Final Report

Title of the project

Development of Iron Oxide Nanoparticle probes for organ specific molecular MR Imaging

(Sanction No.2009/34/06/BRNS/288)

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Submitted to

BOARD OF RESEARCH IN NUCLEAR SCIENCES

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DEPARTMENT OF ATOMIC ENERGY (DAE)

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Principal Investigator(s) and Co- Investigator (s).

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Implementing Institution

Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala, India

Date of commencement. 22-12-2009 (Date of joining of the staff)

Actual date of completion. 31-12-2013

Proposed date of completion 31-12-2013

Objectives as stated in the project proposal.

1. Development of nanoparticles of size 60-70nm (dextran coated iron oxide)
2. Development of nanoparticles of size 8-12 nm (citrate coated iron oxide)
3. Characterisation of the materials using various analytical methods
4. In vitro evaluation of these nanomaterials as probes for molecular imaging
5. Animal model development for atherosclerotic plaque and liver cancer/fibrosis
6. In vivo evaluation of the nanoparticles in rabbit model for molecular imaging
7. Histopathological evaluation of nanoparticle intake by liver and aortic plaque

8. Comparison of the results with available MR contrast materials (Signal enhancement with respect to particle size and dose will be evaluated for immediate and delayed imaging)

Objectives Achieved

1. Development of nanoparticles of size 60-70nm (dextran coated iron oxide)-Completed
2. Development of nanoparticles of size 8-12 nm (citrate coated iron oxide)-Completed
3. Characterisation of the materials using various analytical methods-Completed
4. In vitro evaluation of these nanomaterials as probes for molecular imaging-Completed
5. Animal model development for atherosclerotic plaque –Completed
6. Animal model for Liver fibrosis-Completed
7. In Vivo evaluation of the developed material – completed
8. Histopathological evaluation of nanoparticle intake by liver and aortic plaque-completed

Patent Applied

Patent entitled 'Method of Synthesis of Zerovalent iron Nanoparticles for the use as Positive contrast agent in Magnetic Resonance Imaging and the process involved' has been submitted to BRNS in June 2013, and is under consideration.

Additional objectives achieved

Developed Pullulan, PEG and Alginate coated iron oxide nanoparticles for MR Imaging.

Its characterization and imaging efficacy studies have been completed.

Developed Zerovalent iron nanoparticles for MR angiogram and T1 imaging

PhD Work

Ms.Ariya Saraswathy who is a registered PhD student of Sree Chitra Tirunal Institute for Medical Sciences and Technology, has completed her PhD programme under this project. She has presented the PhD colloquium and ready to submit the thesis.

JRFs who were working in the project have got relieved on 31st of December, 2013

Report of the Work on the project

Introduction

The use of magnetic nanoparticles in the field of biomedical application is a well established area of research. The possibility of tuning the magnetic nanoparticles with various functionalities to incorporate multifunctional property in a single entity has increased its frequency of usage in a wide range of application in the biomedical field [1-5]. Among the magnetic nanoparticles for biomedical applications, iron oxide nanoparticle

gains much importance because of its favourable biocompatibility. [6-8].

In medical diagnostics, MRI has gained wide acceptance because of its accuracy and non invasiveness. This method gives better differentiation between normal and diseased tissue based on the MRI sequences used for the excitation of the water protons. But in many cases, contrast agents are necessary for the accurate diagnosis of the disease. The use of iron oxide nanoparticles as T2 contrast agents can reduce the transverse relaxation of the water protons and hence show reduced signal intensity on MR images. This area has been the interest of research for the past several years. The use of iron oxide nanoparticles is preferred over the commonly used Gd based T1 contrast agents in the early diagnosis of diseases affecting lymph nodes, liver, atherosclerosis, spleen etc, These particles have the properties necessary for the use as contrast agents, which is favoured by the presence of ferritin in the human body [9, 10]. Iron oxide nanoparticles coated with different biocompatible polymers have been already established in this area [11, 12]. However, there are several deciding factors that influence the performance of the iron oxide nanoparticles when it reaches the application side. This includes properties like particle size, surface chemistry of the particles, stability, coating thickness, physicochemical characteristics, biocompatibility and the magnetic properties. [13-21]. Apart from this, the mode of synthesis also is found to play crucial role in the final characteristics of the particles. Even though there exists different routes of synthesis, co-precipitation method is preferred over the other methods when considering the high yield, high magnetic property and the biocompatibility of the product [7]. It is the magnetic properties of the material which makes it most suitable for the use as MRI contrast agents.

The variation in the core size causes fluctuation in the superparamagnetic nature of the nanoparticle, which is one of the most essential properties for the use as negative contrast agent [24]. Considering the variety of magnetic properties exhibited by iron oxide nanoparticles of different size, named as superparamagnetic iron oxide nanoparticles

(SPIONs, 50-150 nm) and Ultra-small Superparamagnetic iron oxide nanoparticles (USPIONs, 10-50 nm), USPIONs are more preferred as contrast agents due to its long circulation time and the blood half-life time [11].

Apart from the use of high molecular weight and long branched polymers, coating with citrate is expected to give excellent magnetic properties so as to use the system as MR contrast agents. Also, considering the release of monomeric content as part of polymer degradation during their interaction with the biological medium, the use of short chained or small ligand citrate ions as shell is considered to be more beneficial [25-27].

The synthesised material also has to be stable over a significant period of time for being considered as a good product which can replace the commercially available negative MRI contrasts. The stability of the particle can be controlled by proper surface modification with the choice of appropriate polymeric coating. The variation in the polymer used for the surface modification as well as the thickness of the polymer layer influences the magnetisation as well as the magnetic relaxivity of the probe significantly[22, 23].

All these factors are considered in the development of USPIONs with particle size of the order of 10-15nm. The surface of the USPIONs are stabilized using citrate and dextran to yield the citrate covered and dextran covered USPIONs (C-USPIONs and D-USPIONs) and the complete characterisation of both C-USPIONs D-USPIONs and their preliminary use as MRI contrast material is also done.

Experiments and Results

Synthesis and surface modification of Ironoxide nanoparticles

Ironoxide nanoparticles were synthesized by co-precipitation method. Briefly, $FeCl_3$ and $FeCl_2.4H_2O$ in 100ml deionized water were mixed in a desired molar ratio. The whole system was then stirred and after adequate mixing, the pH was varied from acidic to basic, as the precipitation starts. When pH reached 14, black precipitate resulted indicating the formation of ironoxide nanoparticles and the system was allowed to continue under the same reaction condition for 2 hours to allow complete precipitation. The precipitate was

magnetically separated, washed and centrifuged. The resultant iron oxide nanoparticles were then dispersed in 0.1 M tri-sodium citrate and stirred for 6 hrs. The citrate coated ironoxide nanoparticle suspension was then washed with deionized water monitoring the pH. The residual sample is then collected and freeze dried and used for further characterization. Similary for getting dextran coated nanoparticles, the ironoxide nanoparticles were dispersed in 3% Dextran and stirred for 6 hrs at 80^{o} C. The suspension was washed with deionized water and centrifuged and collected.

Characterization of particles

Transmission Electron Microscopy

The morphology of bare, citrate and dextran coated nanoparticles were examined by transmission electron microscopy at 100 kV (TEM, JEM-2010, JEOL, Tokyo, Japan). The magnetically separated and washed samples were dispersed in deionized water followed by ultrasonication for 5 min. Small amount of sample dispersed in deionized water was allowed to dry slowly on a formvar-coated copper grid for TEM studies. The particle size and size-distributions were visually analysed from the TEM micrographs.

