Scientific and Technical Progress Report (STPR)

(R & D Projects)

Section A: Project Details

A1. Project Title: **Development of nanomaterial based dual mode contrast agent and their surface mediated conjugation study from first principles**

A2. DBT Sanction Order No. & Date:

BT/357/NE/TBP/2012 dated 21/03/2013

A3. Principal Investigator : Prof. P. Deb, Department of Physics, T.U.

Co-Investigators : Dr. E. Kalita, Department of MBBT, T.U.
: Prof. Abhijit Mookerjee, S N Bose National Centre for Basic Sciences, Kolkata
: Prof. Ranjit Biswas, S N Bose National Centre for Basic Sciences, Kolkata

A4. Institute : TEZPUR UNIVERSITY (CENTRAL UNIVERSITY), TEZPUR

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A6. Total cost : 122.25 lacs

A7. Duration : 2013-16 (Approved extension upto 20th March, 2017)

A8. Approved objectives of the project:

Period of study	Achievable targets
6 Months	❖ Literature survey, Recruitment of manpower and procurement of instrument.
12 Months	❖ Development of T_1 contrast agent using chemical synthesis method and its characterization. ❖ Theoretical and experimental study on surface characteristics and physical properties
18 Months	❖ Development of T_2 contrast agent using chemical synthesis method and its characterization. ❖ Theoretical and experimental study on surface characteristics and physical properties
24 Months	❖ Surface engineering of developed T_1 and T_2 contrast agent and make them biocompatible with suitable hydrophilic coating agent. ❖ Theoretical and experimental study on surface characteristics and physical properties ❖ MRI characterization of T_1 contrast agent which is coated with suitable hydrophilic coating agent. ❖ MRI characterization of T_2 contrast agent which is coated with suitable hydrophilic coating agent.
30 Months	❖ Development of T_1-T_2 monohybrid system ❖ Coating of as prepared monohybrid with suitable hydrophilic agent. ❖ Theoretical and experimental study on surface characteristics and physical properties
36 Months	❖ Study of MRI property of T_1-T_2 monohybrid (in *vitro* and *in vivo*).

DBT has granted sanctions for our project proposal entitled "**Development of nanomaterial based dual mode contrast agent and their surface mediated conjugation study from first principles**" vide letter no. BCIL/NER-BPMC/2013/367 dated 08/04/2013 through sanction (Sanction No. BT/357/NE/TBP/2012 Dated 21/03/2013) for the implementation of the project.

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1. Achieved objectives

(i) Development of library of magnetic nanosystems for both single and multimodal magnetic resonance imaging applications.
(ii) Eleven papers published in international peer reviewed journals
(iii) One patent application filed
(iv) Seventeen conference presentations
(v) One PhD completed, one about to complete

Development of Process/product/technology attempted in this project-

- Development of smart nanosystems having multimodal imaging efficiency is a unique outcome of the project

2. Results and Discussion

Work done in Tezpur University

2.1 Manganese oxide Nanoparticles Encapsulated with Mesoporous Carbon Shell as a Novel Biocompatible T_1 MRI Contrast Probe

Abstract:

Multivalent manganese oxide nanoparticles encapsulated in mesoporous 3D carbon framework (CF) were explored as T_1 MRI contrast agents. This manganese oxide@CF nanosystem was synthesized with controlled microstructure and morphology. Various characterizations (microstructure, surface morphology, composition, stability etc.) were performed to ensure high quality product. Cell viability studies established the applicability of the nanosystem for biomedical purpose. Distinct enhancement of relaxivity and MRI contrast were observed by using this engineered contrast agent.

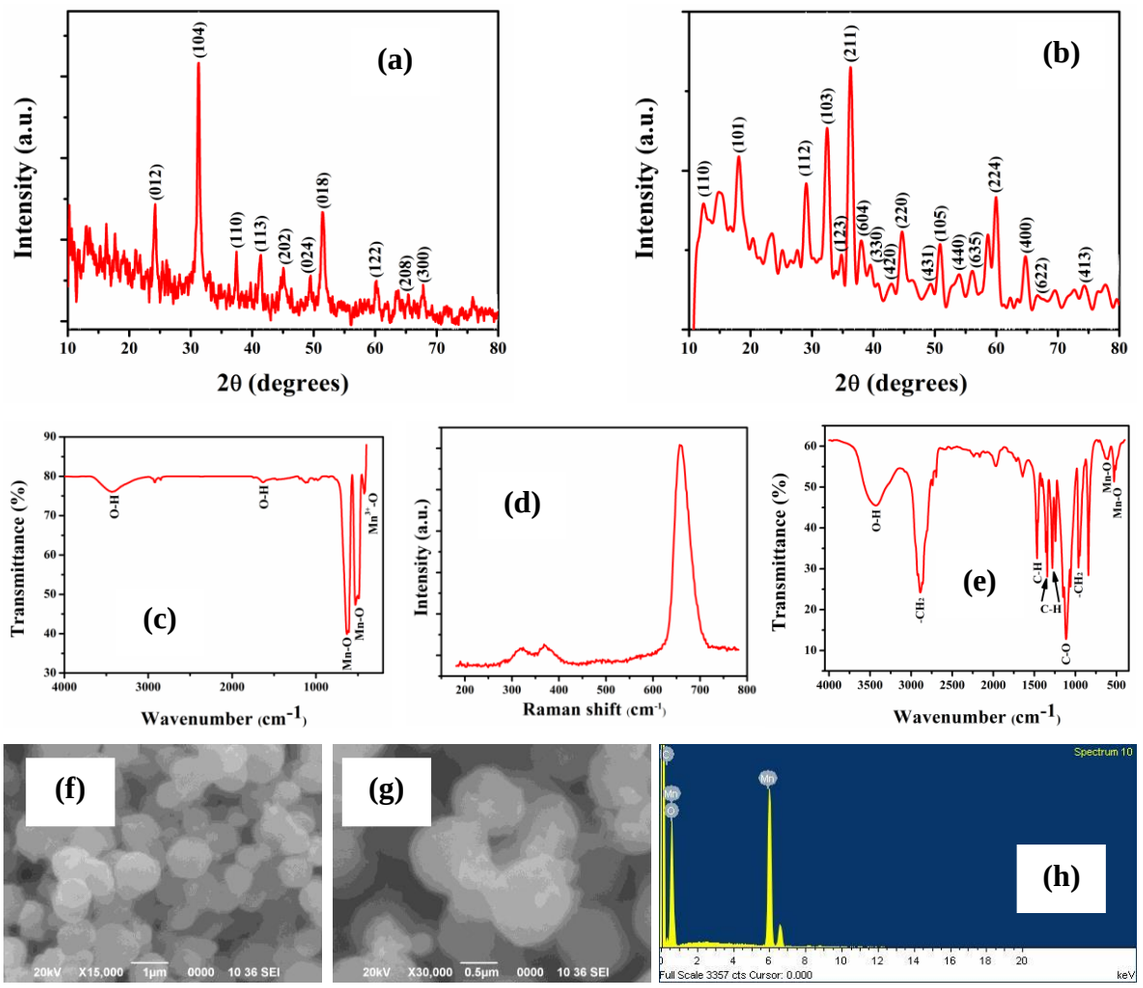


Figure 1 (a) and **(b)** XRD of $MnCO_3$@PVP precursor and Manganese oxide@CF nanosystem **(c)** FTIR of manganese oxide@CF nanosystem **(d)** Raman spectrum of mesoporous 3D carbon framework encapsulated manganese oxide nanoparticles (e) FTIR of PEG functionalized manganese oxide@CF nanosystem **(f)** and **(g)** SEM **(h)** EDS of manganese oxide@CF nanosystem

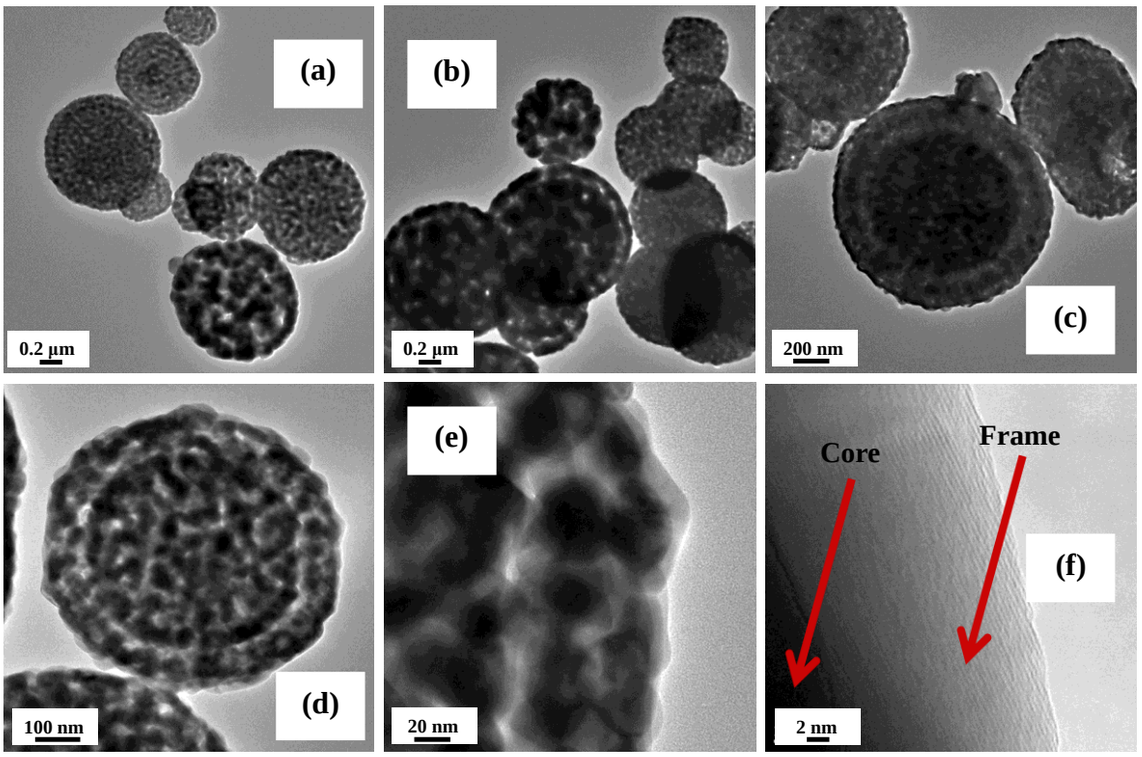


Figure 2 (a) - (f) TEM images of manganese oxide@CF nanosystem at0.2 µm, 0.2 µm, 200 nm, 100 nm, 20 nm and 2 nm resolutions

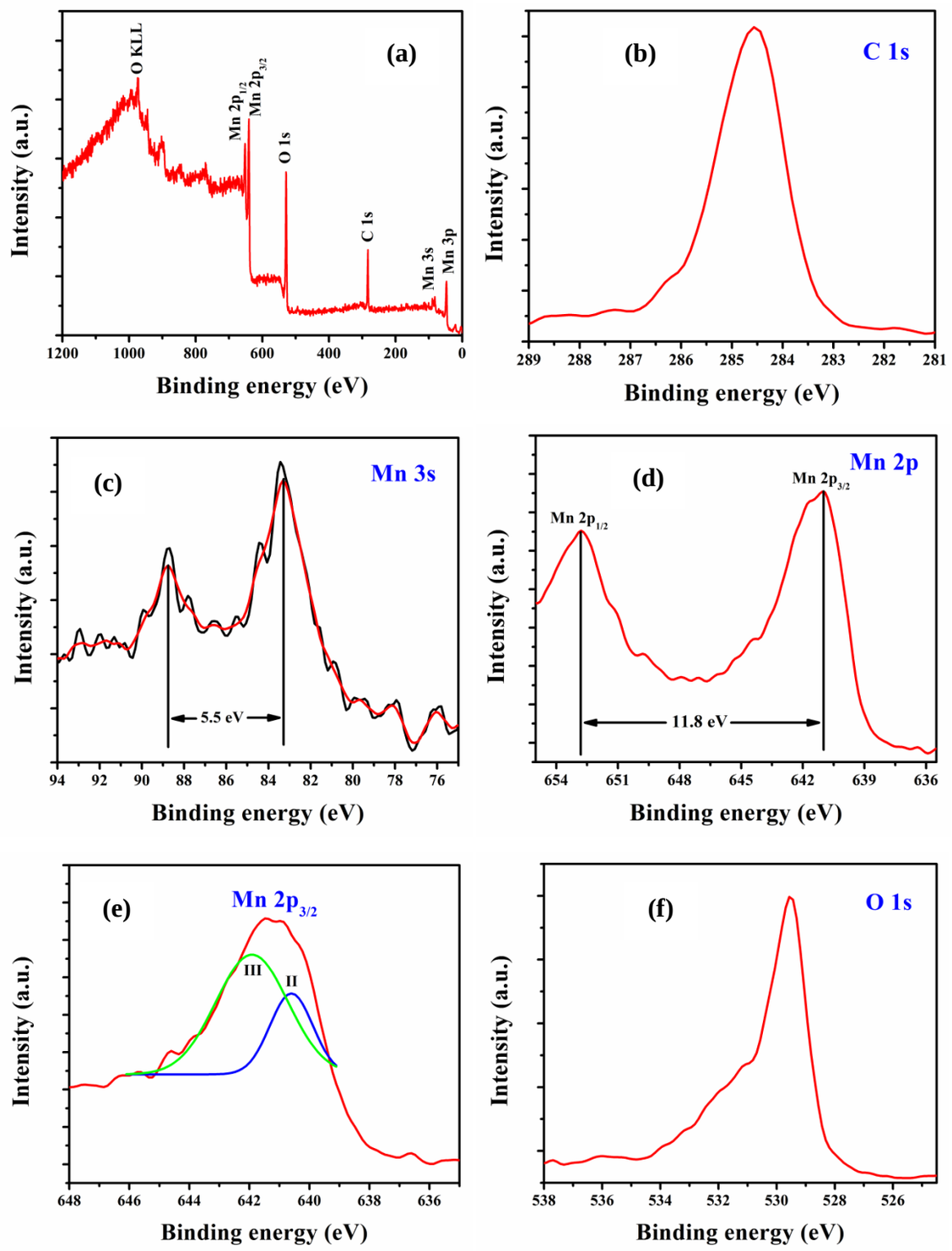


Figure 3 XPS spectra of mesoporous 3D carbon framework encapsulated manganese oxide nanoparticles

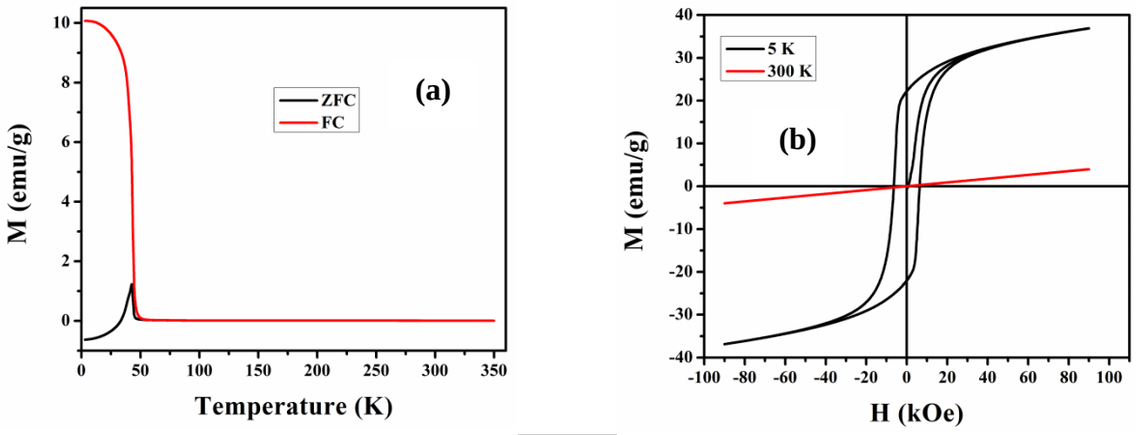


Figure 4 (a) Zero field cooled (ZFC) and field cooled (FC) magnetization curves of manganese oxide@CF nanosystem **(b)** Hysteresis curves of manganese oxide@CF nanosystem

