, it is kept stirring for another 1 hour. The resultant precipitate of MnCO₃@PVP was separated out and dried at 80°C. Finally, three different samples of MOIO@CF nanosystem were obtained by calcination of the dried sample at 700°C for 2
hours, 4 hours and 6 hours followed by cooling inside the furnace till room temperature.

5.3 Results and discussion

5.3.1 Microstructural analysis

5.3.1.1 XRD analysis

XRD pattern of the synthesized MOIO@CF nanosystem (Figure 5.2 (a)) obtained after calcination for 2 hours, 4 hours and 6 hours showed all the major peaks representing Mn$_2$O$_3$ (JCPDS 41-1442; body centered cubic) for (211), (222), (321), (400), (411), (332), (431), (440), (620), (541) and (622) crystal lattice planes [11]. Similarly, peaks corresponding to Fe$_2$O$_3$ (JCPDS 89-0598; Rhomb centered Rhombohedral) were obtained for (012), (104), (110), (006), (113), (024), (214) and (300) crystal lattice planes [12]. Deconvolution of the peak shown in Figure 5.2 (b) for all the samples confirms the presence of individual main peak of Mn$_2$O$_3$ (222) and Fe$_2$O$_3$ (104).

![Figure 5.2 (a) XRD pattern of MOIO@CF nanosystem (b) Deconvolution of the main peak](image-url)
5.3.1.2 SEM and EDS analysis

Figure 5.3 (a), (b) and (c) show SEM images of the MOIO@CF nanosystem obtained after calcination for 2 hours, 4 hours and 6 hours respectively. The micrographs exhibit formation of agglomerated spherical assemblies.

Figure 5.3 SEM of MOIO@CF nanosystem obtained by calcining for (a) 2 hours (b) 4 hours (c) 6 hours

The elemental mapping analysis shown in Figure 5.4 (a)-(c) reveals the presence of Mn, Fe and O in all the samples. It is observed that all the three elements are consistently distributed throughout the developed material.
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5.3.1.3 TEM analysis

Figure 5.5 (a)-(c) show the TEM images of MOIO@CF nanosystem obtained by calcining for 2, 4 and 6 hours respectively. It is observed that with increase of the calcination duration, the carbon frameworks lose their uniform spherical morphology. Therefore, results obtained for the first sample (calcined for 2 hours) only has been considered while presenting various characterizations. Figure 5.5 (d) provides a closer and distinct view of the spherical carbon framework of the first sample where we can clearly see the external porous feature of the framework. In addition, we can observe that the nanoparticles are embedded inside the carbon framework. The average size of the nanoparticles was observed to be ~40 nm.

Figure 5.4 Elemental mapping of MOIO@CF nanosystem obtained by calcining for (a) 2 hours (b) 4 hours (c) 6 hours

Figure 5.5 TEM of MOIO@CF nanosystem obtained by calcining for (a) 2 hours (b) 4 hours (c) 6 hours (d) Higher magnification TEM of the first sample (calcined for 2 hours)
5.3.2 Identification of chemical structure

5.3.2.1 FTIR analysis

Figure 5.6 shows the FTIR spectra of MOIO®CF nanosystem obtained by calcining for 2, 4 and 6 hours respectively. IR bands at 3420 and 1630 cm\(^{-1}\) are attributed to O-H stretching and bending vibrations respectively [13]. A band appears at 674 cm\(^{-1}\) due to Fe-O stretching mode and asymmetric Mn-O stretching vibration [14-16]. The peak located at 511 cm\(^{-1}\) is associated with the bending vibration of Mn-O bond [17]. The peak observed at 567 cm\(^{-1}\) is assigned to symmetric Mn-O stretching vibration and Fe-O stretching vibration [14, 16].

