Facile Development of Iron-Platinum Nanoparticles to Harness Multifunctionality in Single Entity

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Abstract Engineered magnetic nanosystems exhibit attractive options for implementing unique diagnostic options and therapeutic solutions in biomedical applications. Here we report a facile, thermo-free and aqueous synthetic method to prepare ascorbic acid-stabilized iron-platinum nanoparticles. The effects of reducing agent, pH and sequence of precursor addition are investigated, and optimized reaction condition is identified to obtain a unique iron-platinum (Pt-FePt) nanosystems. The multifunctionality of the developed nanosystem has been realized by catalytic efficiency of platinum for therapeutic application and superparamagnetic property of FePt for magnetic resonance imaging contrast enhancement. Moreover, the multifunctional imaging and therapeutic activities have been achieved at physiological pH. The developed multifunctional nanoparticles are monodisperse with uniform morphology as well as stable in solution and non-toxic.

KEY WORDS: Multifunctional; Superparamagnetic; Magnetic resonance imaging (MRI); Therapeutic

1 Introduction

Nanoparticles have been attracting a considerable attention because of their unique physical and chemical properties in multidisciplinary applications. Magnetic nanoparticles have shown to be applicable as magnetic resonance imaging (MRI) T_1 [1] and T_2 [2, 3] contrast agents, targeting and therapeutic agents [4]. To confer multifunctionality to such systems, attempts have been made by coating the magnetic nanoparticles with biofunctional molecules (e.g., antibodies,

ligands or receptors) [5, 6] or by combining magnetic nanoparticles with other functional nanostructures (which may be fluorescent, catalytic or metallic) to generate hybrid nanosystems [7]. For example, simultaneous drug delivery, targeting and contrast enhancement in imaging technique can be obtained from hydroxylated mesoporous nanosilica coated by polyethylenimine coupled with gadolinium and folic acid [8]. Concurrently, imaging and photodynamic therapy are also explored by functionalizing iron oxide nanoparticles with Ir complexes in silica composites [9]. Also, composites of iron-platinum nanoparticles and carbon nanotubes (CNTs) functionalized with folic acid and fluorescein isothiocyanate emerge as a potential candidate for simultaneous diagnosis, therapy and targeting [6]. But a single nanomaterial with multiple functionality is highly desired for simultaneous diagnosis and therapy. This will make the material development process more facile and cost effective. Among magnetic nanoparticles, FePt nanoparticles are superior to conventional superparamagnetic iron oxide nanoparticles (SPIONs) as MRI contrast agents because of their higher magnetocrystalline anisotropy energy, saturation magnetization and chemical stability. In

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addition to this, iron released from fcc FePt at lower pH range can cause tumor cell death by generating reactive oxygen species (ROS) [10, 11].

However, general methods for preparation of FePt nanoparticles involve decomposition of precursors at higher temperatures in nonaqueous medium and use of hydrophobic surfactants viz. oleic acid, oleylamine. [12, 13]. To obtain the multifunctionality required to act as an MRI contrast agent and ROS generator without harsh pH treatments within a single system of FePt nanoparticles, developed through a facile, aqueous, thermo-free synthetic route, is of immense importance. A naturally occurring vitamin, ascorbic acid is used as reducing agent to synthesize metal nanoparticles due to its high water solubility, biodegradability and low toxicity. Because of significant difference in their standard reduction potential, ascorbic acid is a good reducing agent for noble metal precursors ($E^\circ(\text{Pt}^{2+}/\text{Pt}) = +1.2 \text{ V}$, $E^\circ(\text{ascorbic acid}) = +0.06 \text{ V}$). While acting as a reducing agent, its oxidized form is coordinated to the nanoparticle surface which serves to act as a capping agent [14].