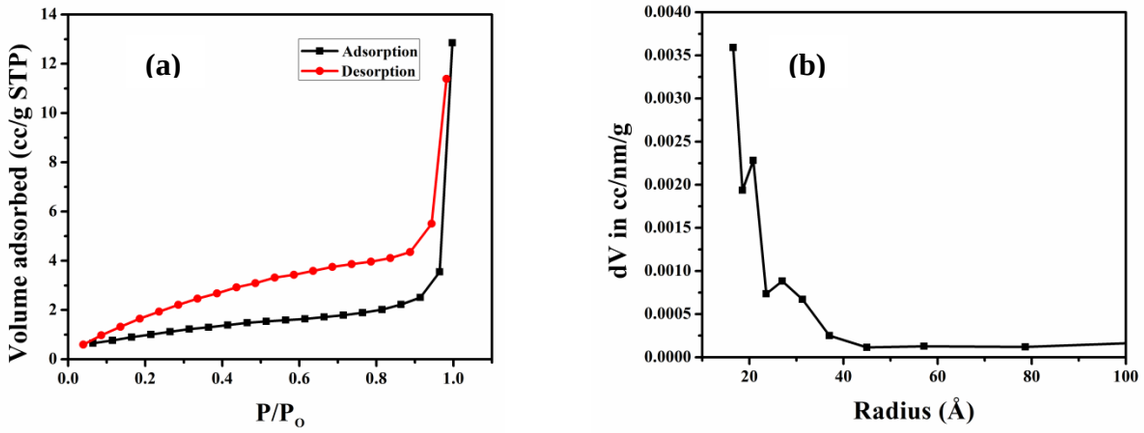


Figure 5 (a) N_2 adsorption-desorption isotherms and **(b)** BJH pore distribution profile of mesoporous 3D carbon framework encapsulated manganese oxide nanoparticles

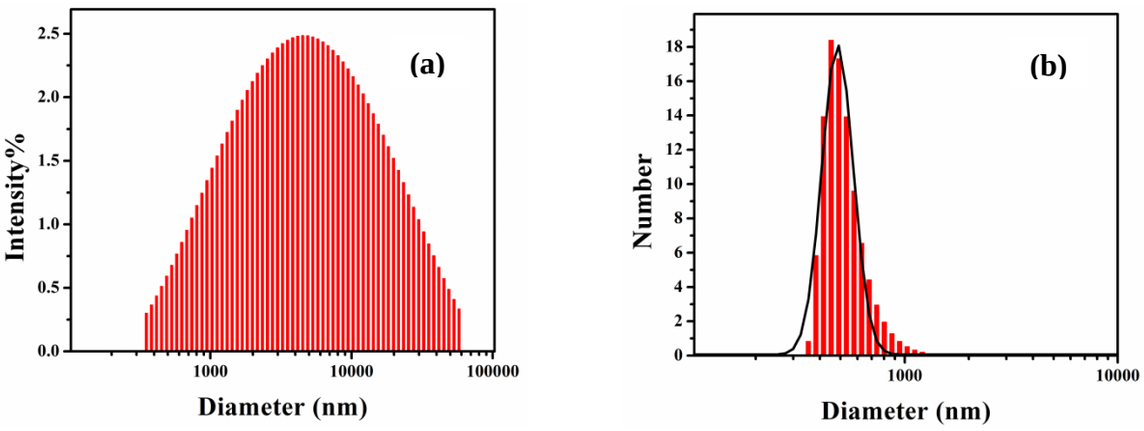


Figure 6 (a) and (b) Intensity and number distribution of particle diameter for PEG functionalized manganese oxide@CF nanosystem

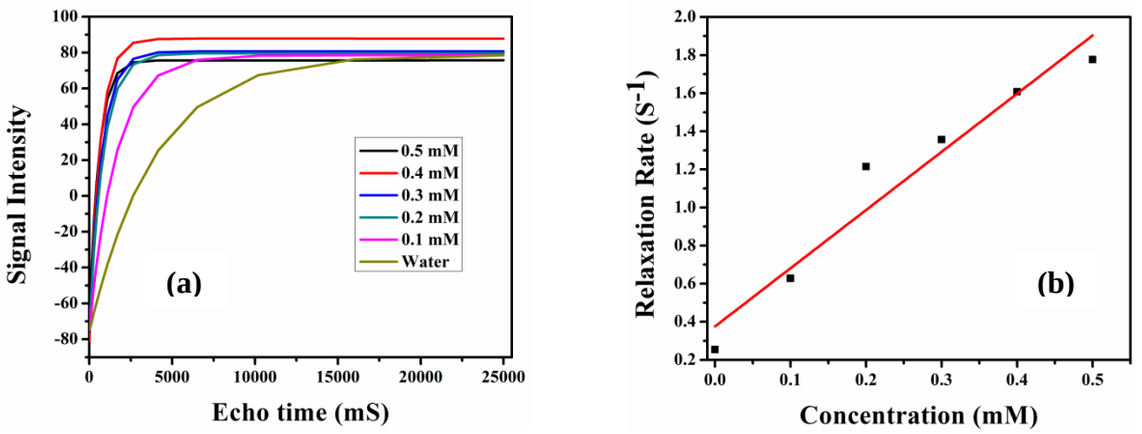


Figure 7 (a) Variation of TD-NMR signal intensity with echo time for PEG functionalized manganese oxide@CF nanosystem having different Mn concentrations **(b)** Plot of Relaxation rate against Mn concentration (The slope provides the relaxivity value)

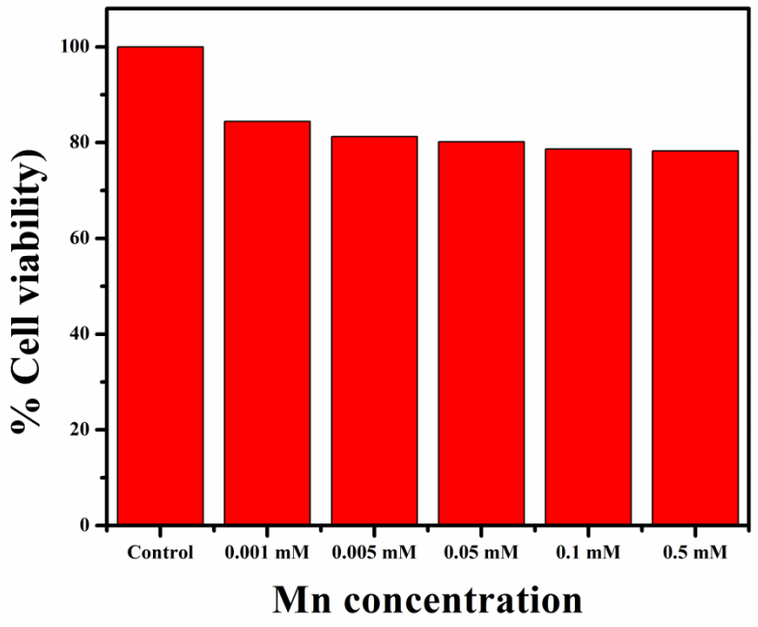


Figure 8 Cell viability profile of RAW 264.7 cell line incubated with PEG functionalized manganese oxide@CF nanosystem of different Mn concentrations

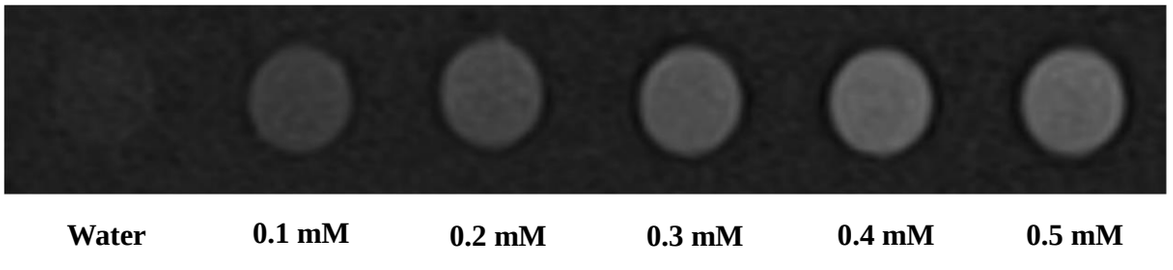


Figure 9 T_1-weighted *in vitro* MR images of aqueous dispersions of PEG functionalized manganese oxide@CF nanosystem with different Mn concentrations

Conclusion: In this study, we have demonstrated a biocompatible approach of employing multivalent manganese oxide nanoparticles as MRI contrast agent. Mesoporous 3D carbon framework encapsulated multivalent manganese oxide nanoparticles were synthesized and characterized for this purpose. We obtained significant enhancement in T_1-weighted relaxivity by using this nanosystem. The carbon framework provides biocompatibility by inhibiting metal ion exposure inside the body and simultaneously enables water exchange through its pores which leads to T_1 contrast improvement.

2.2 Biocompatible Cobalt Based Nanoparticles as Novel MRI Contrast Agent

Abstract:

Water-dispersible PEG stabilised Cobalt oxide (Co_3O_4) nanoparticles were synthesized with controlled microstructure and morphology. Various characterizations (microstructure, surface morphology, composition etc.) have been performed to ensure high quality product through this synthetic strategy. Cell viability studies establish the applicability of these nanoparticles for biomedical purpose. Distinct enhancement of T_2 based relaxivity was observed by using this engineered contrast agent using TD-NMR relaxometer.

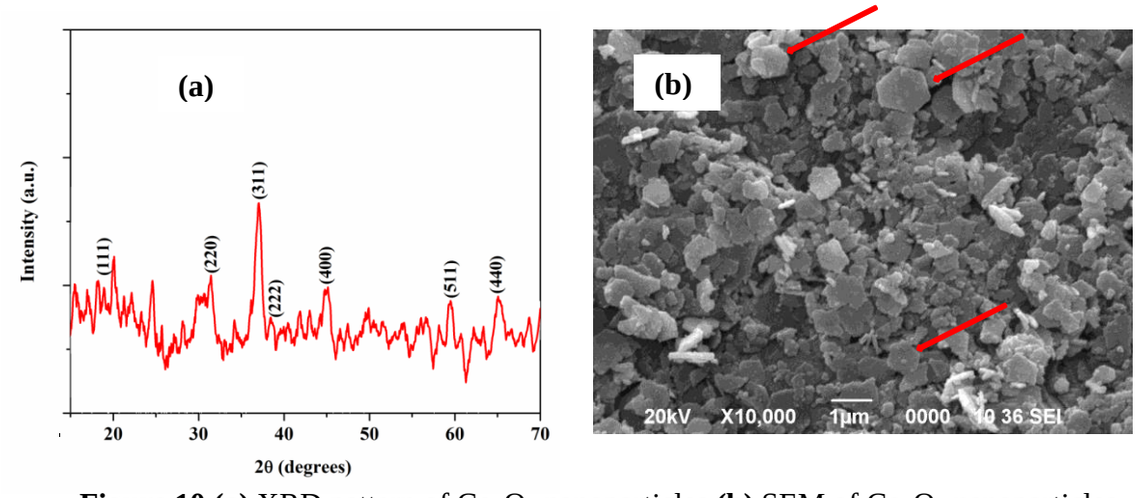


Figure 10 (a) XRD pattern of Co_3O_4 nanoparticles **(b)** SEM of Co_3O_4 nanoparticles

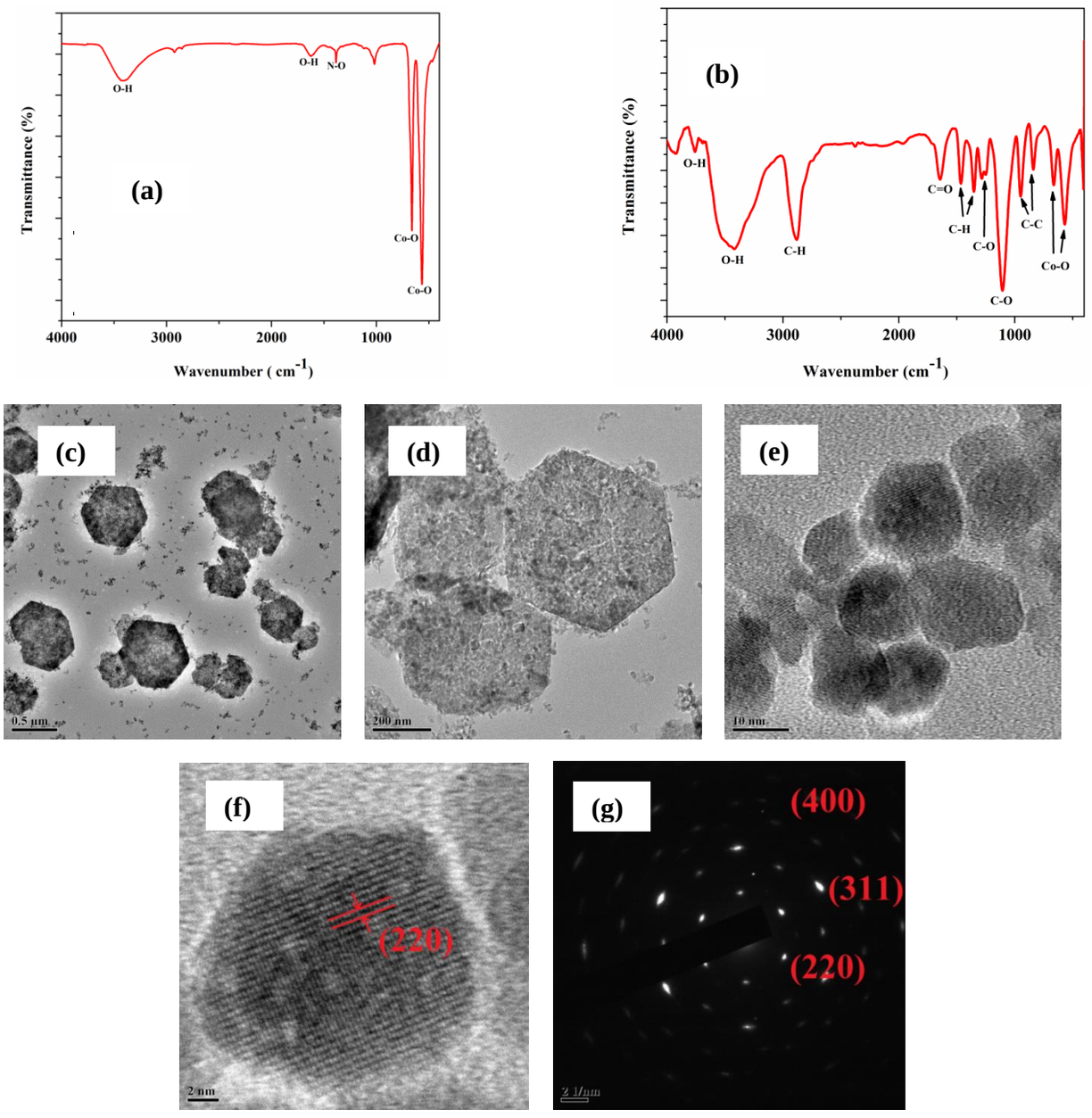


Figure 11 (a) and **(b)** FTIR spectra of Co_3O_4 nanoparticles and PEG stabilized Co_3O_4 nanoparticles **(c) – (f)** TEM images of Co_3O_4 nanoparticles at 0.5µm, 200 nm, 10 nm and 2 nm resolutions **(g)** SAED of Co_3O_4 nanoparticles

