Vitamin E (d-alpha-tocopheryl-co-poly(ethylene glycol) 1000 succinate) micelles-superparamagnetic iron oxide nanoparticles for enhanced thermotheraphy and MRI
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Abstract
We synthesized vitamin E TPGS ([d-z-Tocopheryl-co-poly(ethylene glycol) 1000 succinate]) micelles for superparamagnetic iron oxides formulation for nanothermotherapy and magnetic resonance imaging (MRI), which showed better thermal and magnetic properties, and in vitro cellular uptake and lower cytotoxicity as well as better in vivo therapeutic and imaging effects in comparison with the commercial Resovist® and the Pluronic®/C210 F127 micelles reported in the recent literature. The superparamagnetic iron oxides originally coated with oleic acid and oleylamine were formulated in the core of the TPGS micelles using a simple solvent-exchange method. The IOs-loaded TPGS showed greatest colloidal stability due to the critical micelle concentration (CMC) of vitamin E TPGS. Highly monodisperse and water soluble suspension was obtained which were stable in 0.9% normal saline for a period of 12 days. The micelles were characterized for their size and size distribution. Their morphology was examined through transmission electron microscopy (TEM). The enhanced thermal and superparamagnetic properties of the IOs-loaded TPGS micelles were assessed. Cellular uptake and cytotoxicity were investigated in vitro with MCF-7 cancer cells. Relaxivity study showed that the IOs-loaded TPGS micelles can have better effects for T2-weighted imaging using MRI. T2 mapped images of xenograft grown on SCID mice showed that the TPGS micelle formulation of IOs had ~1.7 times and ~1.05 times T2 decrease at the tumor site compared to Resovist® and the F127 micelle formulation, respectively.

1. Introduction
The surface coating material strongly affect the colloidal stability of nano-sized iron oxides (IOs) suspensions, which also plays key role in determining the adsorption, distribution, metabolism and excretion (ADME) process of the iron oxides after administration. Various macromolecules for IOs coating include simple sugars such as polysaccharide (Dextran) using epichlohydrin (CLIO, Cross linked iron oxide nanoparticles) [1,2], hydrophilic compounds such as poly ethylene glycols (PEG) [3], and high molecular weight amphiphilic polymers such as PLGA [4] and PLA-TPGS [5], which are currently under intensive investigation for biomedical applications such as nanothermotherapy and magnetic resonance imaging (MRI).

Amphiphilic macromolecules have the tendency to self-assemble to form nano-sized colloidal micelles in water at a concentration greater than the critical micelle concentration (CMC). These self-assemblies are oriented in such a way that the hydrophobic part of the amphiphile is kept in the core and the hydrophilic part is in contact with the water. A main concern in the various commercial micellar formulations of imaging and therapeutic agents is their stability. They would disassemble in diluted solution below the CMC. Micelles formed from amphiphilic copolymer may have better resistance to disassembly due to the enhanced interaction among the polymer chains in the micelle core [6–11]. Micelles can be prepared by simply adding the amphiphilic polymer at a concentration above its CMC in water while having higher encapsulation efficiency of the imaging or therapeutic agent in the core.
Miles et al. emphasized the importance and effect of the coating materials on particle formulation in physiological buffer, where the phosphate groups of the buffer solution can interact with the coating, replace them and thus cause instability of the iron oxides [12]. Colloidal stability of iron oxides is a great concern for their biomedical applications. Micellar aggregates can cause blood vessel blockage after administration, which may result in localized hypoxia, necrosis and hypersensitive reactions. Moreover, unstable micelles can be easily recognized by the macrophages, resulting in opsonization. As a result the micelles are readily taken up by the reticuloendothelial system (RES). A possible solution is to make use of surfactant polymers, which can be formulated into nanocapsules, nanospheres and micelles [13]. Nanocapsules contain a hydrophobic core carrying the imaging or therapeutic agent to be encapsulated. The hydrophobic core consists of solvent of high boiling point, which impedes its application in drug delivery due to the toxicity associated with it. Biodegradable nanoparticles, on the other hand, provide an excellent choice for excellent colloidal stability. However, nanoparticles synthesis requires careful optimization and proper selection of solvent and stabilizers. Preparation of micelle is less tedious if the stability problem can be addressed. Diluting the micelle suspension below the CMC, may cause disassembly of the micelle. In many cases, the micelles are cross linked using linker molecules. Unfortunately, most of the linker molecules are not acceptable to be used for biomedical applications [14].