Dynamic light scattering

Hydrodynamic sizes and zeta potentials of the prepared nanoparticles were measured using a dynamic light scattering instrument (DLS, Zetasizer NanoZS90, Malvern Instruments Ltd.,Worcestershire, UK). Small amount of samples were dispersed in deionized water followed by ultrasonication for 5 min and measurements were taken as per standard procedure.

X ray diffraction analysis

The phase analysis of all the nanoparticles was carried out using X'Pert PRO X-ray diffraction (XRD) instrument. Crystal structure was determined by analyzing the position and intensities of diffraction peaks observed in the diffraction angle range, $2\theta = 10 - 80^{o}$ using Cu Kα radiation of 1.5406 Å at 40 kV and a 20 mA current. The diffraction pattern

was also used to yield the crystallite size by analyzing the width and shape of the peak profile using Scherrer equation.

Thermo Gravimetric Analysis

Thermo Gravimetric Analysis (TGA) was practiced for the evaluation of amount of citrate and dextran bound to the ironoxide nanoparticles. The thermal analysis of lyophilized citrate coated ironoxide nanoparticles and the pure tri-sodium citrate used for the coating purpose were performed using SDT 2960 V2.2B (Simultaneous TGA-DTA, TA Instruments, Delaware, USA) instrument. The experiment was performed under nitrogen atmosphere with in a temperature range between room temperature and 1200^{o} C by applying a constant heating rate of 10^{o}C / min. The percentage of weight loss at different stages accounted for the degradation of the organic content present in the sample.

Differential Scanning Calorimetric Analysis

DSC - Q20 V24.4 model instrument was used for the differential scanning calorimetric analysis of lyophilized citrate and dextran coated ironoxide nanoparticles and pure tri-sodium citrate and dextran. Standard aluminium pans were used to encapsulate the samples. The experiment was performed at a heat flow rate of 5^{o}C/min within the temperature range of 25^{o} C to 500^{o}C.

FTIR Analysis

FTIR spectra of USPION, C-USPION, D-USPION, tri-sodium citrate and dextran were recorded by mixing with optical grade potassium bromide using Thermo Nicolet 5700 FTIR spectrometer (USA) in the diffuse reflectance (DRIFT) mode. To obtain high signal to noise ratio, 64 scans were accumulated at a resolution of 4 cm^{-1}. Spectra acquired in the region 400 to 4000 cm^{-1} were plotted and baseline corrected with the assistance of software OMNIC.

Magnetisation Measurements

The saturation magnetization and the superparamagnetic property of the magnetic

nanoparticles were evaluated using a Vibration Sample Magnetometer (VSM), Lakeshore model 7410 using maximum fields of 150 Oe EG&G PAR 4500. The saturation magnetization (Ms) was determined from M versus H plots and extrapolated to infinite fields.

Magnetic Relaxivity Measurements

Phantom Study

Magnetic relaxivity measurements were performed using 1.5 T whole body MR scanner (MAGNETOM Avento Tim System 1.5T, Siemens, Munich, Germany) equipped with a head coil. Phantoms of different concentration of the iron ranging from 0 - 0.45 mM were prepared in deionized water and used. The parameters used for measurements of T_1 and T_2 relaxation times are temperature = 22°C, FOV = 20 X 40 cm, slice thickness = 10 mm.

For measurements of the Longitudinal (T_1) relaxation time, the repetition time (TR) and echo time (TE) were set at values of 4000 ms and 11 ms, respectively, and the MR signal was measured by changing the inversion time (TI), such as 50, 100, 300, 700, 1200, 2000 and 3000 ms. A modified Transverse (T_2) relaxometry spin echo sequence was run at three different planes of the phantoms for T_2 relaxometry measurements. TR was set at 2000 ms and the MR signal was measured by changing the TE values from 15 to 120 ms. From the resulting MRI maps pixel intensity with respect to each concentration was extracted. From the curve fitting, the T_1 and T_2 relaxation time was obtained. This procedure was repeated for five iron concentrations to obtain both T_1 and T_2 relaxation times. Longitudinal and transverse relaxation values (r_1 and r_2) were calculated as per the reported literature [28,29]. T_1 and T_2 relaxation rates were plotted against the iron concentration and relaxivity value was determined by the linear fit.

Cell culture for MRI study

A549 cells (Human Lung Cancer cell lines) were used for in vitro MRI relaxivity studies. The citrate coated nanoparticles at different concentrations ranging from 0 – 100 μg/mL

were added to the cells and incubated for 24 hrs. Then the cells were washed and trypsinized, and the pellets were used for MRI studies.

***In vitro* MR Imaging**

Magnetic relaxivity measurements of the cells incubated with C - USPIONs were performed using a method similar to that of the particle phantoms using 1.5 T clinical MR scanner. For this, the cells were incubated with serial dilution of the particles with deionised water so as to get different concentration of the iron in the range of 0 – 1.8 mM. These samples taken in 1.5 ml microtube phantoms were used for MRI studies. For the T_2 measurement a spin-echo sequence with TE ranging from 50 – 800 ms and a repetition time TR of 6500 was run. Here also the images from 3 different planes were acquired and the pixel intensity with respect to each concentration was extracted.

Animal Models for Liver fibrosis

For chronic CCl4-induced liver injury, a widely adopted standard method reported earlier was followed (31). Briefly, rats were treated once in a week, intragastrically, with CCl_4 in olive oil (0.08 ml CCl4/ml olive oil) for a total of 9 weeks. The initial amount of CCl_4 administered was; 412 mg/kg body wt. To minimize mortality, subsequent doses given were dependent on the amount of weight gained or lost by individual rats during the previous week. If the rats gained 0–5 g, the dose of CCl_4 was not changed. The dose was increased by 32 mg after a weight gain of 6–10 g and by 64 mg if 10 g were gained. If body weight decrease to about 5 g, the dose of CCl_4 was decreased by 32 mg. The dose was decreased by 50% in rats that lost 6–10 g. If the rats lost 10 g, no CCl4 was given, and treatment was resumed with the last dose given when body weight showed indications of regaining.

In Vivo MRI study

Rats were imaged to see the behavior of Fe_3O_4 NPs as a contrast agent in vivo condition. The institutional ethics committee approved all the animal experimental protocols. The animals ranging in body weight from 200-250gm were examined. While the liver fibrosis

models were underdevelopment, MRI imaging of the normal animals were performed using a 1.5 T clinical MRI scanner, using a 12 channel head coil to check the uptake of the prepared materials by the normal liver. Each rats was imaged before and after intravenous administration of the NPs after anesthetizing the animals with ketamine (70 mg/kg), xylazine (5mg/kg) and atropine (0.02 mg/kg). MR imaging included an axial and coronal T1 and T2-weighted sequences Post-NP injection sequences were acquired 20-30 min after its injection. The same procedure was repeated for liver fibrosis induced animals also with citrate coated and dextran coated iron oxide nanoparticles injected intravenously.

Cytotoxicity

Cytotoxicity of USPIONs and C-USPIONs were analysed by MTT assay using rat fibroblast glial cell lines. Cytotoxicity of D-SPIONs was analysed by MTT assay using Hep G2 cell lines of liver origin. Test material of varying concentrations (0.9, 0.6 and 0.3 mg), negative control and positive control were added to the cells and incubated for 24 hrs. The samples were later removed and 3-(4, 5- dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) reagent was added to each well and further incubated for 3 hrs. MTT was then removed and dimethyl sulfoxide (DMSO) was added to dissolve the formed formazan crystals and then incubated for 30 min. Absorbance was quantified by measuring at 570 nm using micro plate reader.