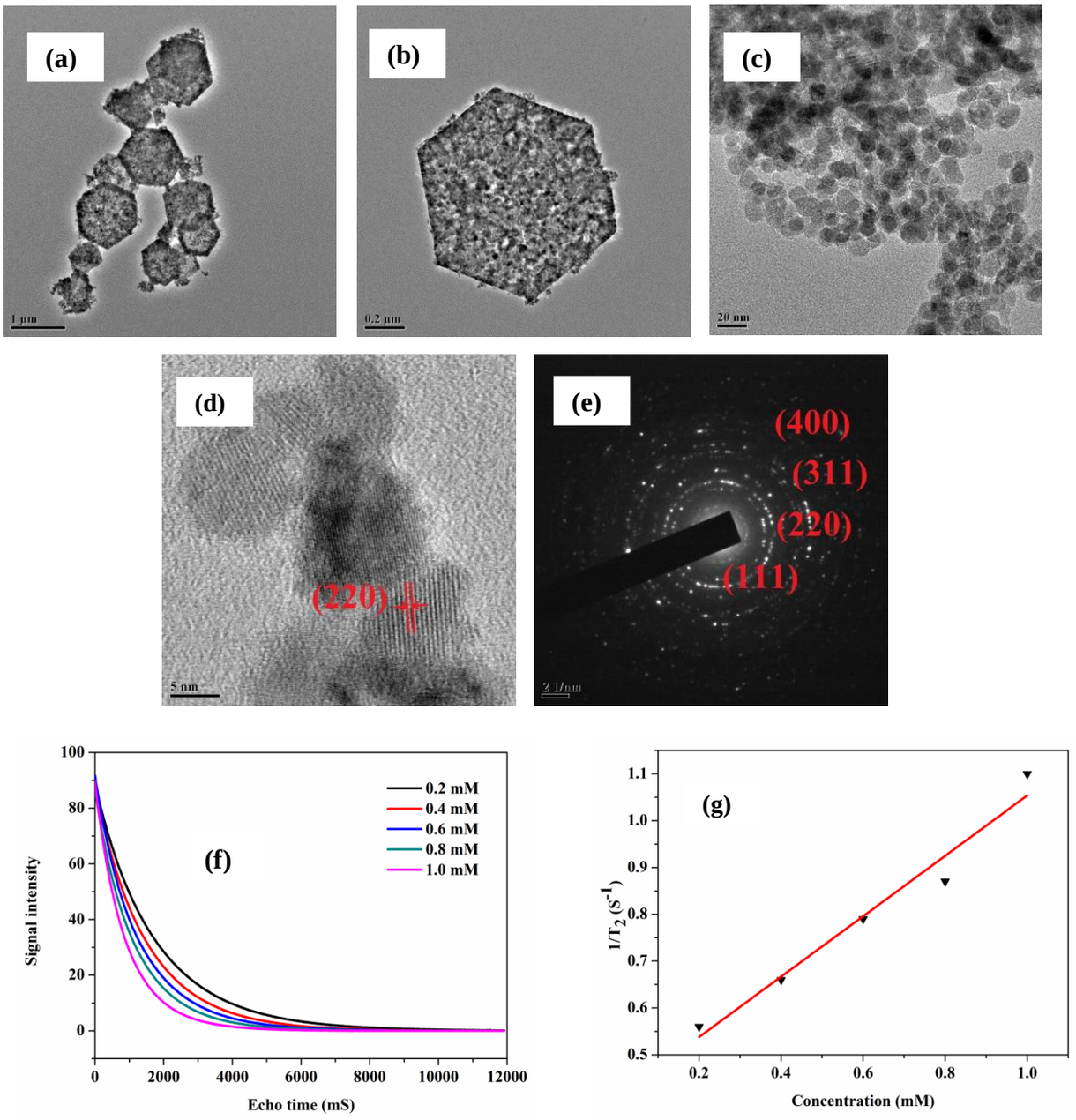


Figure 12 (a) - (d) TEM images of Co_3O_4 nanoparticles at 1µm, 0.2 µm, 20 nm and 5 nm resolutions **(e)** SAED of PEG stabilized Co_3O_4 nanoparticles **(f)** Variation of TD-NMR signal intensity with echo time at different concentration of Co **(g)** Plot of T1 Relaxation rate against Co concentration. The slope provides the relaxivity value

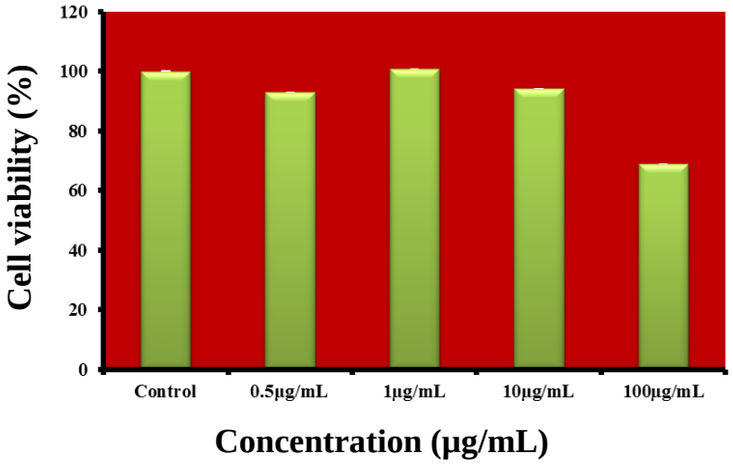


Figure 13 Cell viability profile of L6 rat skeletal muscle cells incubated with different concentrations of PEG stabilized Co_3O_4 nanoparticles

Conclusion: PEG stabilized Co_3O_4 nanoparticles were developed and characterized so as to be used as weight efficient T_2 MRI contrast agents. The nanoparticles enabled in increase in relaxation rates during TD-NMR experiments. Cell viability studies establish the applicability of these nanoparticles for biomedical purpose. In future, comparative study of magnetic property of Co_3O_4 nanoparticles and PEG stabilized Co_3O_4 nanoparticles will be performed. Further studies include DLS measurements to determine hydrodynamic size of the nanoparticles, which is very important for *in vivo* applications. Finally, T_2 weighted MRI experiments will be carried out (both *in vitro* and *in vivo*) to investigate the efficiency of the synthesized nanoparticles as MRI contrast agents.

2.3 Mesoporous 3D Carbon Framework Encapsulated Janus Nanoparticles as Novel Biocompatible Dual MR Imaging Probe

Abstract:

Nanodimensional Janus system of $MnFe_2O_4$@MnO encapsulated in mesoporous 3D carbon framework was synthesized with controlled microstructure and morphology. Various characterizations were performed to ensure high quality product. Distinct enhancement of relaxivity was observed by using this engineered contrast agent.

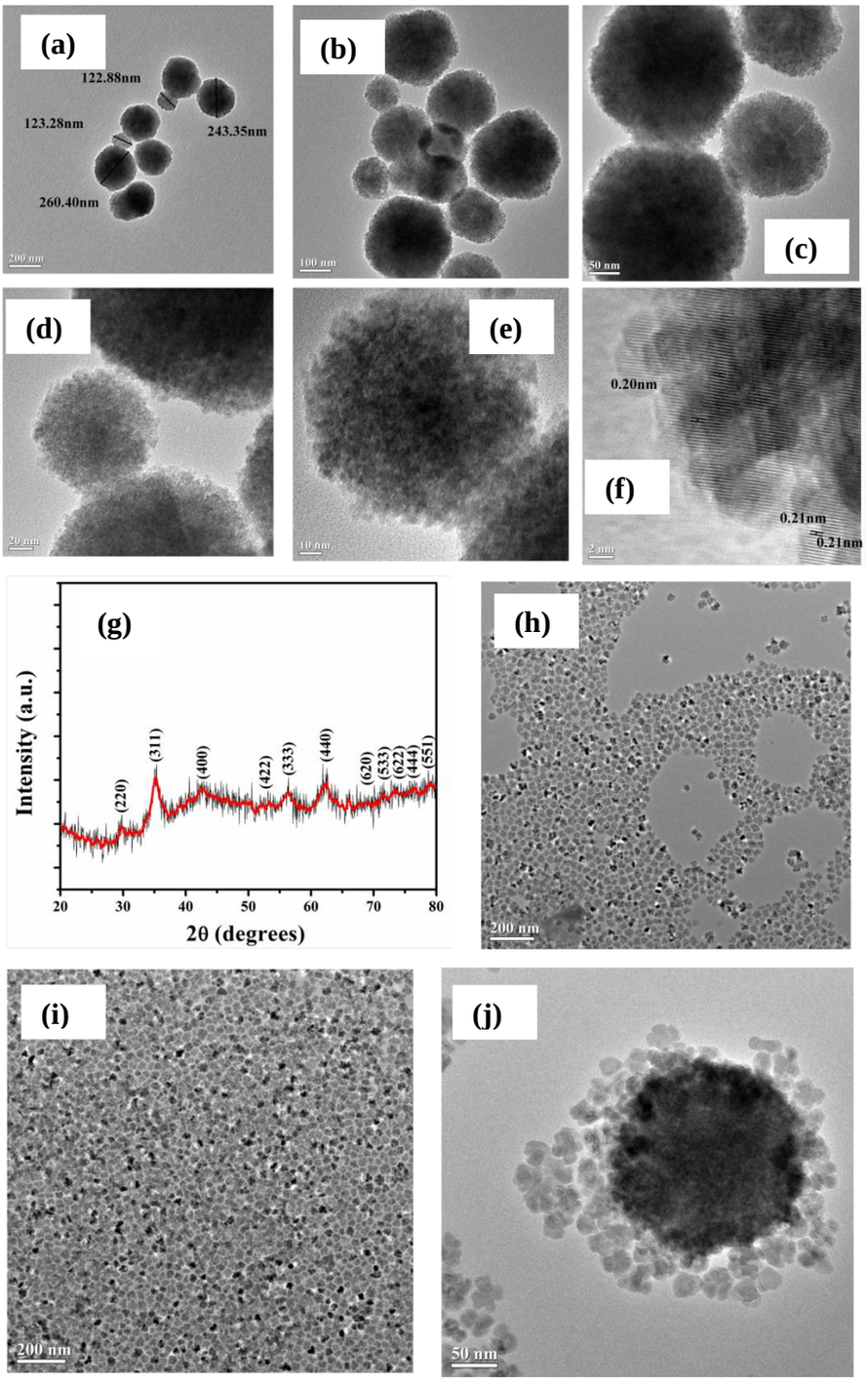


Figure 14 (a)-(f) TEM of $MnFe_2O_4$ **(g)** XRD of $MnFe_2O_4$ **(h)-(j)** TEM of $MnFe_2O_4$@MnO nanoparticles

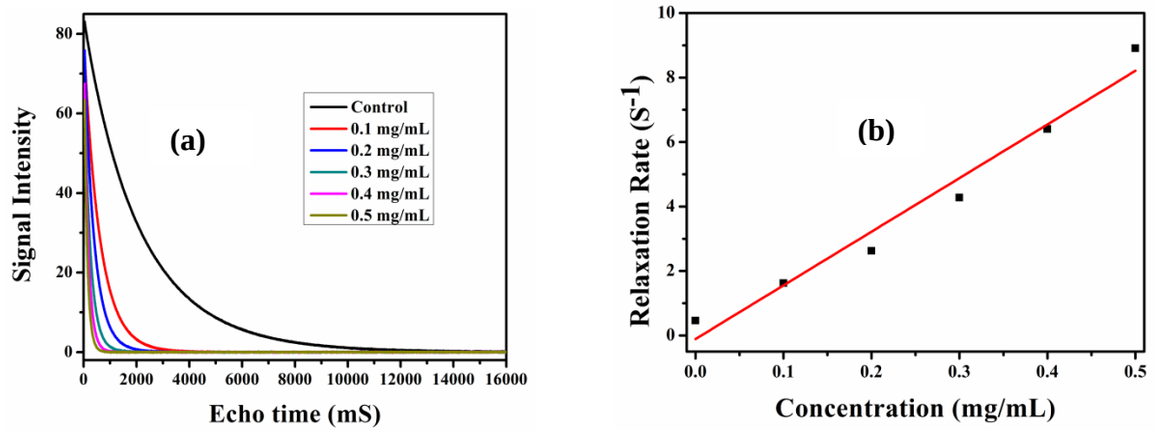


Figure 15 (a) Variation of TD-NMR signal intensity with echo time at different concentrations of manganese oxide@CF nanosystem **(b)** Plot of T_2 Relaxation rate against manganese oxide@CF nanosystem concentration (The slope providess the relaxivity value)

Conclusion: Synthesis of $MnFe_2O_4$@MnO nanoparticles was performed. T_1 based TD-NMR study has also been performed. Crystal structure characterization, hydrodynamic size determination, TD-NMR study (T_1 based), biocompatibility and MRI studies (both *in vivo* and *in vitro*) have to be performed to establish the work.

2.4 Mesoporous 3D Carbon Framework Encapsulated iron oxide and manganese oxide nanoparticles as Novel Biocompatible Dual MR Imaging Probe

Abstract:

Nanoparticles of manganese oxide and iron oxide encapsulated in mesoporous 3D carbon framework were synthesized with controlled microstructure and morphology. XRD, FTIR and SEM characterizations were performed to ensure successful synthesis.