Pluronic®F127 macromolecule surfactant (Poloxamer 407, HLB of 18–23, CMC of 0.05 wt.%) is a block copolymer, which is intensively used for biological application such as for encapsulation of cells [15], for membrane and membrane protein solubilization as well as for biomedical applications for designing drug delivery vehicle [16] and multifunctional nanoparticles [17–19]. δ-δ-Tocopheryl-co-poly (ethylene glycol) 1000 succinate (TPGS, HLB ~ 13, CMC 0.02 wt.%), on the other hand, has been an effective surfactant. TPGS is used in combination with chemotherapeutic drug in order to inhibit P-glycoprotein (P-gp) [20–23] and increase the chemotherapeutic efficacy for cancer treatment. P-gp protein is a class of multi-drug resistance proteins that are present on the cell membrane, which cause increased efflux of drugs, thereby reducing the efficacy of the drug. TPGS has also been used efficiently as an emulsifier for synthesis of nanoparticles of biodegradable polymers, providing a high encapsulation efficiency and cellular uptake of the drug in vivo [5,24,25]. TPGS has also been used for pro-drug design for enhanced chemotherapy [26,27].

In this research, F127 and TPGS are employed to develop micellar formulation of iron oxides for their potential application for nanothermotherapy and magnetic resonance imaging (MRI) in close comparison with the commercial Resovist®. The magnetic micelles of the two macromolecular surfactants of their various molecular weights and the hydrophilic-lipophilic balance (HLB) ratio are investigated. The polymeric surfactants are assigned a HLB, which determines the surfactant characteristics. For instance a lower HLB value means more hydrophobic and higher HLB value indicates more hydrophilic. The colloidal stability of the two magnetic micelles was attributed to the surfactant HLB. The two micellar formulations of iron oxides were investigated in close comparison for their hyperthermia and magnetic properties, in vitro cellular uptake and cytotoxicity, in vivo biodistribution and MRI imaging on SCID mice of xenograft tumor model.

2. Materials and methods

2.1. Materials

δ-δ-tocopheryl polyethylene glycol 1000 succinate (TPGS, C55H96O44 (CH2CHO)23) was from Eastman chemical company (USA). Pluronic®F127 (Poloxamer 407, molecular weight of 12,500) was brought from BASF (Ludwigshafen, Germany). Surfactants were freeze-dried for two days before use. Millipore water was prepared by a Milli-Q Plus system (Millipore Corporation, Bedford, USA). All chemicals including absolute ethanol, dimethylformamide (DMF) and tetrahydrofuran (THF) were of HPLC grade. They were used without further purification. Dialysis membrane was brought from Spectra/Por®. MWCO of 1000. Hydrophobic iron oxides coated with oleic acid and oleylamine were synthesized by decomposition method reported elsewhere [5,28].