![FTIR spectra of MOIO@CF nanosystem](image)

**Figure 5.6** FTIR spectra of MOIO@CF nanosystem

5.3.2.2 XPS analysis

The XPS spectra recorded in the range of 0-1200 eV revealed that the MOIO@CF system contained Fe, O, Mn and C elements (Figure 5.7 (a)) [18-21]. XPS peaks of Fe 2p\(_{3/2}\) and Fe 2p\(_{1/2}\) are obtained in 710.5 eV and 723 eV respectively as shown in Figure 5.7 (b). The Fe 2p\(_{3/2}\) peak possesses associated satellite peak at
approximately 8 eV higher binding energy confirming presence of Fe$_2$O$_3$ [18]. In the O 1s XPS spectrum shown in Figure 5.7 (c), the peaks with binding energies 529.2 eV, 531.3 eV and 532.9 eV can be attributed to Mn−O−Mn, Mn−OH and H−O−H bonds respectively [19]. Figure 5.7 (d) shows the Mn 2p peaks at 641.24 and 652.73 eV which corresponds to Mn2p$_{3/2}$ and Mn2p$_{1/2}$ of Mn$^{3+}$ state respectively [20]. Deconvolution of the C 1s peak (Figure 5.7 (e)) shows the presence of non-oxygenated carbon at 284.5 eV, C=O at 286.1 eV and O−C=O at 288.3 eV [21].
5.3.3 Magnetic property analysis

The field-cooled (FC) and zero-field cooled (ZFC) temperature dependences of magnetization (Figure 5.8 (a)) were carried out on MOIO@CF nanosystem at a magnetic field of 500 Oe from 3 K to 320 K. The M–T curve shows a blocking temperature, $T_B$ near 30 K. Above 30 K, the FC and ZFC magnetization curves
exhibit the same trend and overlap. But, below the blocking temperature, the ZFC magnetization decreases sharply and the two curves split. The plateau in the FC magnetization curve below $T_B$ implies the presence of strong inter-particle interactions. Because, strong interparticle interactions produce a collective magnetic freezing, showing similarities with spin glass freezing [22, 23].

The hysteresis curves were acquired at 3 K and 300 K (Figure 5.8 (b)). At 300 K, the sample shows paramagnetic nature. But, the M-H plot obtained at 3 K shows an unusual elliptical shape. Such kind of hysteresis loop is obtained when the applied magnetic field is not sufficient enough to overcome the magnetic anisotropy energy barrier of the sample. Therefore, it can be concluded that the magnetic anisotropy energy barrier of the MOIO@CF nanosystem was so high that even magnetization provided by 20 kOe magnetic field could not surmount it resulting in an elliptical hysteresis loop [24].

Figure 5.8 (a) Zero field cooled (ZFC) and field cooled (FC) magnetization curves of MOIO@CF nanosystem (b) Hysteresis curves of MOIO@CF nanosystem

5.3.4 Porosity analysis

Figure 5.9 (a) shows the N$_2$ gas adsorption–desorption isotherm of MOIO@CF nanosystem. The adsorption–desorption isotherm resembled to Type IV as stated in the International Union of Pure and Applied Chemistry (IUPAC) classification [25], confirming a mesoporous material. The Brunauer-Emmett-
Teller (BET) specific surface area was measured to be about $40.76 \text{ m}^2\text{g}^{-1}$ with a pore volume of $0.036 \text{ cm}^3\text{g}^{-1}$. On the other hand, the Barrett Joyner Halenda (BJH) pore radius distribution was obtained from the desorption profile (Figure 5.9 (b)) and BJH pore diameter for the sample was calculated to be 3.5 nm. The pore diameter of the sample is within the characteristic size range of mesoporous materials as specified by IUPAC reference (2-50 nm).

![Figure 5.9](image)

**Figure 5.9** (a) N$_2$ adsorption-desorption isotherms and (b) BJH pore distribution profile of MOIO@CF nanosystem

### 5.3.5 Hydrodynamic size measurement

DLS measurement was conducted to determine the distribution of hydrodynamic size of the water dispersed particles of MOIO@CF nanosystem. Intensity, number and volume distributions corresponding to particle diameter (Figure 5.10 (a)-(c)) were obtained through this measurement. It can be observed that the system explored in this work has nanoparticles encapsulated within mesoporous carbon shell which are too large to cross the blood brain barrier abolishing any possibility of brain toxicity [26].
Cell viability assay