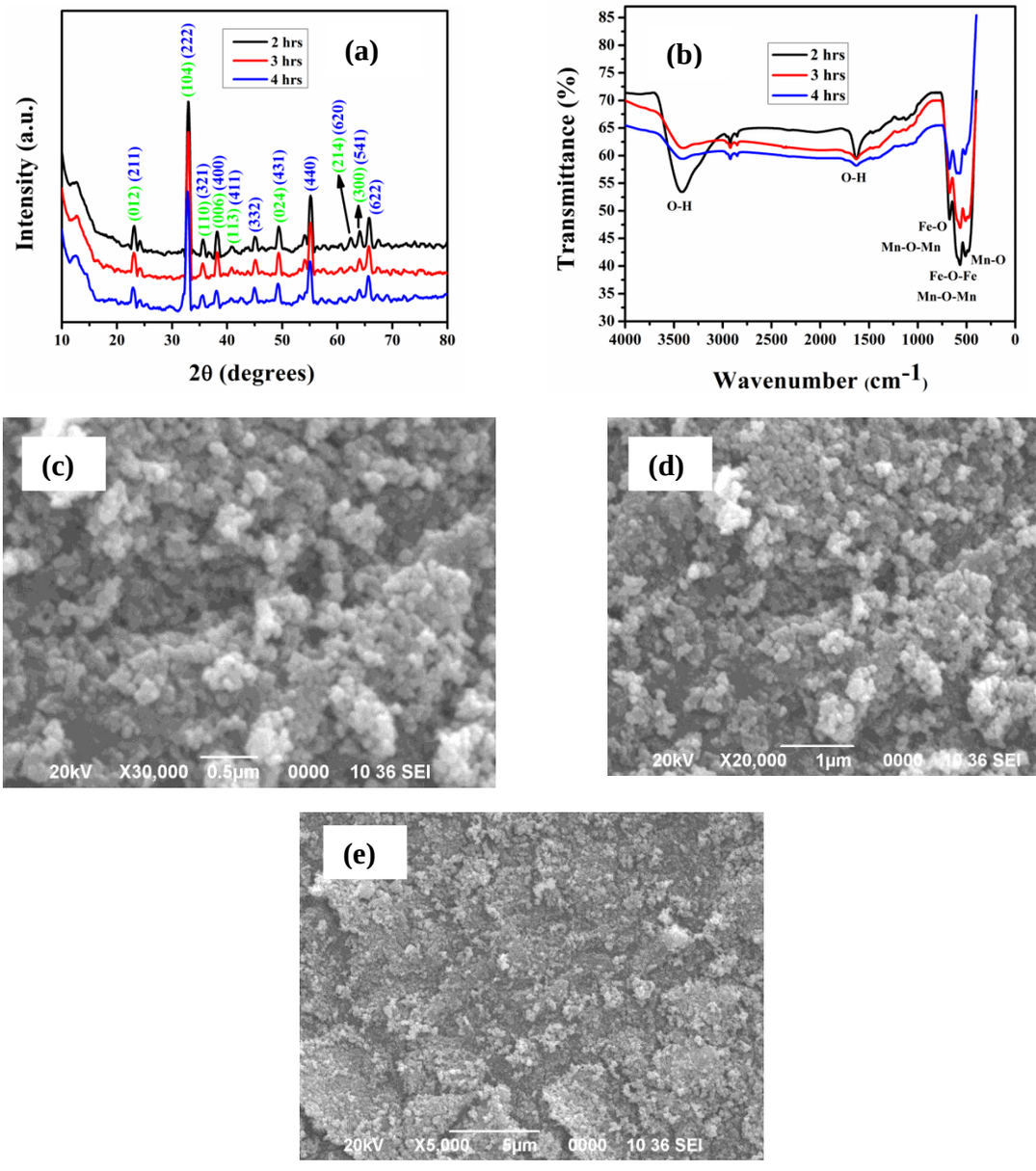


Figure 16 (a) XRD **(b)** FTIR **(c) – (e)** SEM of Mesoporous 3D Carbon Framework Encapsulated iron oxide and manganese oxide nanoparticles

Conclusion: Synthesis of Mesoporous 3D Carbon Framework Encapsulated iron oxide and manganese oxide nanoparticles was performed. XRD, FTIR and SEM characterization has also been performed. Other experiments to see microstructure, composition, stability, biocompatibility, relaxivity and MRI contrast enhancement has to be performed.

2.5 Direct synthesis of water dispersible superparamagnetic TGA capped FePt nanoparticles as a T_2 Contrast Agent

Abstract:

Thioglycolic acid (TGA) capped hydrophilic fcc-FePt magnetic nanoparticles (MNPs) were directly synthesized by a facile one pot polyol method. Thioglycolic acid (TGA) was used to functionalize the nanoparticles by incorporating thiol group onto the surface. It helped in the preparation of highly stable dispersions of nanoparticles with spherical morphology. A possible formation mechanism for these FePt MNPs, depending on the role of TGA, was proposed. The as-prepared FePt MNPs possessed a face centered cubic structure with an average size of 6±1 nm and superparamagnetic property at room temperature. MRI study showed that these MNPs exhibited a transverse relaxivity of ~ 600 mg^{-1} ml s^{-1}, superior to that of reported iron oxide nanoparticles.

Table 1

Variation of precursor in synthesis processes A, B, & C

Si. No	Process	Fe Source [$Fe(acac)_3$]	Pt Source [$Pt(acac)_2$]	Reducing Agent [$NaBH_4$]	Capping Agent [TGA]	TGA/Fe [Ratio]	Product
1	Process-A				2.75 mmol	1.37	FePt-A
2	Process-B	2 mmol	1 mmol	4 mmol	4.13 mmol	2.06	FePt-B
3	Process-C				5.51 mmol	2.75	- - - -

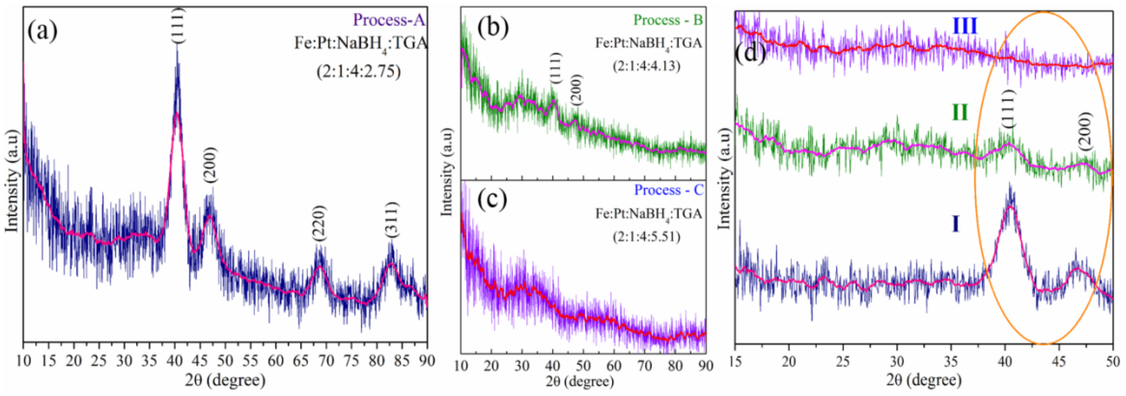


Figure 17 XRD patterns of as prepared samples obtained from (a) process-A (b) process-B (c) process-C. The magnified patterns (2θ = 15 to 50 degree) of the samples, process A (I), B (II), and C (III), are shown in (d)

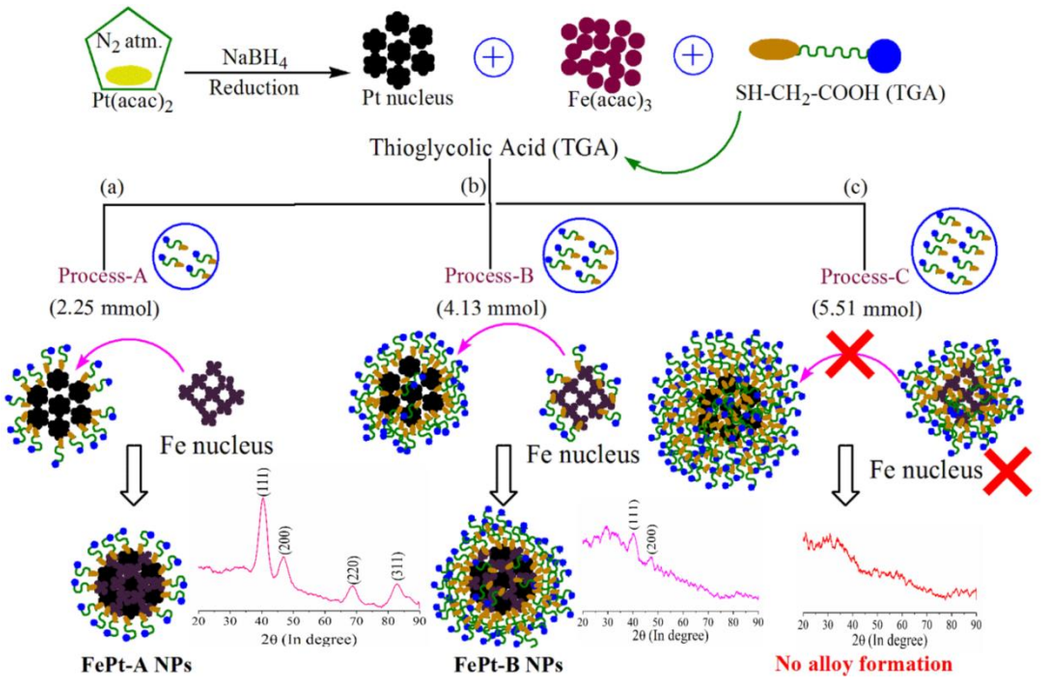


Figure 18 Schematic mechanistic diagram representing the effect of capping agent (TGA) on the synthesis of FePt MNPs

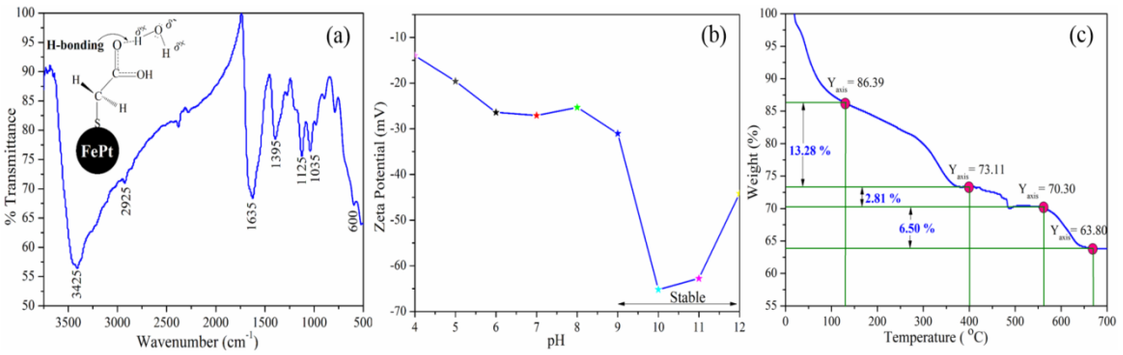


Figure 19 (a) FTIR spectrum (Inset model shows the arrangement of TGA molecule on FePt-A MNPs) (b) zeta potential and (c) thermogravimetric (TG) analysis of as prepared FePt-A MNPs

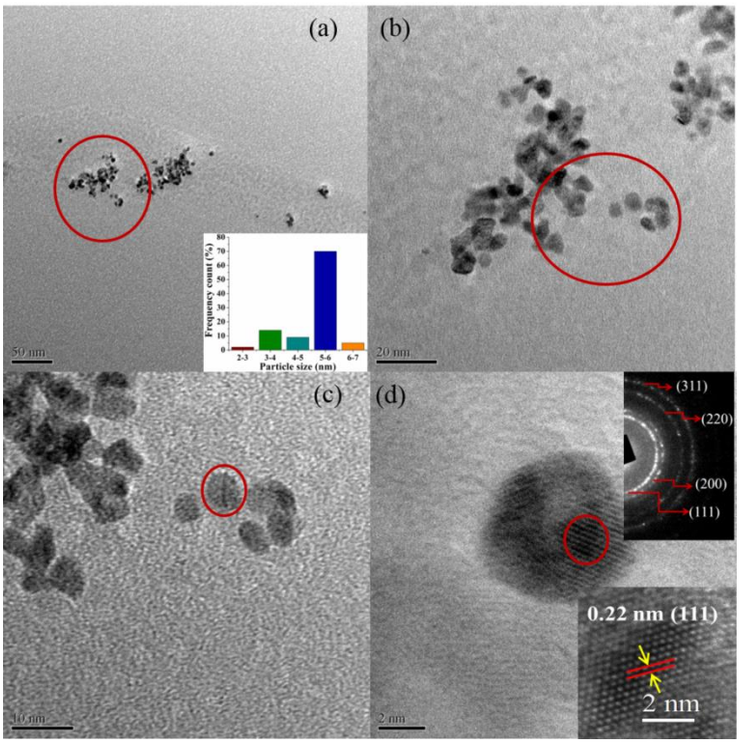


Figure 20 TEM image of FePt-A MNPs. The red circle marked image part is magnified in subsequent figures (a) FePt MNPs, inset shows the particle-size distribution histogram, after counting 500 individual particles, (b) and (c) magnified part of previous image (a) and (b) respectively, (d) the single particle. The upper and lower inset of (d) indicate selected area electron diffraction (SAED) pattern and lattice plane of FePt nanocrystals

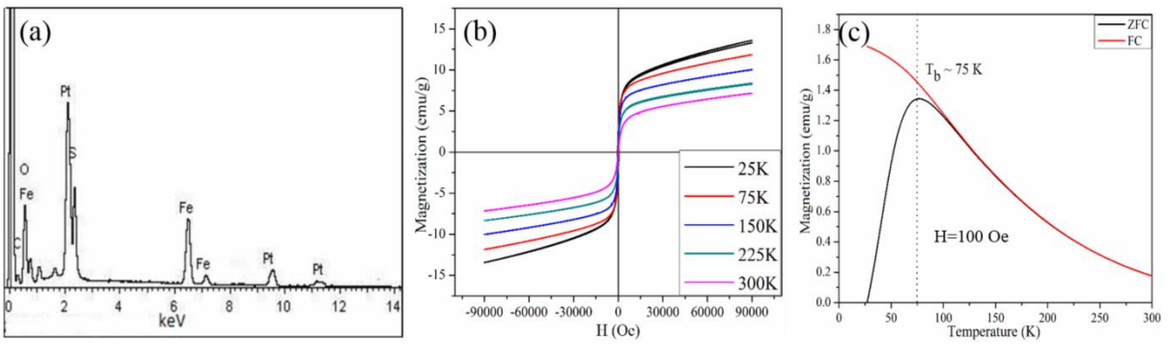


Figure 21 The compositional and magnetization characterization of FePt-A MNPs (a) EDX spectrum (b) M-H curves at different temperatures of as prepared FePt-A MNPs. (c) magnetization versus temperature curve measured under ZFC and FC condition

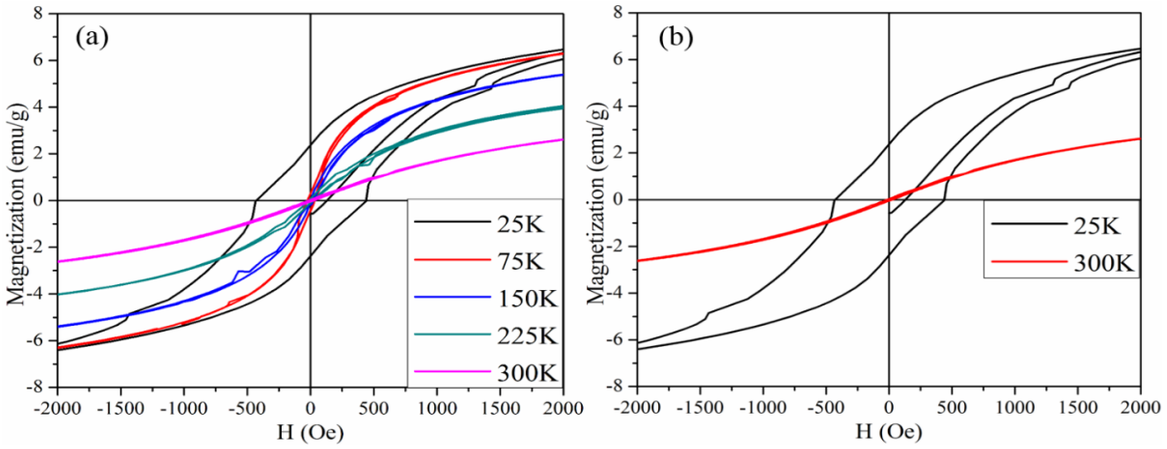


Figure 22 The field dependent magnetization (M-H) of FePt-A nanoparticles (magnified view) at (a) different temperatures (b) 25 K and 300K