In this work, we present a facile, one-pot, aqueous and thermo-free chemical route for the synthesis and stabilization of iron-platinum nanoparticles using ascorbic acid as capping agent. We observed that the use of reducing agent, pH and sequence of precursor addition plays key roles in the formation of the nanoparticles with different magnetic properties ranging from soft ferromagnetic to paramagnetic through superparamagnetic. Presence of the cubic Pt phase along with the FePt phase imparts the capability to be used as an MRI contrast and therapeutic agent. Herein, contrast enhancement in MRI by using the iron-platinum nanoparticles as contrast agent and ROS generation from H_2O_2 (mimicking the cellular production of H_2O_2) at physiological pH by employing the catalytic property of Pt were studied.

2 Materials and Methods

2.1 Materials

$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, ascorbic acid, NaBH_4 , H_2O_2 and NaOH were purchased from Merck. The Pt precursor $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ and the dye 2', 7'-dichlorofluorescein diacetate were procured from Sigma-Aldrich.

2.2 Synthesis of Nanoparticles

P1 A facile direct aqueous synthesis procedure is performed as follows. Ascorbic acid (10 mL, 0.1 M) was dissolved in water and mixed with $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (2 mL, 0.033 M in water) solution at room temperature by magnetic stirring. After 1.25 h, $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ (2 mL,

0.005 M in water) was added to the above mixture in a dropwise fashion and then the reaction mixture was aged for 24 h without stirring. Afterward, the black precipitate was obtained by centrifugation at 14,000 r/min for 15 min. Purification of the product was done by washing with water, ethanol and acetone for several times.

P2 To see the effect of coreduction method in the synthesis of nanoparticles, a secondary reducing agent, NaBH_4 was introduced along with ascorbic acid. Hence, keeping the molar ratio of precursors same as before (P1 synthesis), only NaBH_4 (0.033 M, 0.2548 g) was newly introduced to the reaction mixture after all other precursors are added.

P3 In this method, ascorbic acid (10 mL, 0.1 M) and $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (2 mL, 0.033 M) solutions were mixed at room temperature. This mixture was kept on stirring for 20 min, and then 0.2548 g or 0.033 M of NaBH_4 was added to it. Instantly, black-colored precipitate was formed which was characterized to be iron particles from energy dispersive X-ray spectroscopy (EDS) spectra (data not shown). But, this reaction continued for very short period of time and the solution became completely clear within 30 min, i.e., the formed iron particles dissolute. After that, the platinum precursor, $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ (2 mL, 0.005 M) was added to the above mixture dropwise. The reaction mixture was left as such for 24 h, and then the black precipitate was obtained by centrifugation at 14,000 r/min for 15 min. The purification of the precipitate was done by water, ethanol and acetone washing for several times.

2.3 Cell Culture

Hella cells (obtained from NCCS, Pune, India) were cultured in Dulbecco modified Eagle's medium (DMEM) supplemented with L-glutamine, penicillin, streptomycin and 10% fetal bovine serum. The cells were incubated in a 5% CO_2 incubator at 37 °C.

2.4 Cytotoxicity Assay of Iron-Platinum Nanoparticles

The cytotoxicity of iron-platinum nanoparticles was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay in which tetrazolium salt, MTT was reduced to purple-colored formazan by living cells. About 1×10^4 cells/well were seeded in 96-well plate and were incubated in DMEM media for 24 h in presence of 5% CO_2 at 37 °C. Then nanoparticles of various concentrations (4–20 $\mu\text{g/mL}$) were added to the cells and kept for 24 h under similar conditions. After that, 7.0 μL of MTT was added to each cell and kept for 2 h incubation. Finally, the medium was replaced with dimethylsulfoxide (DMSO) for the development of formazan which absorbs at 550 nm.

All the experiments were carried out in triplicate, and the percentage of cell viability of control was taken as 100%.

2.5 Catalytic Activity of the Nanoparticles in Generation of ROS

To mimic the cellular production of ROS, the primary defense of the body, exogenous oxidant H_2O_2 was used. Fenton's reaction employs Fe-catalyzed H_2O_2 decomposition in which highly reactive oxygen species such as OH radicals are generated to cause death of tumor cells, in which acid etching of Fe-based nanoparticles was carried out for Fe release to take place [10]. To achieve similar kind of therapeutic activity at physiological pH avoiding harsh processes like acid etching, the catalytic property of Pt to decompose H_2O_2 generating ROS was examined.