2.2. Synthesis and characterization of IOs-loaded micelles

Iron oxides-loaded micelles of TPGS and F127 were synthesized by a dialysis method. 150 mg of TPGS or 200 mg of F127 surfactant was taken in a 10% ethanol solution. 50 mg of iron oxide in THF was added into the 10% ethanol solution and sonicated by a probe sonicator at 25W for 2 min. The solution was transferred into a dialysis bag with an MWCO of 1000 and dialyzed against 5% ethanol for 2 h with change of solvent every 1 h and then with Milli-Q water for 6 h with changes of water every 1 h. Dialyzed solution was concentrated using a rotary evaporator system under reduced pressure. A certain amount of the sample was freeze-dried. The liquid sample was then analyzed for micelle size by Zetasizer (Nano ZS, Malvern Instruments Ltd, UK), morphology by transmission electron microscopy (TEM, JEOL JEM-2100F, JEOL, Japan). The amount of coating was calculated by thermogravimetric analysis (TGA) using dry sample. The iron content of the magnetic micelles was determined using inductively coupled plasma mass spectroscopy (ICP-MS).

2.3. ICP-MS analysis

A known volume of IOs-loaded micelle suspension or Resovists® were taken in glass test tube, 2 ml of concentrated nitric acid was added and heated to 90 °C for 45 min and the samples were analyzed using ICP-MS (Agilent ICP-MS 7500 Series) after sufficient dilution with Milli-Q water. The analysis of sample was done in comparison with the ICP-MS standard (Sigma).

2.4. Hysteresis

The hysteresis curve and the magnetic saturation (σs) of the dry IOs-loaded micelles were determined using a vibrating sample magnetometer (VSM, Lakeshore 7300 Series, USA). Dried sample of known mass was taken in non-magnetic glass test tube, 2 ml of concentrated nitric acid was added and heated to 90 °C for 45 min and the samples were analyzed using ICP-MS (Agilent ICP-MS 7500 Series) after sufficient dilution with Milli-Q water. The analysis of sample was done in comparison with the ICP-MS standard (Sigma).

2.5. Cell viability assay

MCF-7 cancer cells were seeded in 96 well plates (Costar, IL, USA) at the density of 20,000 viable cells/well using Dulbecco’s Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% antibiotics, and incubated for 24 h to allow cell attachment. The media was removed and then replaced with fresh media with IOs-loaded micelle suspension or Resovists® at the various concentrations. Untreated wells were used as control. Five hours prior the time point, 10 μl of MTt (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyloxazolium bromide, a yellow tetrazole, 5 mg/ml in PBS) was added into the 96 well plates. The cells were incubated till the designated time point. After incubation, 100 μl of a stop mix solution composing of 20% SDS in 50% DMF was added into each well. The plate was incubated for an hour to dissolve the formazan crystals that are formed and the absorbance in each well was measured using a microplate reader (GENios, Tecan, Switzerland) at 550 nm and 630 nm as reference wavelength. The cell viability was then calculated as:

\[
\% \text{ Cell viability} = \frac{\text{Absorbance of sample well}}{\text{Absorbance of control well}} \times 100
\]

2.6. Determination of SAR value and cell hyperthermia

Determination of the specific absorption rate (SAR) and cell hyperthermia was performed as described elsewhere in our earlier publication [29]. In brief, heating ability of the magnetic micelles was determined from the time-dependent calorimetric measurements using a RF generator (EASYHEAT-5060, Ameritherm) operating at 240 kHz frequency. One milliliter of aqueous suspension of magnetic micelles with the Fe concentration of 5 mg/ml were subjected to 89 kV/m AC field and time-dependent temperature rise was monitored for various designated times using an optical fiber based temperature probe (FLUOTEMP Series, FIP-LN2). The SAR was calculated using the following equation [29]:

\[
\text{SAR} = \frac{C \Delta T}{\Delta t \rho_{\text{Fe}}}
\]

where C is the specific heat of the solvent (here Cwater = 4.18 J/g°C), ΔT/Δt is the initial slope of the time-dependent temperature curve and ρFe is weight fraction of magnetic element (i.e. Fe) in the sample.