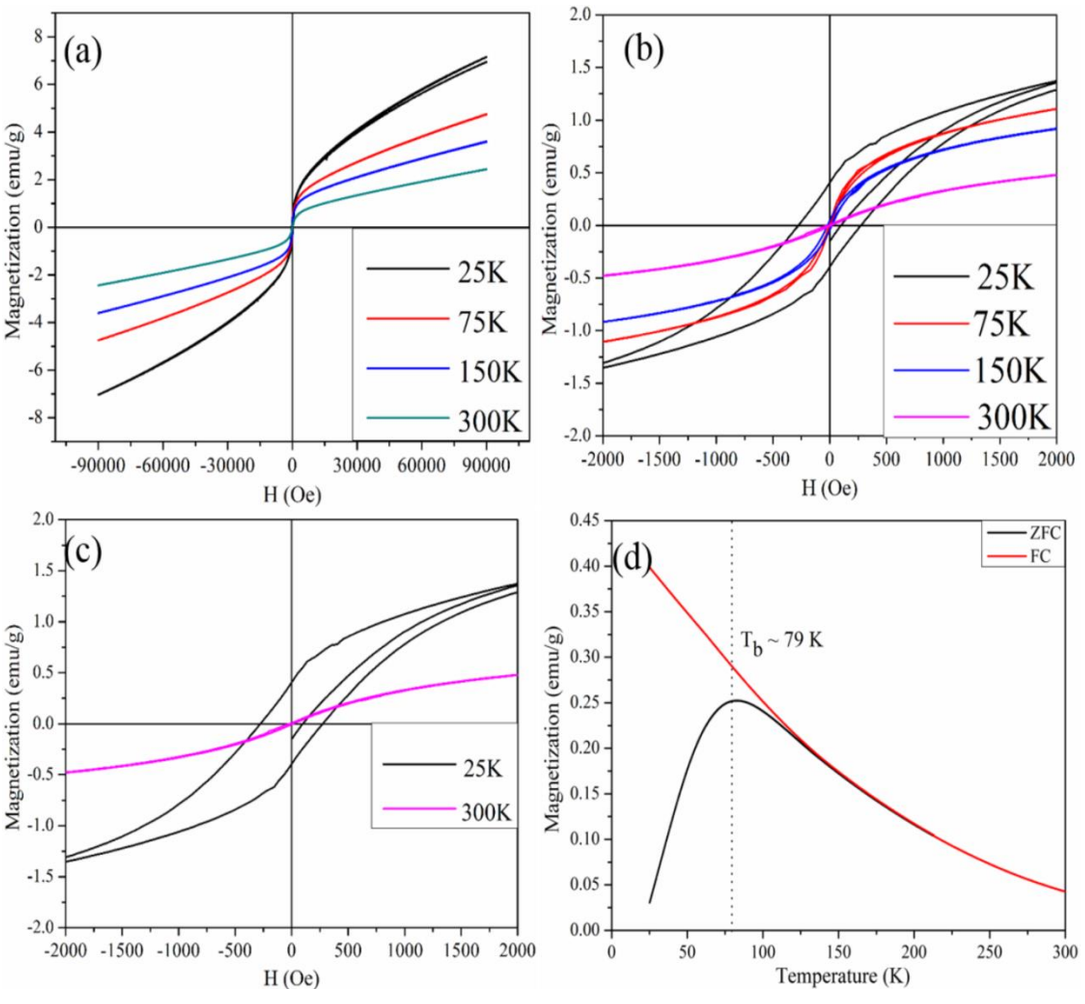


Figure 23 The magnetization of FePt-B nanoparticles (a) M-H curves at different temperature (b) magnified view of M-H (c) magnified view (M-H) at 25 K and 300K and (d) magnetization versus temperature (M-T) curve measured under ZFC and FC condition

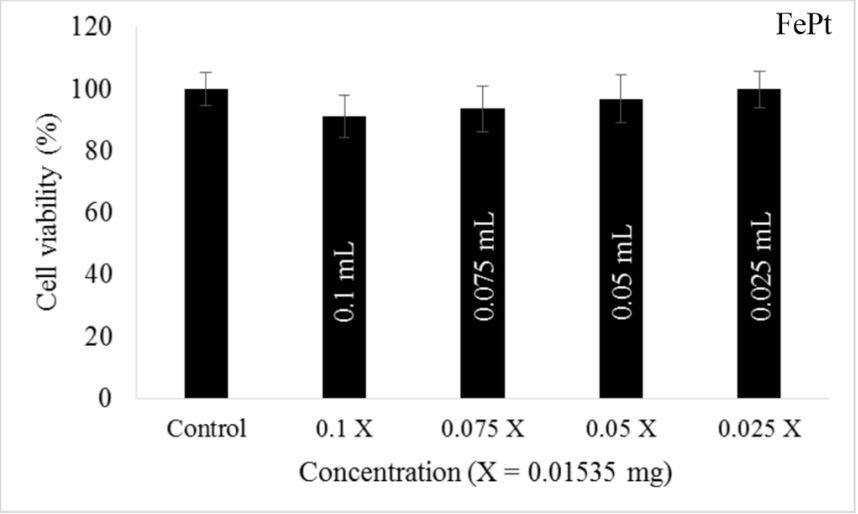


Figure 24 Viability of RAW 264.7 macrophage cell line compared to control indicated by AB assay when the cells were exposed to TGA capped FePt-A MNPs

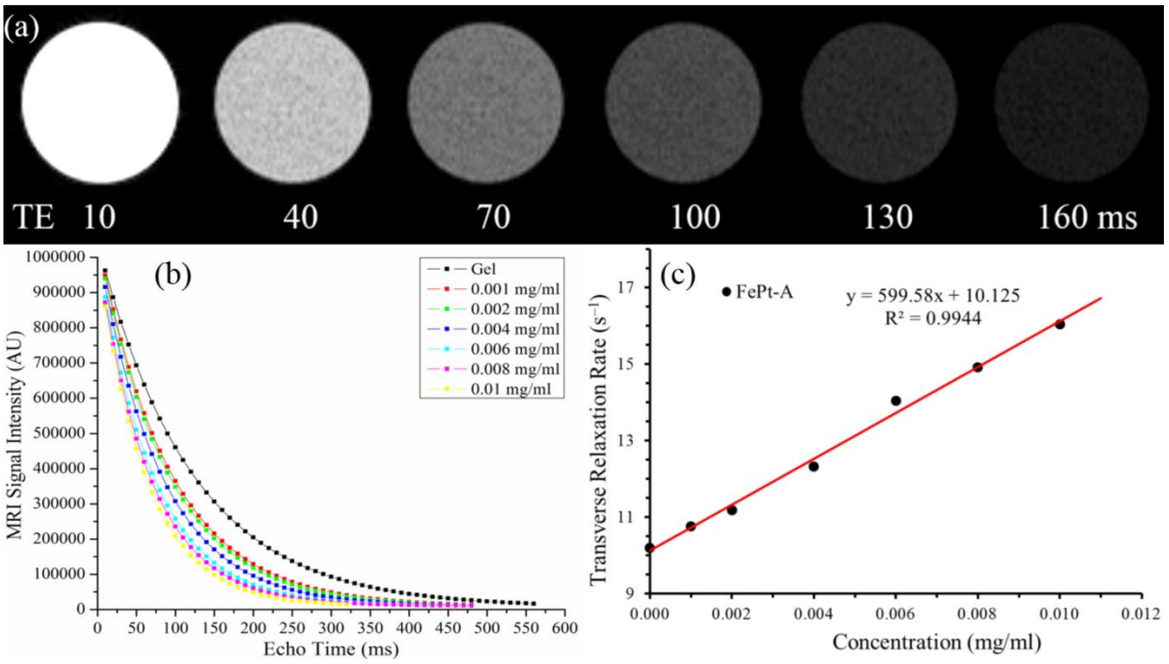


Figure 25 Transverse relaxivity characterization (a) typical MRI images of microfuge tube containing FePt (0.01 mg/ml in 0.5% agarose gel), (b) estimation of transverse relaxation time (T_2) of water, (c) transverse relaxivity of FePt-A in 0.5% agarose gel

Conclusion: Superparamagnetic hydrophilic FePt MNPs with spherical morphology were synthesized via a facile one pot method, in which TGA was used as reducing agent, capping agent and stabilizer simultaneously. This direct synthesis procedure offers several advantageous feature like cost effectivity due to reduced number of steps, better aqueous dispersion with high stability and scope for large scale synthesis in a single step. The as prepared FePt-A nanoparticles was characterized to evaluate microstructure, functionality and magnetic

properties. Moreover this MNP system exhibited notable MRI transverse relaxivity 600 mg^{-1} ml s^{-1}. The development of such a simple and more economical method for hydrophilic FePt MNPs, with high quality and high performance will enhance the scope for their biomedical applications.

2.6 Development and characterization of MnO nanosystems as a MRI T_1 contrast agent

Abstract:

Manganese Oxide (MnO) nanoparticles (NPs) has attracted attention in recent time in the field of biomedical imaging due to their interesting physical properties. The formation of the single phase without any impurity (like Mn_2O_3, Mn_3O_4) is one of the important challenges in the development of MnO nanosystem. Manganese oxide nanoparticles have been prepared by a thermal decomposition method using manganese precursor in an organic solvent under open environment. In the three different synthesis routes, oleic acid, oleic acid (OA) and oleyamine (OAm), and octadecyl phosphonic acid (ODPA) respectively acts as surfactant. All the synthesis processes have resulted in hydrophobic fcc-MnO nanoparticles. These as-prepared hydrophobic MnO particles have been characterized using different techniques, revealing its microstructural, surface and magnetic properties. The as-prepared MnO NPs are hydrophobic and paramagnetic in nature. For biomedical applications, the hydrophilicity of the nanosystem is an important requirement. The experimental research is ongoing for their surface modification and hybrid formation. This MnO nanoparticle is expected to be possessing potential T_1 contrast property and will be suitable for application in clinical magnetic resonance imaging (MRI).

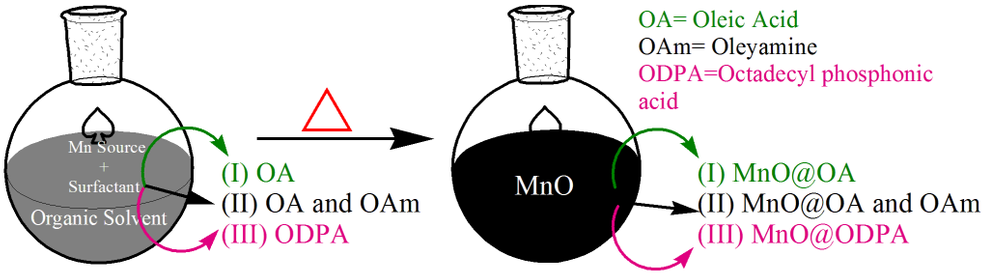
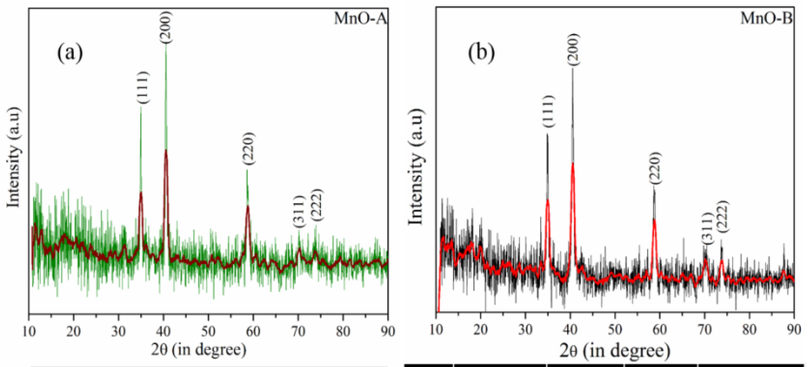


Figure 26 Generalized procedure of MnO NPs synthesis using different surfactant



Sl. No	Sample Name	(hkl)	2Θ	Phase
1	MnO-A	(111)	34.9	Face centered cubic (fcc)
		(200)	40.5	
2	MnO-B	(220)	58.6	
		(311)	70.1	
3	MnO-C	(222)	73.7	

Figure 27 XRD patterns of as prepared MnO-A, MnO-B, and MnO-C obtained from different routes (a-c). Table represent (d) the (hkl), 2θ value and phase of MnO nanosystem

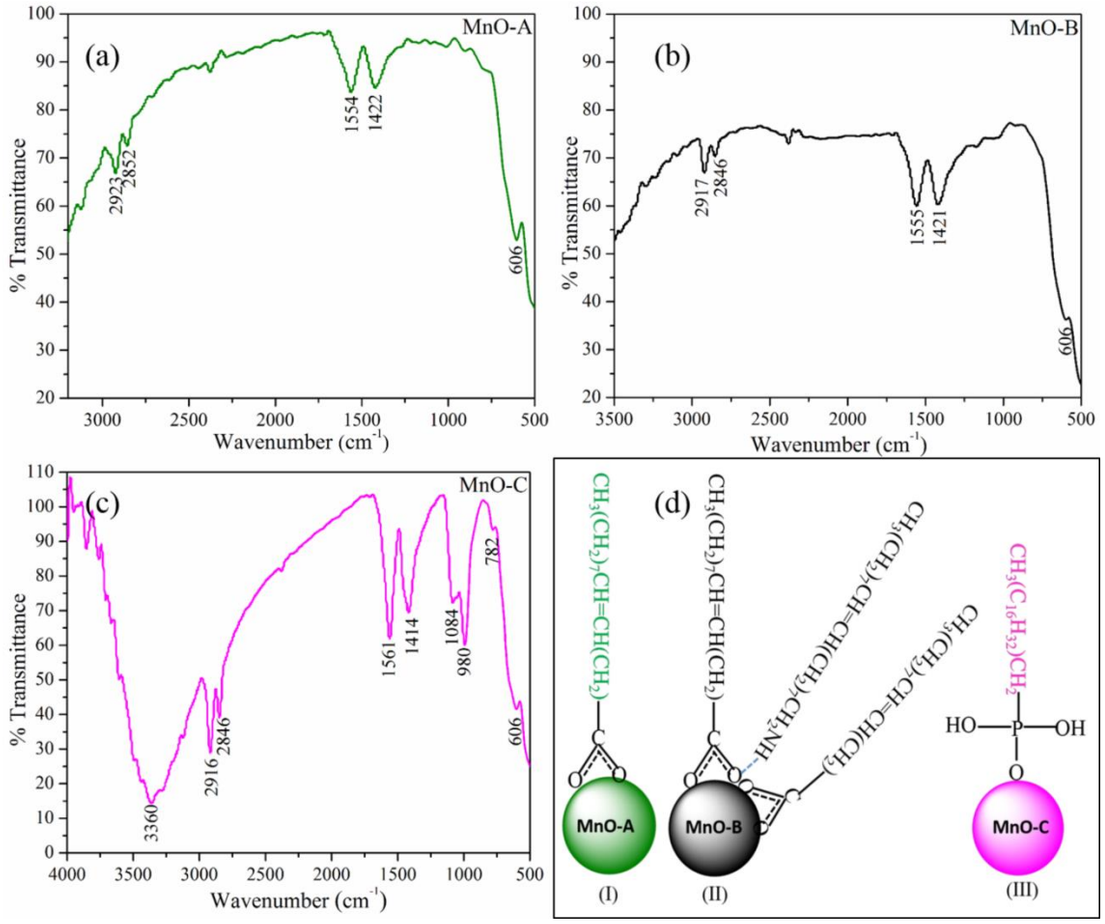


Figure 28 FTIR spectra of (a) MnO-A, (b) MnO-B, (c) MnO-C, and (d) structure of oleic acid (OA) and oleyamine (OAm), and octadecyl phosphonic acid (ODPA) represented by (I), (II), and (III) respectively