Generation of ROS from Pt-catalyzed H_2O_2 decomposition was evaluated by using 2',7'-dichlorofluorescein (DCFH) as the indicator dye. DCFH was produced immediately before each assay by incubating 2',7'-dichlorofluorescein diacetate (DCFHDA) in NaOH (0.1 M) for 20 min. After incubation, basic DCFH solutions were diluted in phosphate buffer at neutral pH, for the experiments. To carry out the H_2O_2 decomposition experiment, six reaction flasks were taken. To each reaction flask, 0.125 mL of 0.01 M DCFH, 0.05 mL of 0.32 M H_2O_2 and 4.825 mL of Tris-HCl buffer solutions were added. The pH of the solution was checked to be 7.4. Then, five different concentrations of nanoparticle solution in water were added to five flasks and one was the control reaction flask without nanoparticles. Then, room temperature shaking was applied for 12 h, followed by centrifugation to separate the nanoparticles. With the supernatant solutions, UV-visible absorption spectroscopy was carried out. All the reactions and characterizations were carried out in the dark (light-protected chamber).

For fluorescence microscopic study of ROS generation from H_2O_2 with DCF (oxidized form of DCFH) as the indicator, solutions from control as well as supernatants of the reaction mixtures were drop-casted over glass slides and then covered with cover slips. They were allowed to dry in air for some time, and then observed under fluorescence microscope, excited with wavelength 340–380 nm.

2.6 Contrast Property of Nanoparticles in MRI

Superparamagnetic FePt nanoparticles can be used as transverse relaxation (T_2) time contrast agent in MRI technique. When targeted to malignant tissue, the superparamagnetic materials should be accumulated at a lesion site and decrease the T_2 of water in the same region. This leads to reduction in MRI signal in T_2 -weighted image in

malignant tissue, and hence enhanced contrast between malignant and normal tissue is obtained. T_2 of water with different concentrations (0.05, 0.10, 0.25 and 0.5 mg/mL) of nanoparticles was measured using a spin-echo sequence.

The contrast property of nanoparticles is evaluated in vivo in Balb C mice. Mice were anesthetized with isoflurane (2.0%–2.5%) mixed in air and O_2 (70%:30%) and positioned inside a ^1H volume coil. T_2 -weighted magnetic resonance images of the abdomen of mouse were obtained before and after (45 min) administration of nanoparticles using the parameters: $T_R = 4000$ ms, $T_E = 14$ ms, slice thickness = 0.5 ms, FOV = 28 mm $\times$ 28 mm; matrix size = 128 $\times$ 128 and number of averages = 2. The ascorbic acid-capped FePt nanoparticles were injected through tail vein, 5 mg/kg (0.5 mg/ml) in mouse (~ 30 g). The acquisition of the MRI was synchronized with the respiration of the animals (measured by placing a balloon under the lung) to correct the artefacts related to the respiration.

2.7 Characterization

Phase, composition and microstructure of the synthesized nanoparticles were evaluated by X-ray diffraction (XRD, Rigaku diffractometer with $\text{CuK}\alpha$ radiation) and further corroborated by high-resolution transmission electron microscopy (HR-TEM, Jeol JSM-2200FS operated at 200 kV). The compositional characterization of the nanoparticles was carried out with the help of EDS (7582, Oxford Instrument, UK) and atomic absorption spectroscopy (AA240 Varian) techniques. The magnetic properties were analyzed by a vibrating sample magnetometer (Lakeshore 7410). Nanoparticles were also studied through Fourier transform infrared (FTIR) spectroscopy (Nicolet Impact I-410). The zeta potential measurement of the samples was carried out using a Malvern Zeta Size Nano ZS-90 instrument at room temperature. The optical properties of the indicator dye were analyzed by UV-visible absorption spectroscopy (Shimadzu, UV-2450), and the fluorescence micrographs were taken by an inverted microscope LEICA DMI6000B using excitation filter of wavelength 340–380 nm (A filter). MRI reflexivity was measured with a 14.1 T NMR micromagnet using repetition time T_R -6 s and echo time T_E in the range of 7–600 ms. MRI was done using a Bruker 89 mm WB Ultra shield plus 600 MHz Avance II imaging system.