Blood aggregation studies

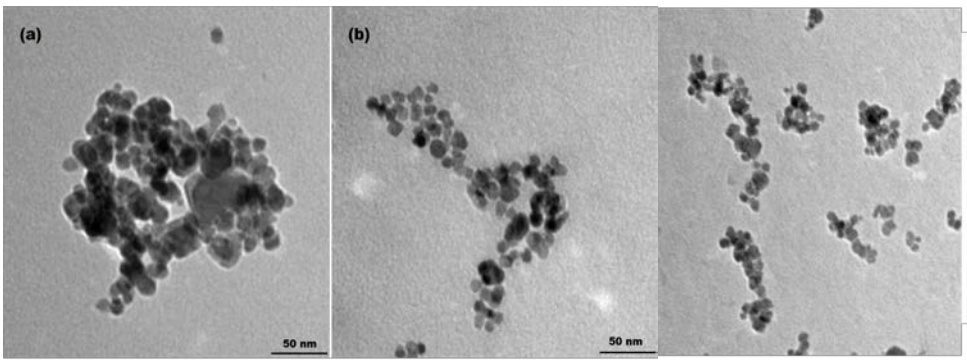
RBC, WBC and Platelet Aggregation

The blood aggregation studies of the developed nanoparticles are important for their application in the biomedical field. For this, the interaction of the different particles with RBC, WBC and platelets were studied. Blood aggregation studies and hemolysis assay of C-USPIONs and D-USPIONs were evaluated on red blood cells (RBC), white blood cells (WBC) and platelets, as per standard protocols. Saline was used as negative control and

polyethyleneimine (PEI) as positive control. Aggregation was assessed by checking the morphology of the cells using phase contrast microscopy (Leica DM IRB, Germany). For hemolysis assay, the acquisition of absorbance spectra in the range 400to 600 nm was carried out and absorbance at 541 nm was measured on the supernatants of RBCs incubated with samples of different concentration. The photographs of the samples after centrifugation are shown in (Fig.) Saline and water were used as negative and positive controls respectively.

Results

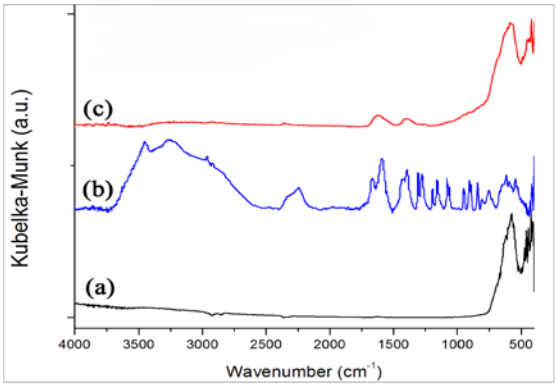
Surface coating of citrate and dextran was confirmed using Thermo Gravimetric Analysis and Zeta potential. The size of the resultant particles were then analysed with Dynamic Light Scattering technique and Transmission Electron Microscope. Magnetic properties of the developed probes were characterized using Vibration Sample Magnetometer and Magnetic Resonance Imaging relaxivity measurement.



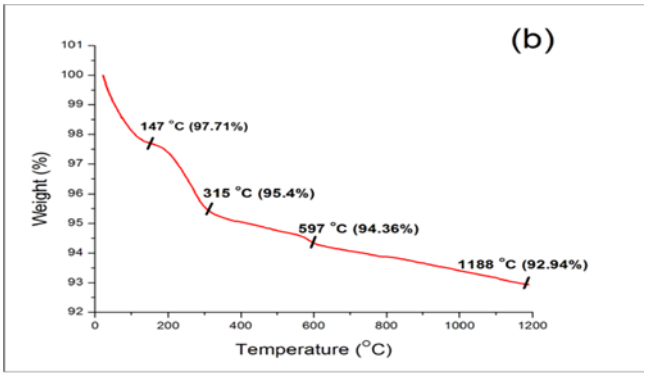
TEM of USPION and CUSPION

TEM and DLS of DUSPION

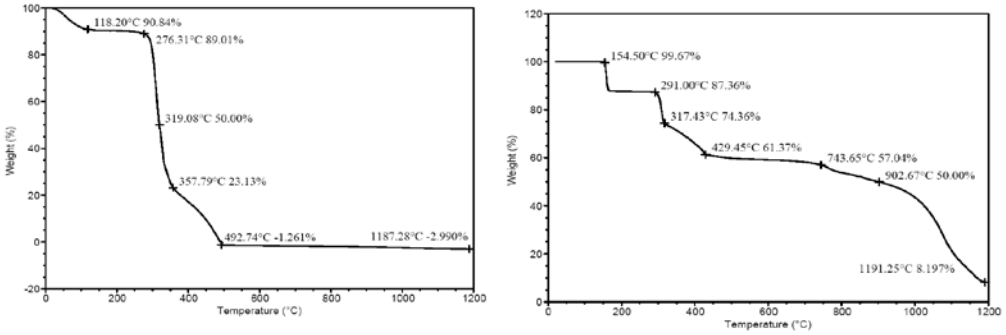
XRD patterns obtained for the particles shows characteristic peaks of iron oxide. Crystallite size obtained using XRD measurement were 15 and 20nm for bare and citrate coated iron oxide nanoparticles. FTIR spectra shows characteristic peaks of iron oxide, citrate and dextran coatings.



FTIR spectra of CUSPION (c),citrate(b) and USPION(a)

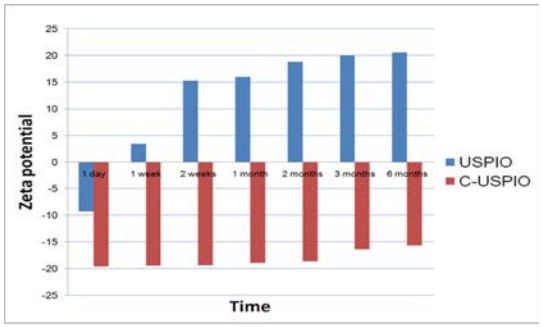


TGA analysis of cUSPION

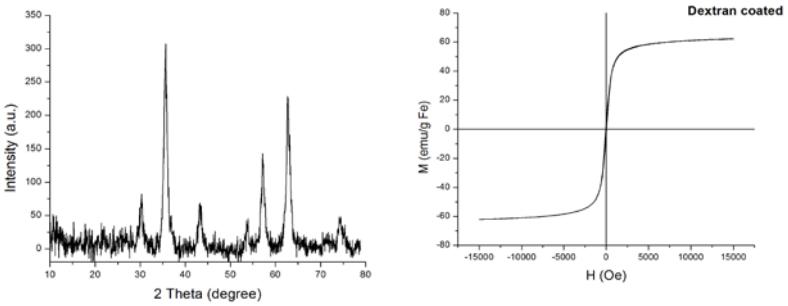


TGA of Dextran and DUSPIO

Thermo Gravimetric Analysis confirms the citrate coating on iron oxide by the shift in thermal decomposition temperature of citrate from 154^{o} C to 127^{o} C. Dextran coating was confirmed by the shift in thermal decomposition temperature of dextran from 276^{o} C to 160^{o} C. A total mass loss of 5% and 40% occurred for citrate and dextran coated iron oxide respectively. Increase in Zeta potential values compared with bare iron oxide to citrate and dextran coated iron oxide nanoparticle supports the TGA result of coating conformation.