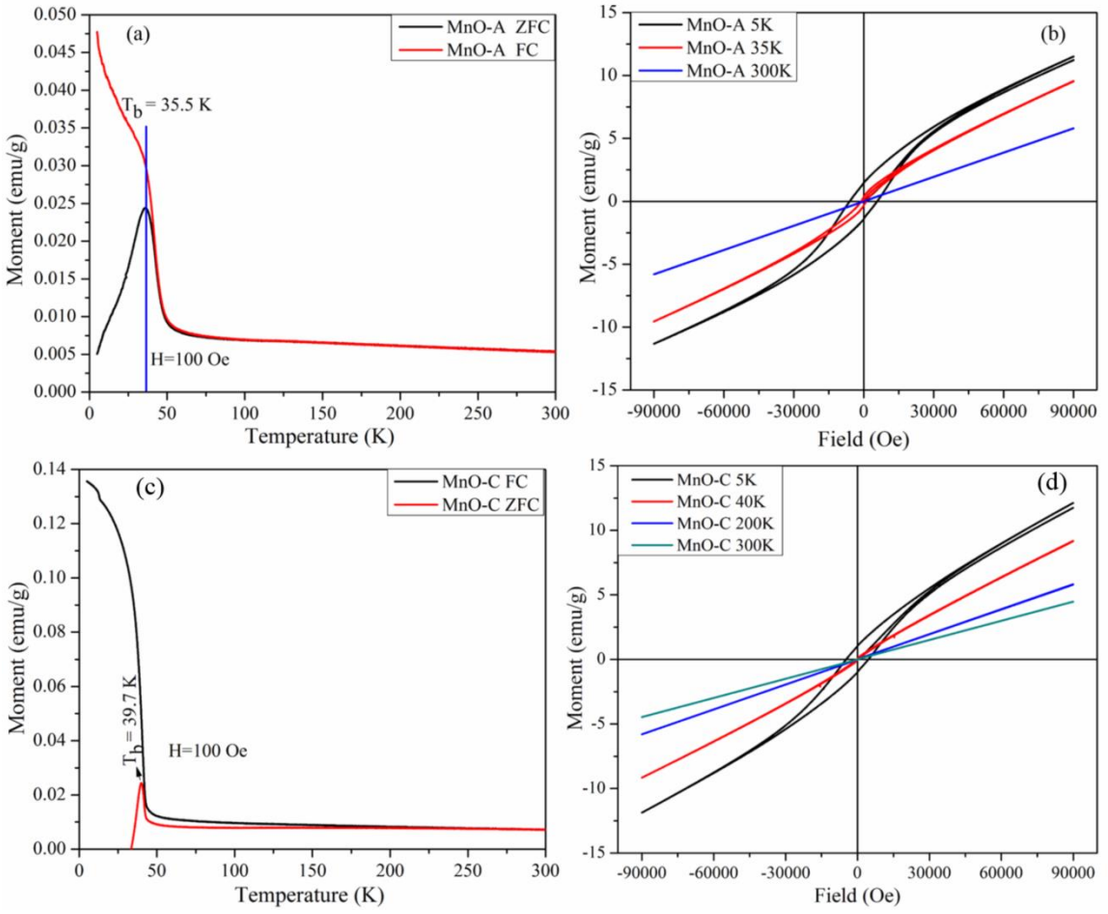


Figure 29 Magnetization vs. temperature curves (a & c) of MnO-A and MnO-C nanoparticles under FC and ZFC conditions using a magnetic field of 100 Oe. Magnetization vs. field of MnO-A and MnO-C nanoparticles (b & d) at room temperature and low temperatures

Conclusion: MnO nanoparticles have been synthesized by a novel chemical route with different types of surfactants. The reaction conditions are optimized for the development of a single phase MnO nanosystems. Magnetic property study reveals that both MnO-A and MnO-C nanoparticles have antiferromagnetic property below Tb whereas paramagnetic at room temperature. The magnetic results strongly indicate that these particles can have high T_1 contrast property for MRI characterization.

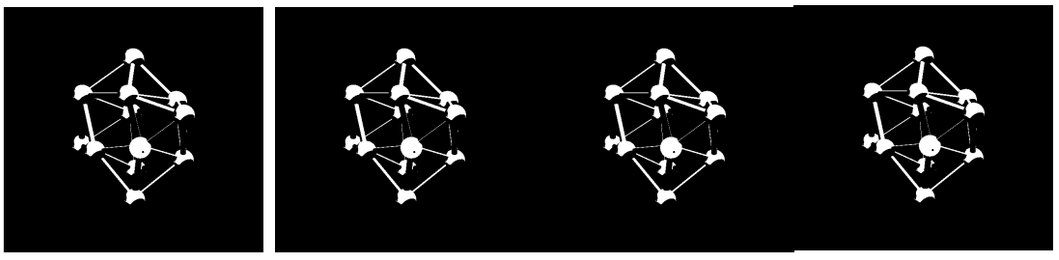
Work done in S.N Bose National Centre for Basic Sciences

2.7 Electronic Structure, Binding energies and Stabilities of Nano-particles

Extensive simulations were carried out using the Quantum Espresso software available with the Centre on the Electronic Structure, binding energies and stable geometries of nano-particles from sizes about 4 to 150 nm. The calculations were based on the Density Functional Approach augmented with U (LDA+U) or using the Harbola-Sahni (HS) exchange. The two substances chosen were Fe_2O_3 and Fe_3O_4. The choice of material was guided by the fact that the Tezpur group had already worked on these materials before and our preliminary confidence in the methodology was the comparison with their experimental results.

2.8 Structure and stability of stearic acid

In the next step, using the same technique we looked at the structure of stearic acid molecule. Initially we had divided the molecule into a head and a tail. We considered the head accurately and replaced the long tail by an effective potential available with quantum chemists. However, recently with the availability of powerful Cray computers we can now simulate the exact molecule. The nano-particles are then activated by decoration the surface with stearic acid molecules. We located the most probable bonding atom on the nano-particle surface. Upto five such molecules have been attached to the nano-particles.



2.9 First principle study based on ground state geometry optimization

We performed simulation based density functional calculations using generalized gradient approximation (GGA) with Perdew–Burke–Ernzerhof (PBE) functional to describe the electron–electron exchange and correlation effects. The geometrical optimization procedures for the local orbitals or non-orthogonal generalized Wannier functions (NGWFs) were solved via a plane-wave pseudo potential (PWPP) formulation using plane-wave accuracy in terms of a minimal basis of *in situ* optimized NGWFs interfaced with linear scaling platform. The NGWFs are themselves expanded in terms of a fixed underlying basis of periodic sine functions equivalent to a systematic plane-wave basis. In the course of calculation, the total energy is minimized with respect to both density kernel and local orbitals in two nested loops, subjected to the constraints of normalization and idempotency. All the systems were calculated on a real space grid defined

with a plane-wave cut-off energy of 800 eV. Our first principle calculations have been focused to carry out geometry optimized structure, energy evolution and maximum rms force per ions with respect to iteration steps. The two substances chosen in pristine form were iron-platinum alloy (FePt) and manganese oxide (MnO). We have calculated these two pristine systems in three parts. First part deals with pristine FePt and FePt capped with thioglycolic acid (TGA) molecules. Second part covers pristine MnO and MnO capped with Oleic acid and Octadecyl phosphonic acid separately. Third part or the last part contains pristine MnO and MnO capped with both Oleic acid and Oleylamine together. The choice of material was guided by the fact that the group had already the experimental results.

(A) FePt capped with thioglycolic acid (TGA)

Geometry optimization is one of the primary tasks in quantum simulation to get idea about the ground state calculations. The essence of the calculation is that the constituting atoms to be moved to the positions where the total energy is minimal with stable structure. In general, this can be tackled efficiently if the forces on the atoms can be computed. The geometry optimization process implemented in first principle calculations relies on the isolation of the atomic and electronic subsystems (i.e. the Born-Oppenheimer approximation). For a given configuration of the ionic positions, the electronic degrees of freedom are completely relaxed so that the electronic subsystem stays on the Born-Oppenheimer surface. All the possible configurations of the ionic positions therefore define a multi-dimensional potential energy surface for which we want to find the global minimum. The structure of FePt and TGA are shown in figure 30. Initially, the structures are revealed taking single point energy calculation with cut-off energy 600 eV and then optimized with cut-off energy 800 eV using Cartesian or delocalized internal coordinates. The atomic forces are calculated by application of the Hellmann-Feynman theorem and the ionic positions are moved around by means of the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method in order to find the minimum of the potential energy.

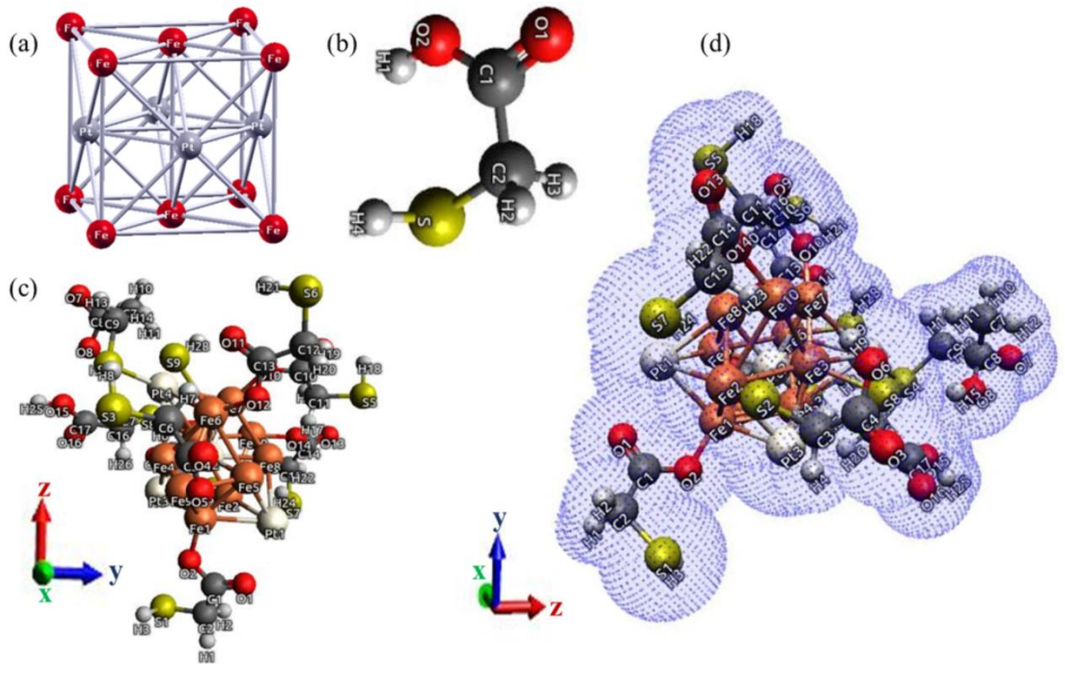


Figure 30 Structure of **(a)** FePt, **(b)** TGA, **(c)** FePt capped with TGA molecules and **(d)** electrostatic potential surface structure of FePt capped with TGA molecules

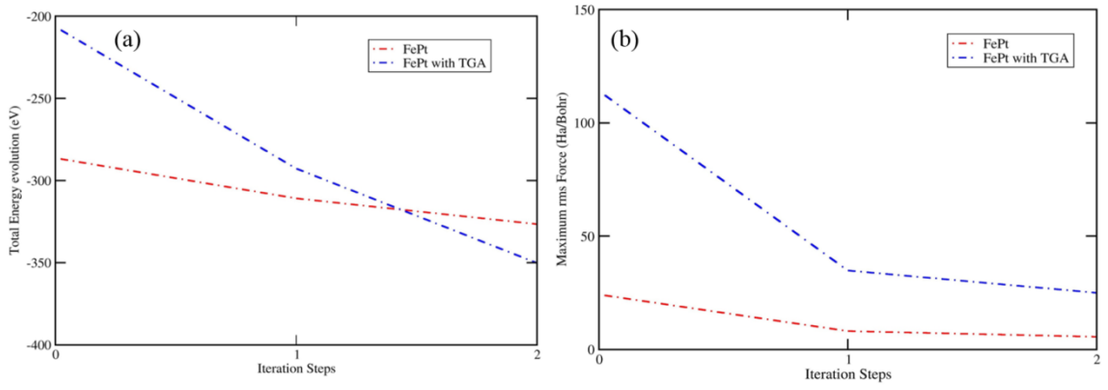


Figure 31 (a) Total energy evolution curve w.r.t iteration steps and **(b)** maximum rms force change w.r.t iteration steps

The square simulation cell is used having 800 eV cut-off energy with truncation radius of 7.5 Bohr. At this point, it is to be noted that several local minima may be present in the configuration space and the algorithm can get trapped in one of those. Though the relaxation provides us with crucial information such as the appearance of dissociation and symmetry breaking.

It is usually a good practice to keep track of the energy and forces at each iteration in order to assess the relaxation process. It is observed that the total energy of the system (Fig. 31 (a)) decreases monotonically indicating the formation of minimum energy state. FePt with TGA system achieves lower minimum energy state and compared with pristine FePt system. Similarly, we can keep track of the maximum rms force on the ions at each iteration by loading the

structure parameters with BFGS algorithms. Like all the quasi-Newton schemes, the BFGS algorithm accumulates information about the Hessian matrix. As the number of iteration increases, BFGS improves its knowledge of the potential energy surface around the minimum and the matrix used to build the quadratic model of the potential energy surface converges towards the true Hessian matrix corresponding to the local minimum. The evolution of forces (Fig. 31 (b)) is decreasing monotonically and shows the presence of local minimum after two iterations.

(B) Oleic acid capped and Octadecylphosphonic acid capped MnO

The required optimised structure of molecules has been simulated with an energy cut-off of about 800 eV, NGWF radii of about 7.5 bohr and a cubic simulation cell of side-length 30 bohr. The crystal structure of MnO (fig. 32(a)) is solved using FCC recursion map interfaced with linear scaling platform and visualized in XcrysDen package. The chain like molecular structure of Oleic acid (fig. 32 (b)) and Octadecylphosphonic acid (fig. 32(e)) are drawn and optimised to get the coordinates of the molecules keeping the NGWFs at maximum limit. The capping process of oleic acid with Manganese oxide (MnO) and octadecyl phosphonic acid with MnO has been estimated using a force field having 500 times iterations. This reaction process is controlled by conjugate gradient algorithm to get the geometry optimization with convergence limit of $10e^{-7}$. The optimised structures of capped MnO revealed from the above process for oleic acid with MnO (fig. 32(c)) and octadecyl phosphonic acid with MnO (fig. 32(f)) are supported by the experimental FTIR result. The Van der Waals surface of the oleic acid capped with MnO system (fig. 32(d)) and octadecyl phosphonic acid capped with MnO (fig. 32(g)) has been drawn using the color pattern of Molecular Electrostatic Potential (MEP).