3 Result and Discussion

3.1 Microstructural and Compositional Analysis

The samples were prepared with slight differences in reaction conditions. Sample P1 was prepared by direct

reduction of $\text{Fe}(\text{NO}_3)_3$ and Na_2PtCl_6 by ascorbic acid in aqueous medium. Fe and Pt precursors were added in a stepwise manner with almost an hour gap. Samples P2 and P3 were synthesized by adding the reducing agent NaBH_4 in addition to ascorbic acid. P2 synthesis consists of simultaneous reduction of Fe and Pt precursors while in P3, Fe particles were formed initially and re-dissolved before addition of the Pt precursor due to the acidic pH of the reaction medium, which was substantiated by the disappearance of black precipitate followed by formation of a clear solution within 15 min. The initial pH of the ascorbic acid and $\text{Fe}(\text{NO}_3)_3$ solution mixture was found to be 2.3, while after NaBH_4 addition, pH increased to 4. Studies on pH-dependent dissolution of iron particles reveal the possibility of dissolution at such pH [15]. When the Pt precursor was added to the reaction mixture containing the reducing agent, rapid formation of Pt nanoparticles occurs, while Fe particles slowly reform after initial dissolution.

In this regard, it is worthwhile to mention that ascorbic acid is a good reducing agent for the synthesis of noble metals [16] and hence the reduction of the noble metal precursor becomes faster. In case of P1, though ascorbic acid induces faster nucleation of Pt slow formation of Fe finally ends up in larger size of FePt nanoparticles with soft ferromagnetic character, whereas in P2 synthesis NaBH_4 plays the role of primary reducing agent which can efficiently cause fast nucleation of both Fe and Pt leading to smaller sized FePt nanoparticles. In this regard, it is already reported that rapid nucleation results in smaller nanoparticles [17]. However, in case of P3 synthesis, initial dissolution of Fe nuclei at acidic pH and later reformation finally yields a smaller sized P3 than P2. The details of size distribution and magnetic characterization of three types of nanoparticles are discussed in subsequent sections. Ascorbic acid and NaBH_4 perform coreduction in samples P2 and P3. Nucleation is initiated by the action of NaBH_4 , while growth is followed by the synergistic reduction by ascorbic acid at room temperature. Ascorbic acid also plays the role of antioxidant by scavenging the free radicals during nuclei formation [18].

Compositional analysis of the nanoparticles has been carried out with the aid of EDS, and the results are shown in Fig. 1. The average Fe and Pt atomic compositions for the three samples as determined from 5 to 6 different locations are found to be $\text{Fe}_{0.04}\text{Pt}_{0.95}$ (P1), $\text{Fe}_{0.14}\text{Pt}_{0.86}$ (P2) and $\text{Fe}_{0.12}\text{Pt}_{0.88}$ (P3), respectively. To see the effect of pH on the composition of FePt system, we have synthesized P3 sample in higher pH (~ 10), and from the EDS analysis, the composition is found to be $\text{Fe}_{0.88}\text{Pt}_{0.12}$. It is also observed that the solution became black after addition of NaBH_4 . The formed particles remain stable afterward unlike P3 sample synthesis. The average weight ratio of metal ion concentrations in the nanoparticles obtained from

EDS result was found to be the same as the atomic ratio between metal ions from atomic absorption spectroscopy (AAS) result.