The *in vitro* cytotoxicity of MOIO@CF nanosystem with different metal concentrations was evaluated via MTT assay of viability of HEK 293 cell line (Figure 5.11). It was observed that incubation of the cells with the nanosystem did not result in any significant decrease in cell viability even up to a concentration of 0.5 mM.
Figure 5.11 Cell viability profile of HEK 293 cell line incubated with MOIO@CF nanosystem

5.3.7 Relaxivity profile

In order to estimate the $T_1$ and $T_2$ dual contrast efficiency, the relaxivities ($r_1$ and $r_2$) of aqueous dispersions of MOIO@CF nanosystem were measured by a TD-NMR system at room temperature. $T_1$ and $T_2$ based TD-NMR signal intensity profiles with time for various metal concentrations are shown in Figure 5.12 (a) and (b). The nanosystem enhanced the $1/T_1$ and $1/T_2$ values with a concentration dependent manner (Figure 5.12 (c) and (d)). The $r_2$ value was calculated to be 3.03 mM$^{-1}$s$^{-1}$. However, the $r_1$ value was not satisfactory for $T_1$ based MRI contrast enhancement (0.019 mM$^{-1}$s$^{-1}$).
Figure 5.12 (a) $T_1$ based (b) $T_2$ based TD-NMR signal intensity variation with time for MOIO@CF nanosystem having different metal concentrations. (c) $T_1$ based and (d) $T_2$ based relaxation rate plot against metal concentration.

5.3.8 MR Imaging

$T_1$ and $T_2$ weighted *in vitro* phantom images of dispersions of MOIO@CF nanosystem in water acquired through a 3 T MRI scanner have been shown in Figure 5.13 (a) and (b). In each image, the top row is the grey scale view and the bottom row is the colour mapped profile. The metal concentrations in the dispersions were varied from 0.1 mM to 0.5 mM. It is observed that the developed system could not contribute significantly in enhancement of contrast in $T_1$ and $T_2$ based MR images. The poor performance by the MOIO@CF nanosystem in terms of MRI contrast enhancement observed from Figure 5.13 can be attributed to interferences of $T_1$ and $T_2$ effects resulting in quenching of signals [27]. Though relaxivity studies on Mn$_2$O$_3$ is still not reported, contrast enhancement property of Fe$_2$O$_3$ is well established and it is observed that the MOIO@CF nanosystem shows less $r_2$ (Sub-section 5.3.7) compared to reported
Fe$_2$O$_3$ based agents [Theranostics 2013, Vol. 3, Issue 8]. This again verifies the possibility of signal quenching which led to decrease of contrast efficiency of Fe$_2$O$_3$.

**Figure 5.13** (a) $T_1$ weighted and (b) $T_2$ weighted *in vitro* MR images of MOIO@CF nanosystem with different metal concentrations; Top row: Grey scale, Bottom row: Colour mapped

### 5.4 Concluding remarks

This study was an approach to combine $T_1$ and $T_2$ active moieties and to examine the efficiency of the dual system as a $T_1$-$T_2$ dual mode MRI contrast agent. Paramagnetic nature and low toxicity of manganese has already established its compounds as suitable $T_1$ based MRI contrast agents. On the other hand, iron oxide nanoparticles are the most preferred $T_2$ based MRI contrast agents used for clinical purposes due to their appreciable
biocompatibility. Therefore, in this study, we have demonstrated development of a biocompatible nanosystem having Mn$_2$O$_3$ and Fe$_2$O$_3$ nanoparticles embedded within mesoporous carbon frameworks. The carbon encapsulation rendered biocompatibility to the nanosystem while the pores on it ensured accessibility of the nanoparticles to the surrounding water molecules in spite of the encapsulation. Potential of the developed nanosystem for biomedical applications was established by the satisfactory biocompatibility acquired through cell viability studies.

It was observed that the degree of contrast enhancement shown by the developed system during both T$_1$ and T$_2$ based in vitro MRI scans was not adequate enough so as to establish it as a T$_1$-T$_2$ dual mode MRI contrast agent. This was caused by the quenching of signals due to the interactions occurring between the T$_1$ and T$_2$ effects of Mn$_2$O$_3$ and Fe$_2$O$_3$ nanoparticles respectively. This observation illustrates scope of further research in quest of other novel chemically designed structures showing proficient T$_1$-T$_2$ dual contrast enhancement.

References