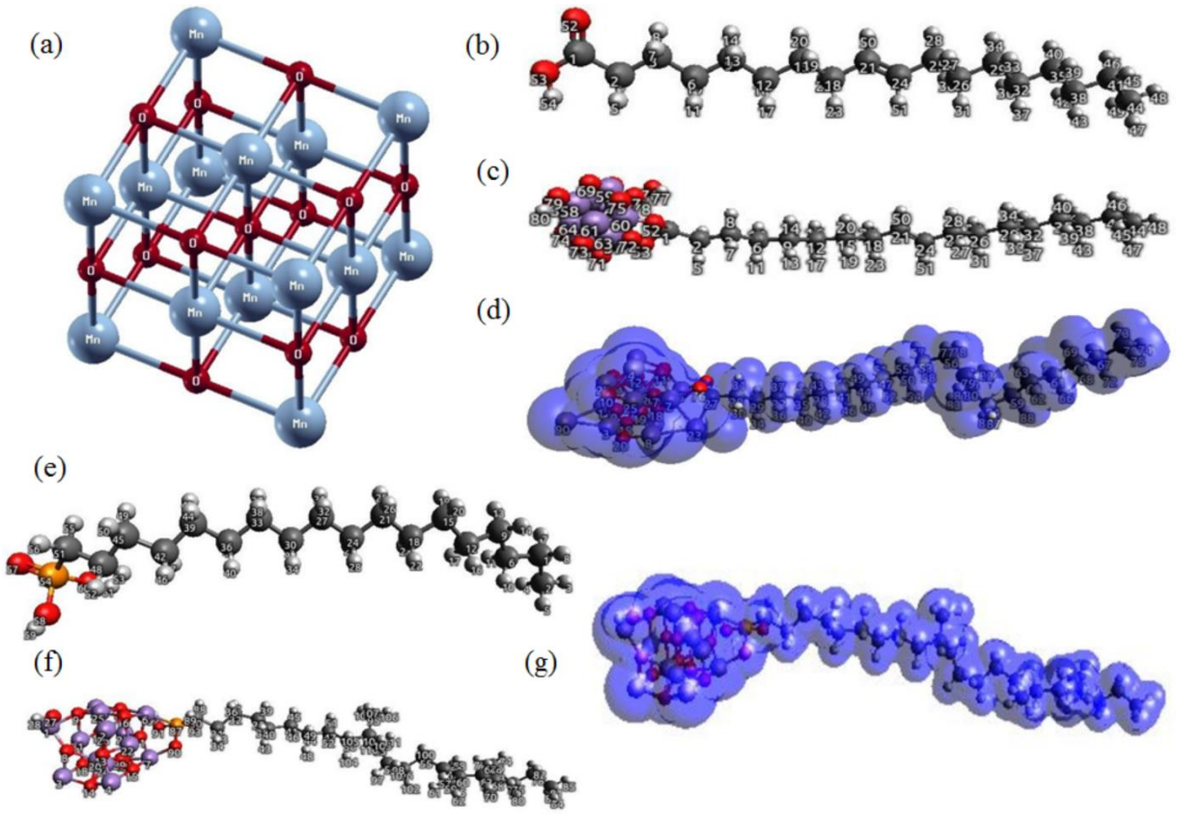


Figure 32 (a) Crystal structure of MnO. The molecular chain structure of **(b)** oleic acid **(c)** oleic acid capped with MnO, **(e)** octadecyl phosphonic acid and **(f)** octadecyl phosphonic acid capped with MnO. Van der Waals surface plot of **(d)** oleic acid capped with MnO and **(g)** octadecyl phosphonic acid capped with MnO.

The atomic forces are calculated by application of the Hellmann-Feynman theorem and the ionic positions are moved around by means of the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method in order to find the minimum of the potential energy. The square simulation cell is used having 800 eV cut-off energy with truncation radius of 7.5 bohr. It is observed that the total energy of MnO, Oleic acid capped MnO and Octadecyl phosphonic acid capped MnO systems (Fig. 33 (a)) decreases monotonically indicating the formation of minimum energy state. The rate of change of energy evolution is high to achieve stable state in capped molecules comparing to the pristine MnO. It is observed that capping with organic molecules enhances the energy evolution rate to achieve the minimum energy state faster. Similarly, we can track the maximum rms force on the ions at each iteration by loading the structure parameters with BFGS algorithms to accumulate information about the Hessian matrix.

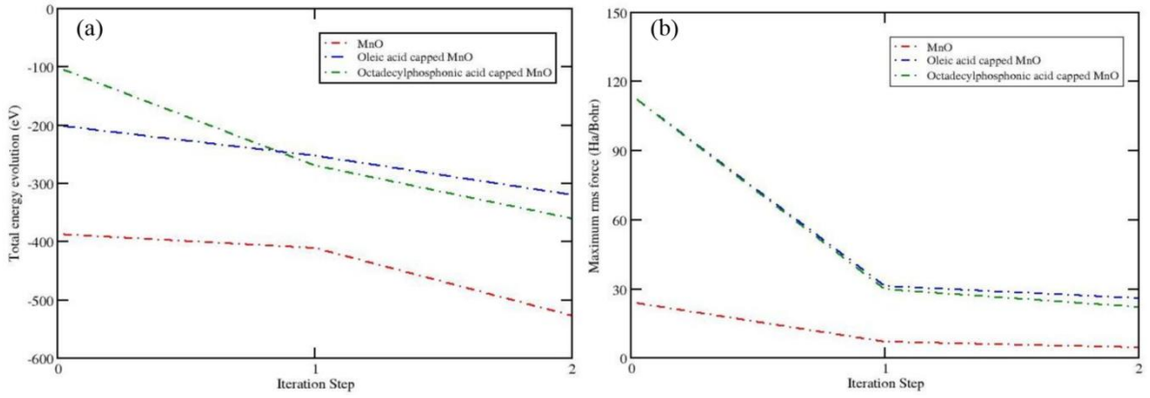


Figure 33 (a) Total energy evolution curve w.r.t iteration steps and **(b)** Maximun rms force change

As the number of iteration increases, BFGS improves its knowledge of the the potential energy surface around the minimum and the matrix used to build the quadratic model of the potential energy surface converges towards the true Hessian matrix corresponding to the local minimum. The evolution of forces (Fig. 33 (b)) is decreasing monotonically and shows the presence of local minimun after two iterations. It is observed that the rate of change of rms force followed the same trend of convergence with iteration processes for Oleic acid capped MnO and Octadecyl phosphonic acid capped MnO. The force gradient of pristine MnO system follows quite linear trend to achieve stable optimised configuration. Thus, the force gradient curve shows enhanced change rate in case of oleic acid capped MnO and octadecyl phosphonic acid capped MnO molecules compared to pristine MnO indicating better structural stability and supporting the experimental results.

(C) Oleic acid and Oleylamine capped MnO

We followed the same method as before to get the optimised structure of molecules has been simulated with an energy cut-off of about 800 eV, NGWF radii of about 7.5 bohr and a cubic simulation cell of side-length 30 bohr. The crystal structure of MnO (fig. 34 (a)) is solved using FCC recursion map interfaced with linear scaling platform and visualized in XcrysDen package. The chain like molecular structure of Oleic acid (fig. 34 (b)) and Oleylamine (fig. 34 (c)) have been drawn and optimised to get the coordinates of the molecules keeping the NGWFs at maximum limit. The capping process of oleic acid and oleylamine with Manganese oxide (MnO) has been estimated using a force field having 500 times iterations. This reaction process is controlled by congugate gradient algorithm to get the geometry optimization with convergence limit of $10e^{-7}$. The optimised structures of capped MnO reveiled from the above process for oleic acid and oleylamine with Manganese oxide (MnO) (fig. 34 (d)) are supported by the

experimental results. The Van der Waals suraface of the capped MnO systems (fig. 34 (e)) has been drawn using the color pattern of Molecular electrostatic potential (MEP).

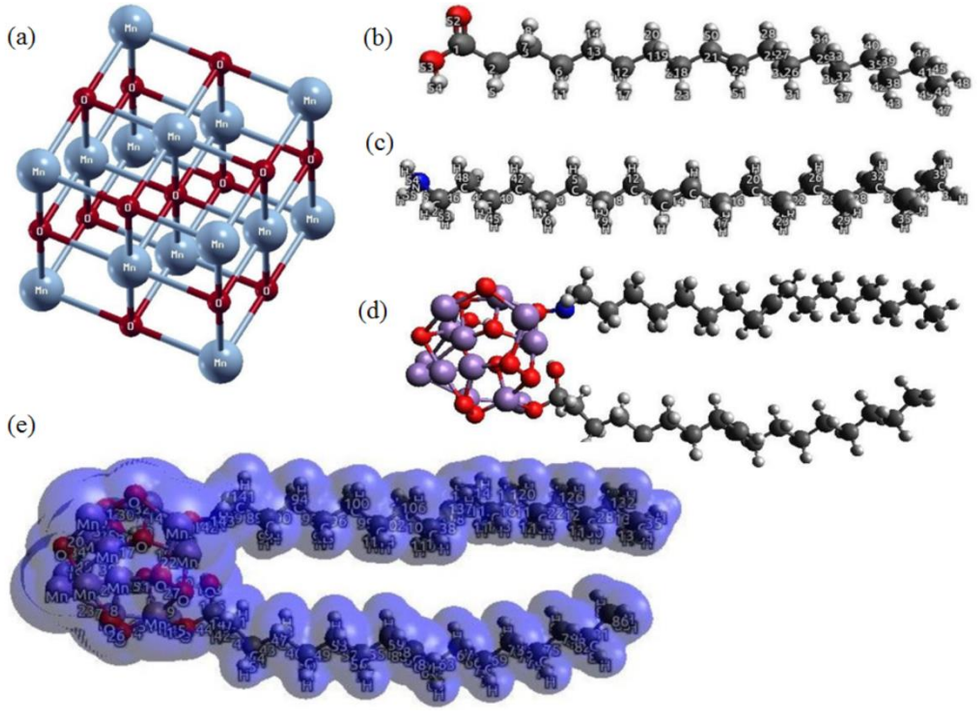


Figure 34 (a) crystal structure of MnO. The molecular chain structure of **(b)** oleic acid, **(c)** oleylamine **(d)** oleic acid and oleylamine capped with MnO. Van der Waals surface plot of **(e)** oleic acid and oleylamine capped with MnO.

The atomic forces are calculated by application of the Hellmann-Feynman theorem and the ionic positions are moved around by means of the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method in order to find the minimum of the potential energy. The square simulation cell is used having 800 eV cut-off energy with truncation radius of 7.5 bohr. It is observed that the total energy of MnO, Oleic acid and oleylamine capped MnO systems (Fig. 35 (a)) decreases monotonically indicating the formation of minimum energy state. Similarly, we can track the maximum rms force on the ions at each iteration by loading the structure parameters with BFGS algorithms to accumulate information about the Hessian matrix. As the number of iteration increases, BFGS improves its knowledge of the the potential energy surface around the minimum and the matrix used to build the quadratic model of the potential energy surface converges towards the true Hessian matrix corresponding to the local minimum.

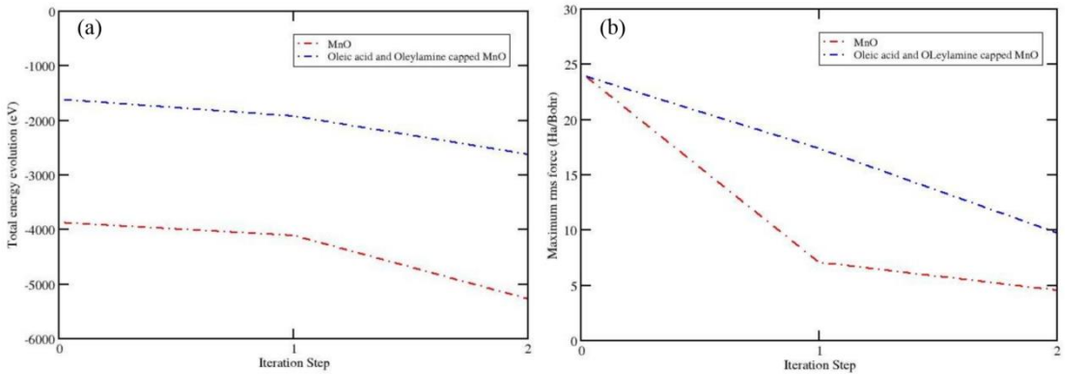


Figure 35 (a) Total energy evolution curve w.r.t iteration steps and **(b)** Maximun rms force change w.r.t iteration steps

The evolution of forces (Fig. 35 (b)) is decreasing monotonically and shows the presence of local minimun after two iterations. It is observed that the rate of change of rms force followed the same trend of convergence with iteration processes for Oleic acid and Oleylamine capped MnO. The force gradient of capped MnO system follows linear downfalling trend to achieve stable optimised configuration. The rms force has no saddle point in case of capped system in order to achieve stability. Thus, the force gradient curve shows enhanced change rate in case of capped MnO and compared to pristine MnO indicating better structural stability and supporting the experimental results.

3. Summary & Conclusion

The scientific objectives outlined for the project included the characterization of developed nanomaterial based systems along with their relaxivity properties. Further, it also involved the assessment of biocompatibility and contrast property optimization of the well characterized nanomaterials. In this direction, a set of single and dual mode MRI contrast agents have been developed. Two different MnO based nontoxic T_1 MRI contrast agents were developed. On the other hand an iron rich FePt system was developed with high relaxivity for MRI T_2 contrast property. Another T_2 MRI contrast agent was developed based on Co_3O_4. The relaxivity and contrast property of all these systems were estimated. As proposed in the work plan, two different dual mode MRI contrast agents were also developed based on MnO and $MnFe_2O_4$. Both these components were brought under a single nanosystem in the form of nanocomposite and Janus nanoparticles.

4. Details of New Leads Obtained

The integration of both T_1 and T_2 contrast enhancement property in a single moiety is a prominent issue explored in the project and the concept can be extended to several other nanosystems. Development of both single and multimodal MRI contrast agents capable of executing water solubility and biocompatibility is a significant outcome of the project. The convincing efficiency in enhancement of MRI contrast is an important achievement of the project. The studies performed under the project were published in reputed international journals and some of the studies are already communicated for publication.

5. Outcomes of the project

Papers published in refereed journals

1. S K Behera, P Deb, A Ghosh, *ChemistrySelect*, **2** (2017) 3657
2. D K Jha, K Saikia, S Chakrabarti, K Bhattacharya, K S Varadarajan, A B Patel, D Goyary, P Chattopadhyay, P Deb, *Materials Science & Engineering C*, **72** (2017) 415
3. K Bhattacharya, V Dupuis, D Le-Roy, P Deb, J. Phys.: Condens. Matter **29** (2017) 045002
4. S K Behera, P Deb, A Ghosh, *Phys. Chem. Chem. Phys.*, **18** (2016) 23220
5. K Bhattacharya, D Parasar, B Mondal, P Deb, *Scientific Reports*, **5** (2015) 17072
6. D K Jha, K S Varadarajan, A B Patel, P Deb, *Materials Chemistry and Physics*, **156** (2015) 247
7. K Bhattacharya, P Deb, *Dalton Trans*., **44** (2015) 9221
8. K Bhattacharya, B Gogoi, A K Buragohain, P Deb, *Materials Science & Engineering C*, **42** (2014) 595
9. S K Behera, K Saikia, P Deb, *AIP Conf. Proc*., **1832** (2016) 050036
10. K Bhattacharya, M Gogoi, P Deb, *AIP Conf. Proc*. **1731** (2015) 050105
11. K Deka, N Nath, B K Saikia, P Deb, *J. Therm. Anal. Calorim* (Accepted manuscript; DOI 10.1007/s10973-017-6476-0)

Patents applied

1. P Deb, K Saikia, Magnetic secondary nanostructure as contrast agent for Magnetic resonance imaging (Application no. 201731009379)

Conference Papers

1. "Mesoporous 3D Carbon Framework Encapsulated Janus Nanoparticles as Novel Biocompatible Dual MR Imaging Probe" at 7th Asia Pacific NMR and 23rd NMRS Symposium held at IISc, Bangalore in 2017.

2. "Mesoporous carbon shell encapsulated manganese oxide nanoparticles as novel biocompaticle MR imaging probe" at Materials research society of India (MRSI) symposium and 27th annual general meeting on MRSI in CSIR-NEIST, Jorhat in 2016.

3. "Strain induced structural and electronic property of graphene/ h-BN heterostructures: combined linear scalling density functional approach and molecular dynamic simulations" at Materials research society of India (MRSI) symposium and 27th annual general meeting on MRSI in CSIR-NEIST, Jorhat in 2016.