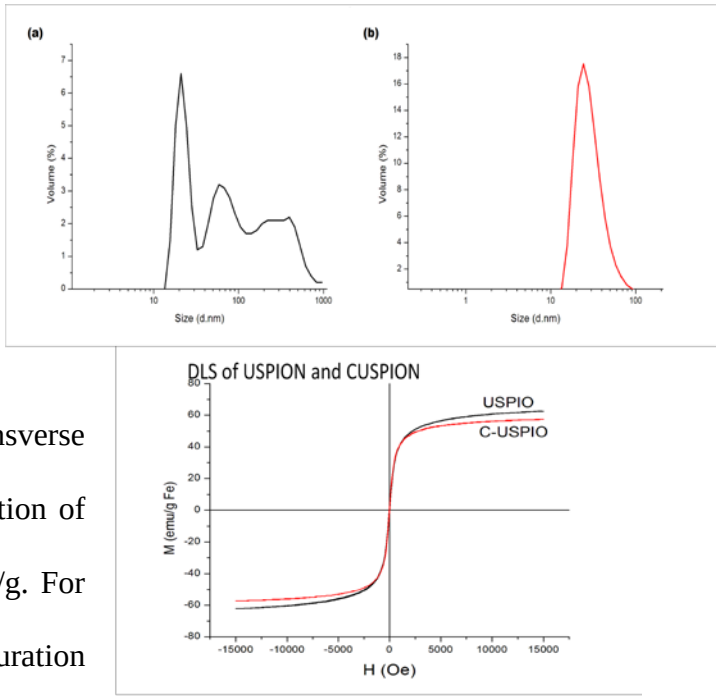
Zeta potential Variation over six months



XRD and Hysteresis of Dextran coated USPION

Using TEM, average particle size of about 12 nm has been obtained for iron oxide nanoparticles. The hydrodynamic size of bare, citrate and dextran coated iron oxide nanoparticles were also done using DLS. Hydrodynamic size obtained for bare, citrate and dextran coated iron oxide was 28, 32 and 50nm respectively.

Magnetic properties of developed materials are confirmed by magnetic hysterisis using Vibration Sample Magnetometer. Super paramagnetic property of the synthesised particles is clearly demonstrated by the zero coercivity in the VSM study. This property is essential for the material to be used as transverse MR contrast agent. The saturation magnetization of the bare USPION was found to be 62.7 emu/g. For C-USPION and D-USPION, saturation magnetizations obtained were 57.5 and 49.74 emu/g. These values indicate the preservation of magnetic properties of C-USPIONs and D-USPIONs even after the surface coating. The observed pattern of the hysteresis curve is an indication of the particle's single domain existence with only one orientation of magnetic moment.

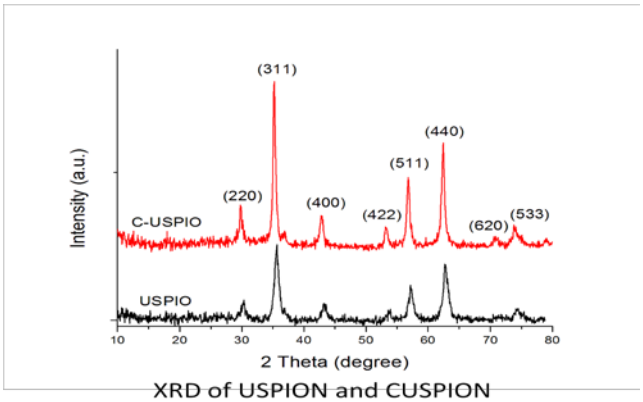


Hysteris curve of USPION and CUSPION showing zero coercivity and hence super paramagnetic nature for the material

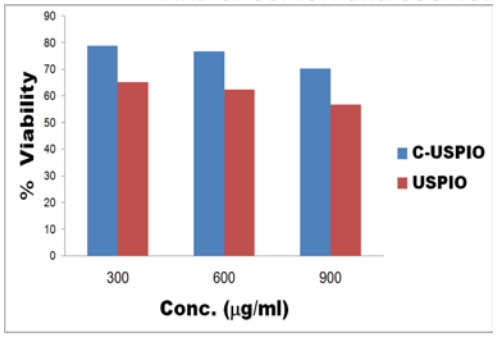
Using 1.5T MR Imaging system, transverse (r_2) and longitudinal (r_1) relaxivity for bare, citrate coated and dextran coated USPIONs were obtained (Table1). Transverse relaxivity (r_2) of 57, 102 and 126.6 mM^{-1} s^{-1} was obtained respectively for bare, citrate coated and

dextran coated USPIONs. Longitudinal relaxivity (r_1) of 1.53, 2.3 and 1.92 mM^{-1} s^{-1} was obtained for bare, citrate coated and dextran coated USPIONs.

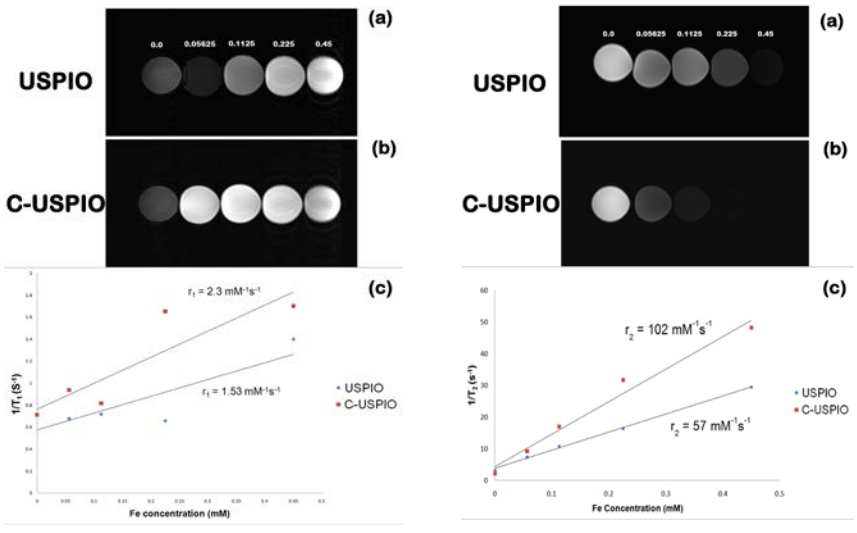
Cytotoxicity of USPIONs and C-USPIONs was analysed by MTT assay using rat fibroblast glial cell lines (C6 cell lines). Cell viability using for C-USPIONs is about 78.8% for 300 µg/ml to that of 70 % viability is observed for bare-USPIONs. A slight decrease in cell viability is only observed for C-USPIONs for high concentrations. But for bare-USPIONs, a rapid decrease in cell viability is observed for higher concentrations. As the aim of the study is to use the developed nanoparticles as MR contrast materials for liver fibrosis, it is important to see the toxicity effect of the particles on liver cells. For this, cytotoxicity of the nanoparticles was evaluated on human hepatocellular cells, HepG2 using MTT assay (Fig. 6). 82 to 100% viability of the cells was observed for 100 to 25 µg/ml concentrations of the D-SPIONs. Compared to previous reports on magnetic nanoparticles, D-SPIONs in the present study showed better cell viability at the respective concentrations.