The XRD pattern of three samples shows the highest intensity peak $\sim 39^\circ$ for 2θ assigned to (111) planes of cubic Pt phase (PDF: 88–2343). The XRD pattern of P1, shown in Fig. 2a, indicates the presence of both tetragonal L1_0 and cubic A1 phases of FePt along with cubic Pt phase. But in samples P2 and P3, the L1_0 phase of FePt is not observed. Under the condition of rapid reduction of Pt precursor in P1 synthesis, there will be sufficient Pt atoms leading to fast surface growth (as evident from microstructural analysis) and also resulting in evolution of ordered L1_0 phase in P1 sample. P2 synthesis incorporates the simultaneous reduction of Fe and Pt precursors resulting in less intense and broad XRD peaks for cubic Pt phase as seen in Fig. 2a. This is also evident from EDS analysis that Fe composition is the highest in P2; therefore, the XRD peak corresponding to cubic Pt evolves out with more intensity in P1 and P2. The reaction conditions of sample P3 are similar to sample P1, but the coreduction by ascorbic acid and NaBH_4 may be responsible for the absence of L1_0 FePt phase, in contrast to sample P1. The XRD analysis data of three samples are presented in Table 1.

To assess magnetic property of the samples, M – H characterizations of the three samples were carried out and the results are shown in Fig. 2b–d. The coercivity of magnetic nanoparticles is mainly dependent on two factors: (1) particle size and (2) ordering [19]. The P1 sample is soft ferromagnetic in nature, as observed from its M – H curve having coercivity ~ 240.5 G (Fig. 2c). The ferromagnetic behavior is quite obvious since the ordered L1_0 fct phase of FePt is present in this sample, along with the A1 fcc FePt phase with disorder in lattice site occupation, and cubic Pt phase. It is noteworthy to mention here that pure Pt particles were synthesized following the same procedure and their magnetic properties were evaluated. From Fig. 2b, diamagnetic behavior of pristine Pt particles is clearly visible. Therefore, the cubic Pt phase in the nanoparticle should not contribute to the soft ferromagnetic property of FePt. The M – H curve of P1 sample is replotted within magnified X and Y range around the origin (Fig. 2c). From this plot, shifting of the hysteresis loop away from the origin is noticeable which is the signature of the exchange bias phenomenon [20]. As estimated from the M – H plot, the exchange bias field is obtained to be 47.5 G for P1. From this, formation of antiferromagnetic L1_2 FePt₃ phase can also be assumed due to the utilization of ascorbic acid as reducing agent (efficient for noble metal reduction) leading to the Pt-rich phase. Sample P2 is superparamagnetic by nature with negligible coercivity as evident from its magnetization curve (Fig. 2d). The magnetic measurement of sample P2 at low temperature

Fig. 1 EDS spectra of a P1, b P2, c P3, d P3 synthesized at alkaline pH

Fig. 2 a XRD patterns of P1, P2, P3, b M – H curves of P1, P3 and Pt particles, c exchange bias in P1, d M – H curve of P2, e M – T curve of P2

(M – T measurement) has been carried out, and the result is shown in Fig. 2e. From the M – T characterization curve of sample P2, the superparamagnetic property is further confirmed by splitting of field cooled (FC) and zero-field-cooled (ZFC) curves at a temperature above blocking temperature, T_B (144.6 K). The superparamagnetic behavior in this nanoparticle can be attributed to their ultrafine dimension. Most interestingly, sample P3 is paramagnetic (Fig. 2b). In alloys like FePt, the magnetization decreases as the atomic percentage of Pt

increases in the overall composition. Therefore, it can be inferred that Pt acts as a diluent of magnetization. In such dilute conditions, Fe atoms (magnetic) are much apart from each other and hence the exchange interaction is unable to align the spins of nearest neighbor Fe atoms in parallel arrangement, resulting in paramagnetic behavior as a whole. Such a diluted composition of P3 sample can be understood from the observations made during its synthesis as described in the first paragraph of Sect. 3.1.