4. "Molecular design of one dimensional magnetic $FeNi_3$ nanochains and their application in oil removal at 60th DAE Solid state physics symposium (DAR-SSPS-2015) in Amity University, Noida during 21-25 December, 2015.

5. "Biocompatible cobalt based nanoparticles as novel MRI contrast agent" at 4th International conference on advanced nanomaterials and nanotechnology (ICANN) in IIT Guwahati in 2015.

6. "Linear scalling density functional approach for realizing pressure effect on anisotropic Co_3O_4 nanostructures" at XXVII IUPAP conference on computational physics (CCP 2015) in IIT Guwahati in 2015.

7. "Investigation on the Nature of Interparticle Interaction in Worm-hole like porous Fe_3O_4 Architecture" at Regional Conference on Young Scientists on Nanoscience and Nanomaterials in JNCASR, Bangalore in 2014.

8. "Hybrid nanosystem in nanomedicine: multimodalities and multifunctionality" at Workshop on Nanoengineering in Medicine in AIIMS, Delhi in 2014.

9. Nanoparticle Catalyzed Biomass Pyrolysis: Kinetic Analysis and Efficiency Assessment at International Symposium on Applications of Thermal Analysis and Calorimetry in ISM Dhanbad in 2014.

10. "Facile One Pot Synthesis of Hydrophilic Superparamagnetic FePt Nanoparticles for Molecular Imaging" at 20th symposium of Nuclear Magnetic Resonance Society, India

(NMRS 2014) in Tezpur University in 2014.

11. "Magnetoporous assembly of iron oxide nanoparticles for MRI molecular imaging" at 20th symposium of Nuclear Magnetic Resonance Society, India (NMRS 2014) in Tezpur University in 2014.

12. "MnO Nanoparticles Encapsulated with Mesoporous Carbon Shell: A Biocompatible MR Imaging Agent" at 20th symposium of Nuclear Magnetic Resonance Society, India (NMRS 2014) in Tezpur University in 2014.

13. "Study on the interparticle interaction of superparamagnetic iron oxide nanoparticles" at 20th symposium of Nuclear Magnetic Resonance Society, India (NMRS 2014) in Tezpur University in 2014.

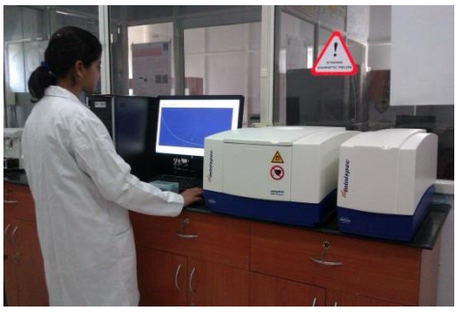
14. "Maglumino hybrid nanosystem as dual contrast agent for magnetic resonance and optical imaging" at 20th symposium of Nuclear Magnetic Resonance Society, India (NMRS 2014) in Tezpur University in 2014.

15. "Superparamagnetic carbon encapsulated magnetite nanoparticles and evaluation of its antioxidant property for biomedical applications" at International conference on Magnetic Materials and applications (MagMA-2013) in Indian Institute of Technology, Guwahati in 2013.

16. "Single Moiety Multifunctional SIPP for dual Imaging and Therapeutic Application" at International conference on Magnetic Materials and applications (MagMA-2013) in Indian Institute of Technology, Guwahati in 2013.

17. "On magnetic property correlation with interparticle interaction in superparamagnetic iron oxide nanosystems" at International conference on Magnetic Materials and applications (MagMA-2013) in Indian Institute of Technology, Guwahati in 2013.

6. Instruments Procured and Installed



Time-domain NMR instrument

Nano Particle Size & Zeta Potential Analyzer

Dip Coater System

(P. Deb)
Principal Investigator,
Tezpur University (Central University)

Sanction no. BT/357/NE/TBP/2012 Dated. 21.3.2013

CONSOLIDATED STATEMENT OF EXPENDITURE (SOE)

Item	Sanctioned outlay	Released fund by DBT				Expenditure as per latest Statement of expenditure (SOE) (Amounts in Rs.)					
		1st year	2nd year	3rd year	Total	1.4.2013 to 31.3.2014	1.4.2014 to 31.3.2015	1.4.2015 to 31.3.2016	1.4.2016 to 31.3.2017	Grand Total expenditure	Balance Rs.
					(3+4+5+6)	1st UC	2nd UC	3rd UC	4th UC	(7+8+9+10)	(6-11)
	Total Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.
1	2	3	4	5	6	7	8	9	10	11	12
Equipment	7500000.00	7500000.00	1302000.00	0.00	8802000.00	0.00	8060513.00	582312.00	0.00	8642825.00	159175.00
Manpower	1320000.00	422000.00	406000.00	832000.00	1660000.00	265866.00	196000.00	588699.00	694245.00	1744810.00	(-)84810.00
Consumable	1600000.00	550000.00	70000.00	354000.00	974000.00	120187.00	0.00	304021.00	97673.00	521881.00	452119.00
Travel	150000.00	50000.00	35000.00	16000.00	101000.00	35111.00	0.00	169769.00	48388.00	253268.00	(-)152268.00
Contingency	300000.00	100000.00	15000.00	0.00	115000.00	15000.00	0.00	207940.00	250651.00	473591.00	(-)358591.00
Overhead	150000.00	100000.00	0.00	0.00	100000.00	75000.00	0.00	25000.00	15625.00	115625.00	(-)15625.00
Total	11020000.00	8722000.00	1828000.00	1202000.00	11752000.00	511164.00	8256513.00	1877741.00	1106582.00	11752000.00	NIL

Total fund released Rs.= 11752000.00

Total expenditure Rs.= 11752000.00

Balance Rs.= 0.00

(P.Deb)
Principal Investigator
DBT Project
Tezpur University

Principal Investigator
"Devel of..... First Principles
DBT Project, Dept of Physics
Tezpur University

27.5.17
Finance Officer
Tezpur University

Finance Officer
Tezpur University

Registrar
Tezpur University

Registrar
Tezpur University

Appendix A

No. BT/357/NE/TBP/2012 DATED.21.3.2013

Assets acquired wholly or substantially out of Govt. Grants Register to be maintained by Grantee Institution

1. Name of the sanctioning authority : Department of Biotechnology, Govt. of India, New Delhi
2. Name of Grantee institution : Tezpur University
3. No. & Date of sanction order : BT/357/NE/TBP/2012 DATED.21.3.2013
4. Amount of the sanctioned grant : Rs. 122.25 lakhs
5. Brief purpose of the grant : Research purpose
6. Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant in aid sanction order . Yes
7. Particulars of assets actually credited or acquired :

Sl.no	Item	Actual cost Rs.
1	Nano Particles size & Zeta Potential Analyzer & accessories	Rs.3262157.00
2	Time Domain NMR & accessories	Rs.5086010.00
3	Laptop Computer	Rs.73100.00
4	Dip Coating System	Rs.221558.00
	Total Rs.	Rs.8642825.00

8. Value of the Assets as on 31st March, 2017 : Rs.8642825.00
9. Purpose for which utilized at present Research work in University level.
10. Encumbered or not
11. Reasons, if encumbered
12. Disposed of or not
13. Reasons and authority , if any disposal N/A
14. Amount realized on disposal
15. Remarks

(P. Deb)
Principal Investigator
DBT Project
Tezpur University

Principal Investigator
"Devel of..... First Principles
DBT Project, Dept of Physics
Tezpur University

27.5.17
Finance Officer
Tezpur University
Finance Officer
Tezpur University

Registrar
Tezpur University
Registrar
Tezpur University

Appendix B

UTILISATION CERTIFICATE

(For the financial year ending 1st April,2016 to 31th March, 2017)

1. The title of the project /Scheme : **Development of nanomaterial based dual mode constrast agent and their surface mediated conjugation study from first principles"**

2. Name of the Organization : TEZPUR UNIVERSITY

3. Principal Investigator : Dr. P. Deb

4. Department of Biotechnology sanction : order no and date of sanction the project — No. BT/357/NE/TBP/2012 Dated. 21.3.2013

5. Amount brought forward from the previous financial year quoting DBT letter no. and date in which the authority to carry of forward the said amount was given — Rs. (-) 95418.00

6. Amount received from DBT during the Financial year (please give no. and dates of sanction orders showing the amount paid. — Rs.1202000.00 No. BT/357/NE/TBP/2012 Dated. 5.9.2016

7. Other receipts / Interest earned, if any, On the DBT grants. — Not Applicable

8. Total amount that was available for expenditure during the financial year (sl.nos.5,6 and 7). — Rs.1106582.00

9. Actual expenditure (excluding commitments) incurred during the financial year (2016-17) (1.4.2016 to 31.3.2017) — Rs.1106582.00

10. Unspent balance refunded, if any / (Please give details of cheque no. etc.) — NIL

11. Balance amount available at the end of the Financial year (2016-17) — Rs. 0.00 (NIL)

12.Amount allowed to be carried forward to the next financial year vide letter no.....date.....

1. Certified that the amount of **Rs.1106582.00** mentioned against col. 9 has been utilized on the project scheme for the purpose for which it was sanctioned and that the balance of **Rs.0.00(NIL)** remaining unutilized ~~at the end~~ of the ~~year~~ has been surrendered of Govt. (Vide no...nil...dated...nil.) will be adjusted towards the grants-in-aid payable during the next year.
2. Certified that I have satisfied myself that the conditions on which that grants –in aid was sanctioned have been duly fulfilled being fulfilled and that I have exercised following checked to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised : 1.Cash books 2.Ladgers 3.Vouchers 4.Bank statements.

27.5.17

(P. Deb)
Principal Investigator
DBT Project
Tezpur University

Finance Officer
Tezpur University
Finance Officer
Tezpur University

Registrar
Tezpur University
Registrar
Tezpur University

(to be counter signed by the DBT Officer - incharge)

Principal Investigator
"Devel of..... First Principles
DBT Project, Dept of Physics
Tezpur University

No. BT/357/NE/TBP/2012 DATED.21.3.2013

Appendix C

Statement of Expenditure referred to in Para 9 of the Utilisation Certificate

Showing grants received from the Department of Biotechnology and the expenditure incurred from 1st April 2016 to 31st March, 2017

Rs. In lakhs

Item	Unspent balance carried forward from previous year 2015-16 Rs.	Grants received from DBT during the year 2016-17 Rs.	Other receipts/ interest earned if any, on the DBT grants	Total of Col. (2+3+4) Rs.	Expenditure Rs.	Balance Rs. (5-6)	Remarks
1	2	3	4	5	6	7	8
1.Non-recurring							
(i)Equipments	159175.00	0.00	0.00	159175.00	0.00	159175.00	
2. Recurring							
(i)Human Resource	(-)222565.00	832000.00	0.00	609435.00	694245.00	(-)84810.00	
(ii) Consumables	195792.00	354000.00	0.00	549792.00	97673.00	452119.00	
(iii) Travel	(-)119880.00	16000.00	0.00	(-)103880.00	48388.00	(-)152268.00	
(iv) Contingency	(-)107940.00	0.00	0.00	(-)107940.00	250651.00	(-)358591.00	
(v) Overheads (if applicable)	0.00	0.00	0.00	0.00	15625.00	(-)15625.00	
(vi)Interest Earned	0.00	0.00	0,00	0.00	0.00	0.00	
Total Rs.	**(-)95418.00**	**1202000.00**	**0.00**	**1106582.00**	**1106582.00**	**0.00**	

Total fund utilized

(a) From 1.4.2013-31.3.2014	Rs. 511164.00
(b)From 1.4.2014-31.3.2015	Rs. 8256513.00
(c)From 1.4.2015-31.3.2016	Rs. 1877741.00
(d) From 1.4.2016-31.3.2017	Rs. 1106582.00
Total	Rs. 11752000.00

Actual Expenditure :

Total fund received	Rs. 11752000.00
Total fund utilized	Rs .11752000.00
Balance	Rs. 0.00

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Tezpur University

Registrar
Tezpur University

Annexure A

No. BT/357/NE/TBP/2012 DATED.21.3.2013

Manpower Staffing Details (From 1st August 2015 to 20th November, 2016)
JRF & SRF salary increased as per DBT circular no. SR/S9/S-09/2012 Dated 21.10.2014.

Name of the person	Name of the post	Date of joining	Date of leaving	Total monthly salary Rs.	Total salary paid during the financial year Rs.	Total salary paid during project period Rs.
Mr. Deepak Kr. Jha	JRF SRF	14.6.2013 15.6.2015	25.10.2016	25000.00 28000.00	302579.00	918344.00
Ms. Kashmiri Deka	JRF	19.6.2013	20.11.2016	25000.00	391666.00	826466.00
Total Rs.					**694245.00**	**1744810.00**

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No. BT/357/NE/TBP/2012 DATED.21.3.2013

Annexure B

Manpower Expenditure Details (From 1st August 2015 to 20th November, 2016)

JRF & SRF salary increased as per DBT circular no. SR/S9/S-09/2012 dated 21.10.2014.

Sanctioned posts	Number	Scale of pay Rs.	Annual outlay	Outlay for the entire period	Revised scale, if any	Revised Project outlay	Actual release by DBT Rs.	Actual expenditure Rs.	Balance Rs	Balance used for adjustment of instrument Rs.	Actual balance available Rs.
Junior Research Fellow	02	JRF Rs.25000.00 SRF Rs.28000.00	--	--	NIL	NIL	609435.00	694245.00	(-)84810.00.00	NIL	(-)84810.00

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No. BT/357/NE/TBP/2012 DATED. 21.3.2013

Head : Manpower

DBT Project Title : Development of nanomaterial based dual mode constrast and their surface mediated conjugation study from first principles"

Monthly stipend : JRF Rs.25000.00 + HRA , SRF Rs.28000.00

JRF& SRF salary increased as per DBT circular no SR/S9/Z-09/2012 dated 21/10/2014

	Year	Month	Post : JRF Name: Mr. Deepak Kr.Jha Date of appointed: 14.6.2013 Post : SRF Date of appointment : 15.6.2015 Date of leaving : 25.10.2016	Post : JRF Name: Ms. Kashmiri Deka Date of appointed:19.6.2013 Date of leaving : 20.11.2016	
Financial Year			Rs.	Rs.	**Total**
2015-16	**2015**	August	0.00	25000.00	25000.00
		September	0.00	25000.00	25000.00
		October	0.00	25000.00	25000.00
		November	0.00	25000.00	25000.00
		December	28000.00	25000.00	53000.00
	2016	January	28000.00	25000.00	53000.00
		February	28000.00	25000.00	53000.00
		March	28000.00	25000.00	53000.00
2016-17	**2016**	April	28000.00	25000.00	53000.00
		May	28000.00	25000.00	53000.00
		June	28000.00	25000.00	53000.00
		July	28000.00	25000.00	53000.00
		August	28000.00	25000.00	53000.00
		September	28000.00	25000.00	53000.00
		October	22579.00	25000.00	47579.00
		November	0.00	16666.00	16666.00
		Total	**302579.00**	**391666.00**	**694245.00**

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तेजपुर - 784028 :: असम

TEZPUR UNIVERSITY (A Central University)
Napaam, Tezpur - 784028, Assam

TO WHOM IT MAY CONCERN

This is to certify that no interest earned during the project period (April, 2013 to March, 2017) in respect of the DBT sponsored project (No. BT/357/NE/TBP/2012 dated. 21.3.2013) entitled *"Development of nanomaterial based dual mode contrast agent and their surface mediated conjugation study from first principles"* funded by Department of Biotechnology (DBT), Govt. of India

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