For in vitro cellular hyperthermia, 105 exponentially growing MCF-7 cancer cells in DMEM cell culture medium containing 10% FBS and 1% antibiotic-antimycotic solution were taken in 15 ml sterile polypolyethylene tubes and centrifuged at
1000 rpm for 5 min to get cell pellet. Then, the cells were re-suspended in 1 ml DMEM medium containing magnetic micelles having 1 mg of total iron (i.e. 1 mg/ml Fe concentration). The tubes were subjected to an initial AC field of 89 kA/m field using the RF generator operating at 240 kHz frequency and temperature rise of the cell suspension was monitored using the optical fiber based temperature probe. After the temperature reached to 45 °C, the field was adjusted accordingly to maintain the temperature at 45 °C for 1 h. For comparison, same numbers of cells were treated using magnetic field alone (no IOs) and another with IOs (1 mg of Fe/ml) without magnetic field. Cells without any treatment were used as control.

After the treatment, the cells were centrifuged and washed with PBS for 2 times. Then, 100 μl of 2 × 10⁵ cells/ml were seeded in 96 well plates in 16 replicates and following 24 h of incubation in 5% CO₂ incubator, MTT assay was performed to evaluate the cell viability. For this purpose, the cell culture supernatants were replaced with 100 μl each of fresh medium containing 10 μl of 5 mg/ml MTT each. The plates were then incubated for 5 h at 37 °C. The purple formazan crystals were dissolved in 50% DMF in water solution containing 5% SDS (sodium dodecyl sulfate). Thereafter, the absorbance was measured at 550 nm wavelength in a plate reader. The percentage of cell viability was calculated using equation:

\[
\text{Cell Viability } \% = \frac{\text{Absorbance from the well treated with IOs or magnetic field or both}}{\text{Absorbance from the well without any treatment}} \times 100
\]

2.7. Quantitative and qualitative cell uptake study

For quantitative cell uptake, MCF-7 cancer cells were seeded in 24 well plates (Costar, IL, USA) at a density 10,000 viable cells/well. The cells were allowed to grow for 24 h. After 24 h the media from the wells were replaced with fresh media with IOs-loaded micelle suspension or Resovists® at the various designated concentration. Wells without magnetic micelles were used as control. After incubating for 24 h the medium was removed. The plates were washed 4 times with PBS, each time with fresh exchange of PBS. The cells were then trypsinated, the number of cell was counted using a hemocytometer. The cell suspension was then centrifuged at 2000 rpm for 5 min. To the cell pellet, 2 ml of concentrated nitric acid was added and was heated to 80 °C. This dissolves and converts the magnetic micelles taken up by the cells into its corresponding ions. The solution was diluted using Milli-Q water, and the samples were analyzed by Inductively Coupled Plasma-Mass Spectrometer (Agilent ICP-MS 7500).

To assess the cell uptake qualitatively, the MCF-7 cancer cells were grown in 175 cm² culture flask at a cell density of 1 × 10⁵ cells/ml. After 24 h, the medium was replaced by fresh medium containing the magnetic micelles at a concentration of 2 mmol of Fe/l. After 4 h of incubation, the medium was removed; the cells were washed 4 times with PBS, and fixed with 2.5% glutaraldehyde at 37 °C. After 2 h, the glutaraldehyde solution was removed and the cells were washed with PBS. The cell was then scrapped and pelleted at 3500 rpm for 10 min. The pellet was post-fixed using 1% Osmium tetroxide at pH 7.4 for 1 h, followed by washing with PBS two times. The cell pellet was then fixed in 6% gelatin. Followed by a series of dehydration process using ethanol and acetone, and finally embedded in araldite resin. The sections were stained using lead citrate prior to viewing. The samples were viewed using Transmission Electron Microscope (JEOL, JEM-1220).

2.8. Magnetic resonance properties and in vivo MRI

Transverse relaxivity measurement and in vivo MRI were conducted on a Bruker 7T Clinscan MRI system.

2.8.1. Calculation of relaxivity

The transverse relaxation time T2 of the iron oxides formulated in the surfactant micelles were measured using phantoms at different [Fe] concentration for the given sample. A multiple spin echo sequence at TR = 5000 ms and TE = 6.8 ms was used. Transverse relaxivity (r2 in mM⁻¹s⁻¹) were obtained from linear least-squares determination of the slopes of 1/T2 versus the concentration of [Fe] plots.