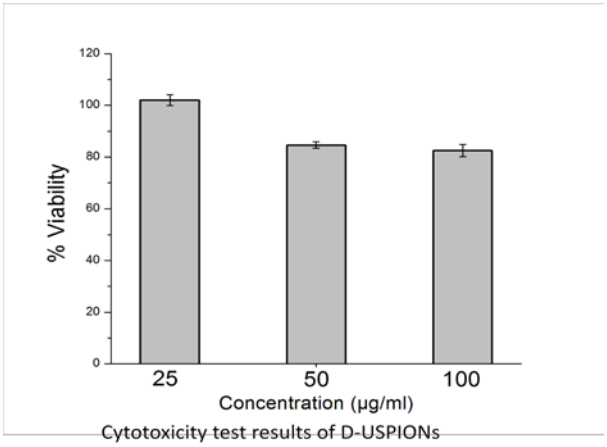
XRD of USPION and CUSPION



Cytotoxicity test results

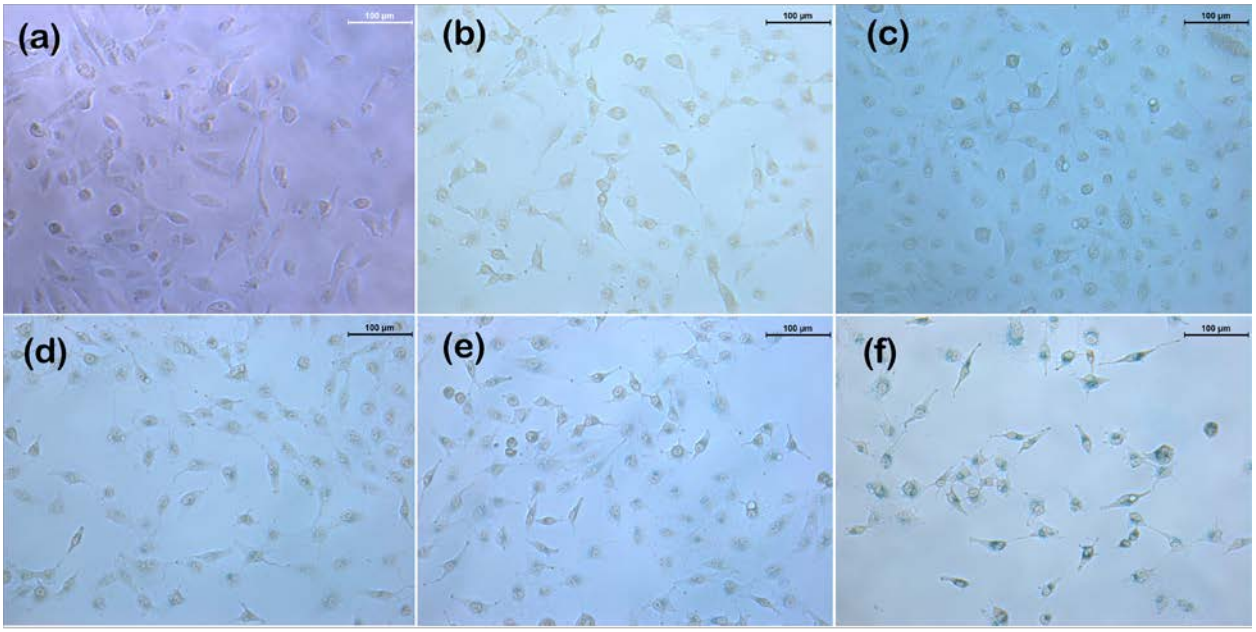


R1 and R2 studies of citrate coated and uncoated



Cytotoxicity test results of D-USPIONs

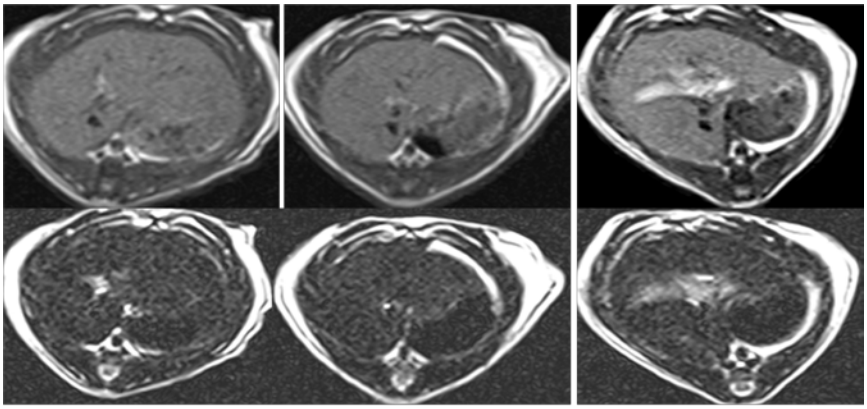
Cell uptake was studied using Prussian blue staining. Uptake on different iron concentrations such as 100, 50, 25, 12.5 and 6.25 µg/ml on A549 cells was clearly visible in the micrograph. These concentrations are also used for invitro MR cell imaging to analyse variation in MR signal. The cellular MR imaging with varying concentration of Fe resulted in the T_2 weighted image signal drop with the increase in the iron concentration. The control samples with the cell alone appear bright and the gradual decrease in the brightness is observed as the Fe concentration gradually increases for each TE.



Cell uptake study –(a) normal cell and b to f different concentrations of CUSPION

In vivo MR imaging of the rat models has been performed using clinical 1.5T magnet. Mild signal variation is observed in the normal animals injected with citrate and dextran coated USPIONs whereas significant contrast is observed in the liver fibrosis models. The preliminary studies have been completed with doses less that of the cytotoxic level as seen

from MTTT assay. Later the concentration of the materials for optimum contrast has also been standardised and the images are shown.



T1(Top row) and T2(bottom row) images of control, cUSPION and DUSPION(left to right)

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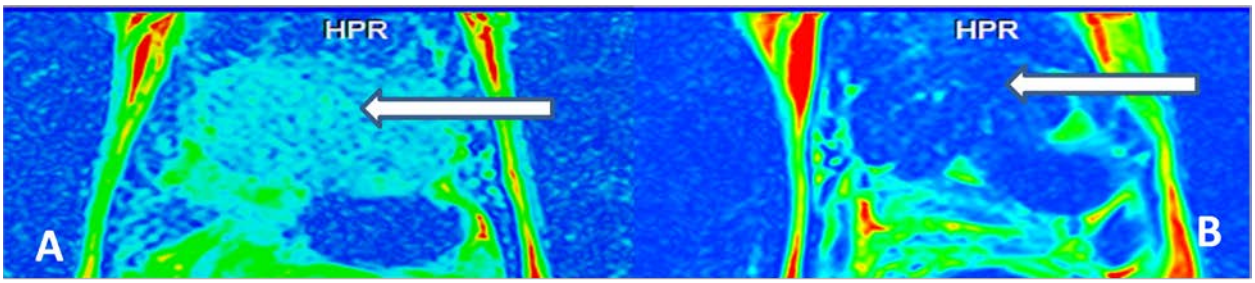
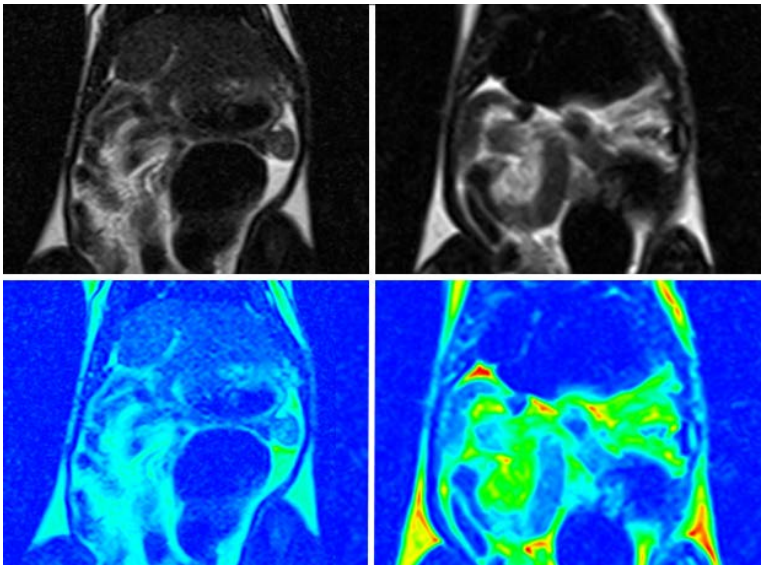