Table 1 Analysis of XRD patterns of three samples

Value of 2 θ	Plane	System	Reference PDF
33°	(110)	L1 ₀ FePt	65-4674
39°	(111)	Cubic Pt	88-2343
44°	(200)	Cubic Pt	87-0644
46°	(200)	A1 FePt	29-0717
65.96°	(220)	Cubic Pt	88-2343
67°	(220)	L1 ₀ FePt	89-2051

HR-TEM images along with fast Fourier transform (FFT) image of the single particle of three samples are shown in Fig. 3a, c, e. Lattice spacing value measured from the FFT images shows the presence of basically L1₀ phase in P1 whereas A1 phase in samples P2 and P3, respectively. Data from HR-TEM micrographs were used to obtain size distribution profiles. Average particle sizes are found as ~ 4.0 , 2.2 and 1.9 nm for P1, P2 and P3, respectively. The selected area electron diffraction (SAED) pattern of P1 (Fig. 3b) clearly shows the ring of bright spots of (100) plane with d -spacing of 0.39 nm, which is a fingerprint of the L1₀ FePt phase. However, (111) plane

with d -spacing 0.21 nm and (200) plane with d -spacing 0.14 nm of disordered A1 phase of FePt are identified in the SAED pattern of P1. Because of very similar lattice spacing characteristics between cubic Pt and A1 FePt phases, it is very difficult to distinguish their presence in the samples, although in P2 and P3 samples, only the planes of A1 and/or pure Pt phases are observed.

The surface characteristics of the synthesized samples are examined by FTIR spectroscopy and streaming potential measurements. The FTIR spectra and streaming potential plots of three samples are shown in Fig. 4. In case of P1 and P3 samples, almost similar bands are observed in the FTIR spectra (Fig. 4a, c). In this regard, it is worthwhile to mention that ascorbic acid acts as a reducing agent by oxidizing its C=C double bond as well as its oxidized form, dehydroascorbic acid, acts as a capping agent through the interaction of its carbonyl group with the FePt nanoparticles [14]. This fact is corroborated by the absence of C=C stretching band at ~ 1650 cm⁻¹ and the presence of a band at 1626 cm⁻¹ due to the adsorption of dehydroascorbic acid carbonyl to the nanoparticle surface [14]. Another band observed at 1725 cm⁻¹ is due to the oxidated ester carbonyl of

Fig. 3 a, c, e HR-TEM images (insets showing the FFT images of single crystalline particles), b, d, f SAED patterns (insets showing the size distribution of particles) of P1, P2 and P3, respectively

Fig. 4 FTIR spectra of **a** P1, **b** P2, **c** P3

dehydroascorbic acid [14]. However, the scissoring, symmetric and antisymmetric stretching of C–H vibrational bands is observed at 1474, 2853 and 2924 cm^{-1} , respectively [21]. Also the bands corresponding to lactone ring deformation as well as C–O stretching vibration are observed at 1030 and 1165 cm^{-1} , respectively [22]. In the spectra of P3 sample, a band at 1379 cm^{-1} is observed corresponding to C–O–H bending vibration (Fig. 4c) [22]. In case of P2 sample, only the 1630 cm^{-1} band is observed prominently corresponding to the coordination of dehydroascorbic acid to nanoparticles (Fig. 4b). Furthermore, the presence of strong O–H stretching mode at around $\sim 3300 \text{ cm}^{-1}$ confirms the presence of hydroxyl groups in all three samples, which provide hydrophilicity to the nanoparticles increasing their water solubility. In case of sample P2, this band has been shifted to $\sim 3500 \text{ cm}^{-1}$. This is because of very dilute nature of the solution, hence, no H-bonding occurs among O–H groups leading to free hydroxyl groups. Consequently, the stretching mode of free hydroxyl group, as well as that from solvent water, masks the other characteristic peaks of the surfactant molecule. From the zeta potential measurement of three samples, the potential values are found to be negative in their as-prepared dispersions in water of pH ~ 6 . The values are obtained as -37.1 , -43.2 and -44.4 mV for P1, P2 and P3 samples, respectively. Hence, it is observed that the nanoparticles form a stable dispersion in water which is important for biological applications.

Being superparamagnetic in nature, the sample P2 (prepared by simultaneous reduction of Fe and Pt precursor at acidic pH using both ascorbic acid and NaBH_4 as reducing agent) is carried forward for examining multifunctionality in a single system suitable for biomedical application.