2.8.2. Xenograft model and in vivo MRI

The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC), National University of Singapore (#802/05/A10/09) and the A*STAR Institutional Animal Care and Use Committee (Protocol #040/10).

![Fig. 1. TEM image of (A) the IOs-loaded TPGS micelles, (B) the IOs-loaded F127 micelles and (C) Resovists®. Scale – 50 nm.](image)

![Fig. 2. The stability study carried out for 12 days by measuring the size of the IOs-loaded micelles and Resovist® by laser light scattering at the various time points.](image)
Xenograft was grown on SCID mice of 7–8 weeks old female (20 g). Mice were anaesthetized, $1 \times 10^6$ MCF-7 cancer cells were injected subcutaneously on the right flank of the mice. The mice were monitored frequently for the tumor growth and the tumor was measured using a vernier caliper. Mice bearing a tumor of 80–120 mm$^3$ were used for MRI study. MRI was performed on mice on a Bruker 7T Clinscan MRI system. Contrast agent was injected (dosage: 5 mg of [Fe]/Kg body weight) through tail veins of the mice under 1% isoflurane anesthesia. $T_2$ mapped images were acquired pre-injection, and 45 min, 4 h, 24 h post-injection of contrast agent using multiple spin echo sequence at TR $= 3000$ ms and TE $= 6.8$ ms (resolution $= 100$ μm, thickness $= 1$ mm). Pixel-based $T_2$ values were calculated using in house program written in Matlab V7.2. MRicro 1.40 (Chris Rorden Copyright 1999–2005) was used to analyze the region of interest (ROI) of the $T_2$ mapped MRI images. The images were colour coded and the scale with the value of $T_2$ was provided.

3. Results and discussion

3.1. Characterization of magnetic micelles

IOs-loaded micelles of TPGS or F127 and Resovist® were characterized using transmission electron microscopy (TEM). Fig. 1 shows their TEM images. It was observed that the micelles of F127 were larger in size than the TPGS micelles. Nevertheless, both were monodisperse and uniform. Resovists® nanoparticles were smaller in size compared to the IOs-loaded micelles of TPGS or F127. The hydrodynamic size of the micelles as measured using the Zetasizer.
was found to be around 145.5 nm (PDI: 0.122) for TPGS IOs micelle, 178.2 nm (PDI: 0.231) for F127 IOs micelle, and 60.78 nm (PDI: 0.185) for Resovist® nanoparticles. Although the size of the IOs core of Resovist® is around 5–10 nm as found from TEM, its coated hydrodynamic size is around 60 nm as measured by Zetasizer. The size of the F127 micelles was consistent with that in a recent publication [19].

The stability study was performed by measuring the size of the micelles in Milli-Q water and 0.9% saline at the various designated time points. The size of the micelles measured by Zetasizer versus time in the two medium, one consisting of Milli-Q water and another with 0.9% normal saline (B.BRAUN, 0.9% w/v Sodium Chloride, Intravenous Infusion B.P.) is shown in Fig. 2 measured under room temperature. In Milli-Q water suspension, the IOs-loaded micelles of TPGS and Resovist® were observed to be very stable for almost 12 days, whereas the F127 micelles lost stability after 2 days and their size increased significantly on the 4th day, and aggregated and settled on the 12th day. In the 0.9% normal saline suspension, the IOs-loaded micelles of TPGS also showed great stability until day 12, after that only a small degree of aggregation was observed. Instead, the IOs-loaded micelles of F127 aggregated and settled in soon and their size were much larger than their size in Milli-Q suspension. Resovist® also showed significant level of instability and an immediate increase in size from 60.78 nm in Milli-Q water to 90.06 nm in 0.9% normal saline was observed. It can thus be concluded that as the colloidal stability regards, our TPGS micelles have great advantages against the F127 micelles reported in the recent literature and the commercial formulation Resovist®.