Pre and Post contrast images of CUSPIO



Pre and Post contrast images of D –USPIO

Comparison of the important parameters of the two systems, C-USPIONs and D-USPIONs

Name	**Coating agent**	**Hydrodynamic Size (nm)**	**R_2 ($mM^{-1}S^{-1}$)**	**R_1 ($mM^{-1}S^{-1}$)**	**R_2/R_1**	**B_0 T**
Bare-USPION	--	20-25	57	1.83	31.14	1.5
C-USPION	Citrate	30	102	2.3	44.35	1.5
D-USPION	Dextran	40-60	126.6	1.92	65.93	1.5

Publications

1. A Saraswathy, SS Nazeer, M Jeevan, N Nimi, S Arumugam, S Shenoy, **R.S.Jayasree,** Citrate coated iron oxide nanoparticles with enhanced relaxivity for in vivo Magnetic Resonance Imaging of liver fibrosis, Colloids and Surfaces B: Biointerfaces,117, 216-224, 2014

2. Ariya Saraswathy, Shaiju S Nazeer, N Nimi, S Arumugam, SJ Shenoy, **R.S. Jayasree**, Synthesis and characterization of dextran stabilized superparamagnetic iron oxide nanoparticles for in vivo MR imaging of liver fibrosis. Carbohydrate Polymers 101, 760–768, 2014.

3. Ariya Saraswathy, Shaiju.S.Nazeer, Nimi.N, Sabareeswaran.A, Sachin.J.Shenoy, Jayasree.R.S Magnetic Nanoparticles for Liver imaging, Highlight about our work in **Nature India**, December, 2013

Best Poster Award

Poster titled "**Super Paramagnetic Iron Oxide nanoparticles for in-vivo Magnetic Resonance Imaging of liver fibrosis**" by Ariya Saraswathy won the Best Poster Prize in the 2nd International Conference on Advanced Functional Materials, ICAFM on 19th Feb 2014, held at NIIST, TRivandrum

Conference papers

1. Ariya Saraswathy presented a poster titled "Super Paramagnetic Iron Oxide nanoparticles for in-vivo Magnetic Resonance Imaging of liver fibrosis", in the 2nd International Conference on Advanced Functional Materials, ICAFM on 19th Feb 2014.
2. Ariya Saraswathy, Shaiju S Nazeer, Harikrishna Varma PR, Jayasree RS. **Ultrasmall superparamagnetic iron oxide nanoparticles with high magnetic properties for biomedical applications.** National seminar on Frontiers in chemistry oraganised by IIST Trivandrum on 7 – 8, December 2011
3. Ariya Saraswathy, Shaiju S Nazeer, Harikrishna Varma PR, Jayasree RS. **Iron oxide nanoparticles with enhanced transverse relaxation rate for MR Imaging** 4th Bangalore nano organised by JNCASR Bangalore on 8 – 9, December 2011
4. Jayasree RS**. Higher transverse relaxivity and stability of iron oxide nanoparticles**

for MR imaging Health Care India -2012 New Delhi on 21-23 January 2012

5. Shaiju.S.Nazeer, Ariya Saraswathy, A.K. Gupta, **R.S. Jayasree,** Fluorescence spectroscopy to discriminate neoplastic human brain lesions: a study using the spectral intensity ratio and multivariate linear discriminant analysis, Laser Phys. 24, 025602 , 2014

6. Ariya Saraswathy, Shaiju S Nazeer, Jayasree RS. **High magnetic properties of iron oxide nanoparticles for improved contrast in Magnetic Resonance Imaging,** DAE-BRNS 4th interdisciplinary symposium on material chemistry, December 11-15th, 2012

7. Ariya Saraswathy, Shaiju S Nazeer, Jayasree.R.S- Oral presentation on "**A Novel Iron Oxide Based Contrast Agent For Magnetic Resonance Imaging Of Liver Diseases"** in 25th Kerala Science Congress, 2013.

8. Ariya Saraswathy, Shaiju S Nazeer, Nimi.N, Jayasree.R.S Poster presentation on "**Alginate stabilized Super Paramagnetic Iron Oxide nanoparticles for in-vivo Imaging of liver fibrosis**" in "Indo-German Conference Laser Applications and Nanoscience, 5th to 7th December 2013

9. Ariya Saraswathy, Shaiju S Nazeer, Jayasree.R.S Oral presentation on "**In-Vivo evaluation of liver fibrosis using Dextran stabilised Super Paramagnetic Iron Oxide nanoparticles"** in NCMST-2013 (IIST)

Total publications that emerged out of the financial support of BRNS during the period

1. Ariya S, Shaiju S N, Nimi N, Sabareeswaran A, Sachin JS, Jayasree R S., Synthesis and characterization of Dextran stabilized superparamagnetic iron oxide nanoparticles for in vivo MR imaging of liver fibrosis., CarbohydratePolymers. 2014.101 (760– 768).

2. Ariya S, Shaiju S N, Madhumol J, Nimi N, Sabareeswaran A, Varma PRH, Jayasree R S., Citrate coated iron oxide nanoparticles with enhanced relaxivity for in vivo Magnetic Resonance Imaging of liver fibrosis., Colloids&Surfaces B: Biointerfaces, 117, 216-224, 2014

3. Shaiju.S.Nazeer, Ariya Saraswathy, A.K. Gupta, **R.S. Jayasree,** Fluorescence spectroscopy to discriminate neoplastic human brain lesions: a study using the spectral intensity ratio and multivariate linear discriminant analysis, Laser Phys. 24 (2014) 025602

4. Nazeer SS, Saraswathy A, Gupta AK, Jayasree RS., Fluorescence spectroscopy as a highly potential single-entity tool to identify chromophores and fluorophores: study on neoplastic human brain lesions. Journal of Biomedical Optics (2013) 18 (6):067002-067002.

5. Nazeer Shaiju S, Ariya S, Asish R, Salim Haris P, Anita B, Arun Kumar G, Jayasree RS. Habits with killer instincts: in vivo analysis on the severity of oral mucosal alterations using autofluorescence spectroscopy. J Biomed Opt., 16, 087006, 2011.