3.2 Generation of ROS from H_2O_2 Decomposition Catalyzed by Iron-Platinum Nanoparticle

Fluorescent product 2', 7'-dichlorofluorescein (DCF) is formed by oxidation of non-fluorescent DCFH [23], caused

by the ROS generated through nanoparticle catalyzed H_2O_2 decomposition. Figure 5a shows an increase in absorbance of DCF at $\sim 502 \text{ nm}$ with increase in nanoparticle concentration, which is elaborated in the inset of Fig. 5a. Increase in nanoparticle concentration causes increase in H_2O_2 decomposition; hence, enhanced oxidation of the DCFH results in increased absorbance of the oxidized form of DCF. The same inference follows from the observed many-fold increase in fluorescence intensity in fluorescence micrographs (Fig. 5b, c).

3.3 Cell Viability Study

As shown in Fig. 6, after 24 h of exposure, the MTT assay shows no significant cytotoxic response (the cell viability $>80\%$) detected at concentration of 20 $\mu\text{g/mL}$ and below for the P2 sample of iron-platinum nanoparticles.

3.4 Iron-Platinum Nanoparticles as MRI Contrast Agent

It is observed from relaxivity measurement of T_2 -weighted proton that T_2 of water decreases drastically (55 vs 200 ms for pure water) at a concentration of 0.28 mM nanoparticles (Fig. 7a). T_2 values are further decrease with increasing concentration of FePt nanoparticles. The transverse relaxivity, r_2 , determined by plotting the transverse relaxation rate, $R_2 = 1000/T_2$, against the concentration of nanoparticles, was found to be 33 $\text{mM}^{-1} \text{ s}^{-1}$ (Fig. 7b) which makes it a promising T_2 contrast agent for MRI application.

To measure in vivo T_2 magnetic resonance image, aqueous sample solution of ascorbic acid-capped FePt nanoparticles was injected into mouse tail vein. As shown in Fig. 7, distinct negative contrast enhancement in liver (Fig. 7e, f, indicated with arrows) following administration of FePt nanoparticles as compared to the corresponding pre-administered images (Fig. 7c, d) was observed. These results imply that the nanoparticles are potential T_2 MRI

Fig. 5 **a** Decomposition of H_2O_2 using P2 indicated by increasing absorbance of ROS-sensitive DCF. Here samples 1, 2, 3, 4 and 5 are designated by the amount of P2 used as 50, 100, 150, 200 and 250 μL of a solution of concentration 1 mg/mL. Inset shows the plot between absorbance of the indicator dye against nanoparticle concentration. **b, c** Fluorescence micrographs of control and sample 5

Fig. 6 In vitro cytotoxicity evaluated by MTT assay using Hella cells in the range of 0–20 $\mu\text{g/mL}$ iron-platinum nanoparticles after incubation time of 24 h

contrast agents with high efficiency both in vitro and in vivo conditions.

4 Conclusion

In this work, we have presented an optimized reaction condition to produce novel superparamagnetic iron-platinum nanoparticles consisting of cubic Pt and FePt phases. The method proposed is facile, easy-to-control, fast, reproducible and up-scalable. The nanoparticle encapsulation by ascorbic acid is the pivotal factor that improves the

Fig. 7 **a** Transverse relaxation time (T_2), **b** transverse relaxation rate ($R_2 = 1000/T_2$) with varying concentration of P2. The slope of the line in panel **b** indicates the relaxivity. T_2 -weighted magnetic resonance images of mouse abdomen pre **c, d**-, post **e, f**-administration of P2. Hypointensity in liver following administration of P2 is indicated with white arrows **e, f**

biocompatibility and colloidal stability of the particles. Due to the presence of cubic Pt phase, the nanoparticles possess the potential for ROS generation by catalyzing H_2O_2 decomposition, useful for tumor cell death. This, along with their T_2 contrast enhancement capability in MRI, ensures their efficacy as both therapeutic and diagnostic agents in biomedical applications. Achieving multifunctionality in a single entity of novel nanosystem for biomedical applications at physiological pH is the key aspect of this study.

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