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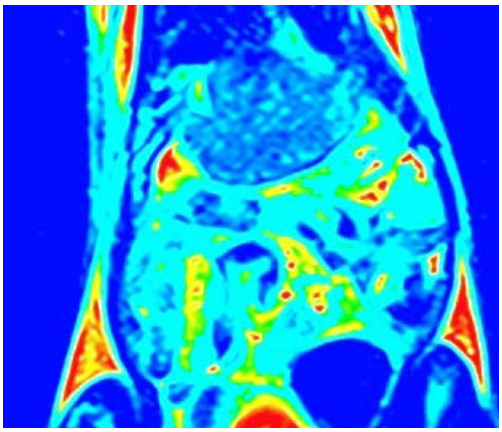
10. C. Venugopal , Shaiju S Nazeer , Anita Balan, R.S. Jayasree, Autofluorescence spectroscopy augmented by multivariate analysis for improved classification efficiency and early diagnosis of oral cavity disorders¸ Photomedicine and Laser Surgery 31(12); 2013

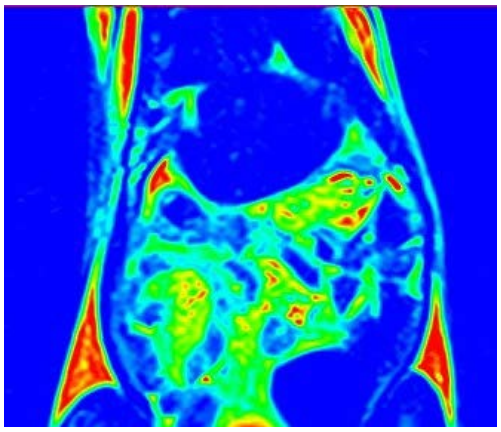
11. Shaiju S.Nazeer, Ariya Saraswathy, P. Venkatachalam, Luxmi Varma, YM. Fazil Marickar,R.S. Jayasree Structural characterization of human urinary stones using Infrared and Raman spectroscopic techniques.
(Under review in Vibrational Spectroscopy)

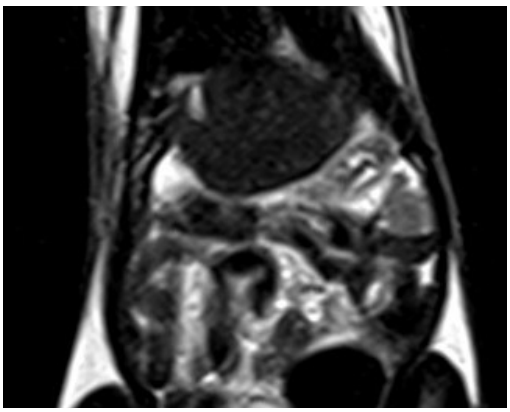
Additional work done

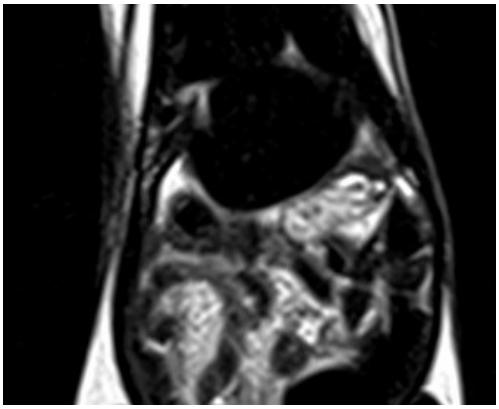
Pullulan coated iron oxide

Final Results



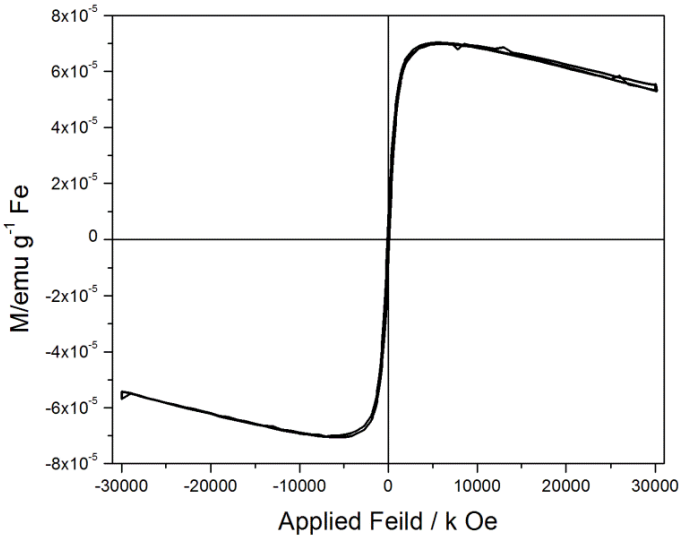


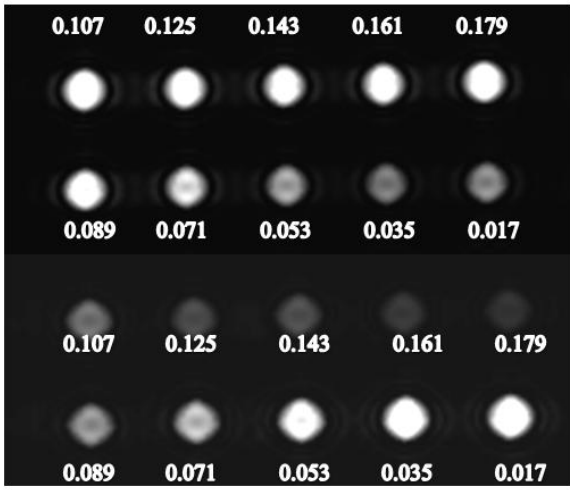




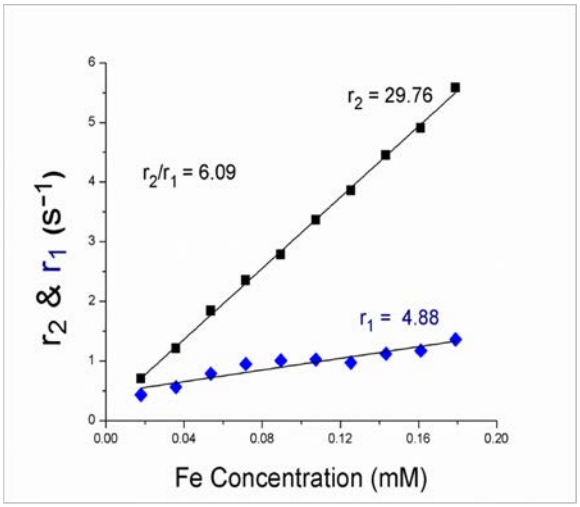
Zerovalent iron nanoparticles for MR angiography

The Magnetic properties of developed Zerovalent iron nanoparticles were determined by magnetic hysteresis using Vibration Sample Magnetometer. The magnetic hysteresis loop of the sample measured at room temperature exhibits a paramagnetic behaviour, with a remnant magnetization of ca. $7E^{-5}$ emu/g which is lower than the superparamagnetic iron oxide nanoparticles (40–70 emu/g). The observed pattern of the hysteresis curve is an indication of the particle's single domain existence with only one orientation of magnetic moment. Paramagnetic property of the developed material enables its use as Ti contrast agent and hence further works were done in this direction





Pixel intensity plot T1 and T2



r1and r2 plot for ZVI nanoparticle

Cytotoxicity of ZVI nanoparticle was determined by MTT assay using HepG2 cells. Cell viability was checked for different concentrations (100 µg/ml, 200 µg/ml, 500 µg/ml and 1mg/ml) and it was found to be about 100% viable for 100 µg/ml concentration and 90% for 200 µg/ml. Only a slight decrease in cell viability was only observed for higher concentrations.

Blood Compatibility Studies

Blood compatibility studies of the particles have been carried out for its application in biomedical field, adopting similar procedures followed for the other materials above.

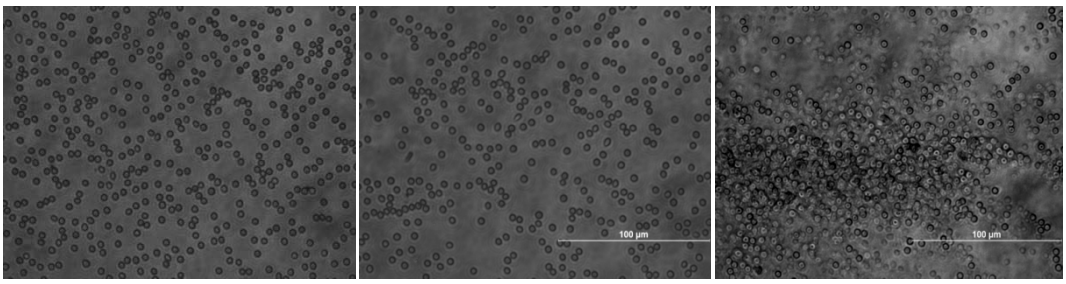
Hemolysis

Hemolysis assay was studied with different concentration of ZVI nanoparticles (50µg/ml, 100µg/ml, 200µg/ml). The percentage hemolysis was determined quantitatively. The results of hemolysis study showed no lysis when the particles were in contact with the erythrocytes related to the controls used. The percentage of lysis was found to be below 1% for the ZVI nanoparticles.

Blood Aggregation studies

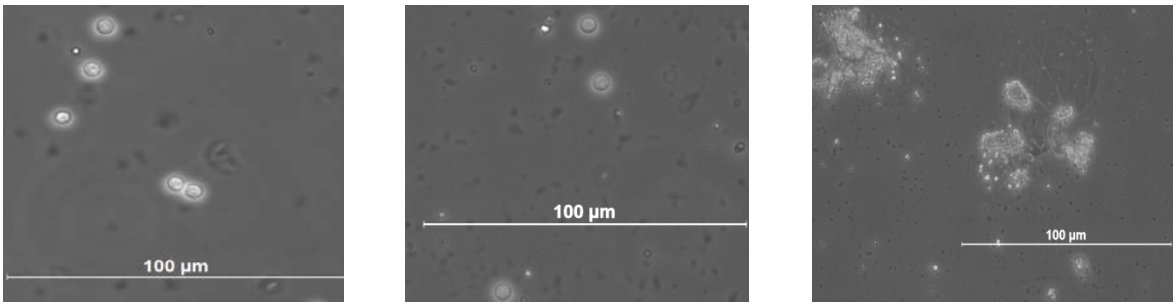
The washed RBC was diluted with saline in the ratio 1:4. The WBC was isolated by centrifuging whole blood layered on the histopaque. To the histopaque solution the whole blood was added carefully and centrifuged for 10mins at 1000rpm. Platelet rich plasma (PRP) was collected by centrifuging the whole blood at 1000 rpm for 20 minutes layered on histopaque solution. 200 µg of the ZVI nanoparticles were taken and to it was added 100ul of the diluted RBC, WBC and PRP separately and was incubated for 30 minutes at 37 ^{0}C. The negative control used was saline and positive control was polyethylene imine (PEI). Aggregation if any was detected through phase contrast microscope (Leica DM IRB, Germany) at 40X magnification. The particles showed no sign of aggregation with respect to the controls used. Saline was used as negative control and PEI as positive control.

RBC Aggregation study



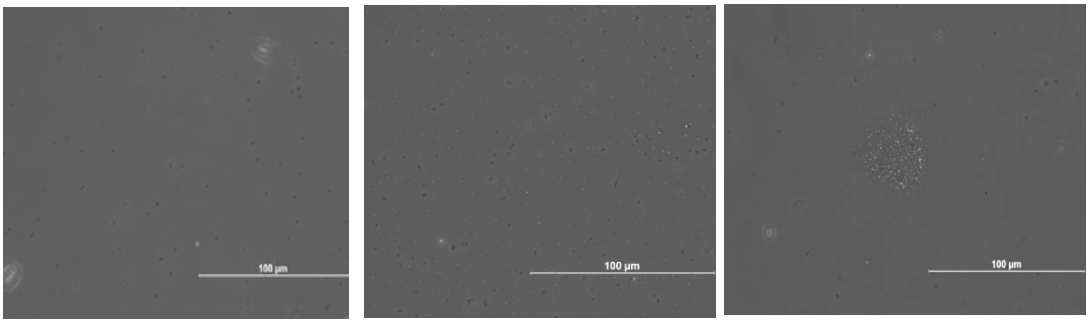
Saline ZVI PEI

WBC Aggregation study



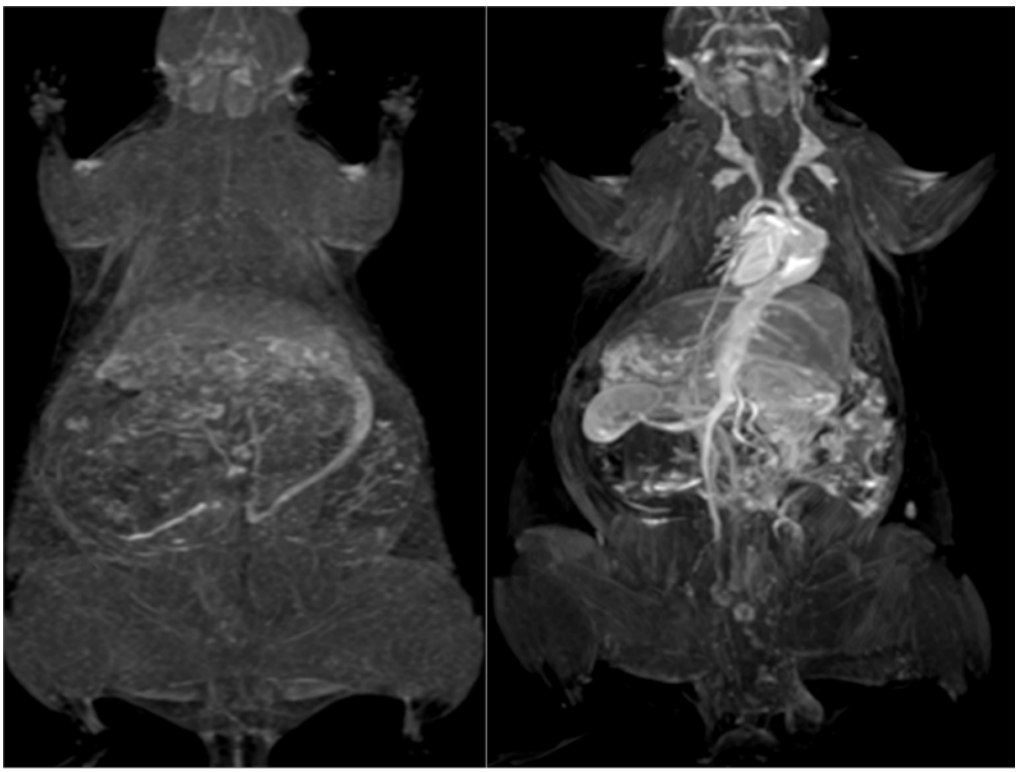
Saline ZVI PEI

Platelet Aggregation study



Saline ZVI PE

As the developed material showed good relaxivity properties for T1 weighted imaging, we have treid the same for MR angiogram by running routine angiogram protocol MR sequences by simultaneous acquisition of image and injection of the contrast agent. This has been performed using clinical 1.5T MRI scanner using standard head coil. The animals were injected intravenously through the tail vein at a dose of 50mg/Kg. Longitudinal relaxivity of iron nanoparticles in 1.5T applied field (r 1) is 4.88 $mM^{-1}S^{-1}$, which is comparable with that of the commercially available Dotarem (r 1= 3.6 $mM^{-1}S^{-1}$) and the particle has got a transverse relaxivity (r2) value of 29.76 $mM^{-1}S^{-1}$. The pronounced longitudinal relaxivity of iron nanoparticles suggests its potential as a positive contrast agent. The results obtained are really encouraging and it is the first of its kind in many ways and hence has been proposed for a patent application.



Pre and post contrast MR angiogram.

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