The micellar stability can be explained in terms of the hydrophilic-lipophilic balance of the surfactant. Pandit et al explains how the salt effect the CMC (critical micelle concentration) and CMT (critical micelle temperature) of the F127 surfactant [30]. Considering F127 as a block copolymer of PEO-PPO-PEO, the PPO groups are less polar and are thus considered as the hydrophobic part of the polymer. Any addition of salt thus results in decrease of PPO solubility in water, which favors micellization and reduces the CMC. It can thus be concluded that if the hydrophobic part of the surfactant is larger, there would be a greater decrease in the CMC of the surfactant, which means increased stability of the micelles. Further, it can be explained in terms of the aggregation number, which is a description of the number of molecules present in a micelle once the CMC has been reached. The aggregation number increases with increase of the number of the hydrophobic group and decreases with increases in number of hydrophilic group of the surfactant [31,32]. Thus in saline, the aggregation number increases for surfactant with HLB ~ 13 to 15 and decreases for surfactant with HLB >15. The surfactant of lower HLB has smaller CMC in saline, its hydrophobic core will protects the hydrophobic material that is encapsulated inside and thereby provides a greater stability. Instead, the surfactant of higher HLB has reduced CMC though, the hydrophilic part of the polymer is well hydrated in saline and thus, the hydrophobic material that is encapsulated inside would be less protected and thereby leads to its agglomeration in the micelle. The optimum HLB of 13 seems excellent for stable micelle formation. Resovist® on the other hand had shorter dextran chain coating, it is possible that the interaction of Resovist® in saline may lead to replacement of the dextran coating with ionic groups and the subsequent particle agglomeration may be one of the reasons for this size increase in saline [12].

Thermogravimetric analysis (TGA) in Fig. 3 shows the percentage weight loss of the IOs formulated in the micelles with temperature. From this figure, the amount of surfactant in the

![Magnetization curve measured using vibrating sample magnetometer (VSM) of the IOs-loaded TPGS and F127 micelles and Resovist® by varying the magnetic field, which shows the superparamagnetic nature of the TPGS micelle formulation had a greater saturation magnetization than the F127 micelle formulation and Resovist®.](image)
micelle was determined. It was observed that 75% of TPGS and 81% of F127 was present in their respective IOs-loaded micelles.

3.2. Magnetic property

IOs-loaded micelles were checked for their superparamagnetic property by using VSM. The amount of magnetization of the IOs in the various formulations is shown against the varying magnetic field in Fig. 4. The saturation magnetization ($\sigma_s$) was determined to be 84.5, 73.9, 54.8, and 57.8 emu/g of Fe for Hydrophobic IOs, TPGS IOs, F127 IOs and Resovists® respectively. The reduction in $\sigma_s$ can be attributed to the dense coating of the non-magnetic polymer of the IOs-loaded micelles. Nevertheless the $\sigma_s$ values in the first three formulations were all greater than that of Resovists®. The low value of $\sigma_s$ when compared to that of the hydrophobic IOs was due to the greater non-magnetic content present over the IOs [33]. The zero coercivity and remanence of the M–H curves indicate that the IOs-loaded micelles are superparamagnetic in nature.

3.3. Cell viability

Cell viability assay performed on MCF-7 breast cancer cells showed that the IOs-loaded micelles showed little toxicity compared to Resovists® after 12 h incubation on MCF-7 cancer cells. At 24 h of incubation, there was a certain degree of decrease in viability of the cells incubated with the IOs-loaded TPGS and F127 micelle at higher concentration of Fe as shown in Fig. 5. This can be due to the higher surfactant content at higher concentration of Fe loading, which can to certain extend disrupt the cell membrane. Nevertheless, all formulations showed insignificant toxicity.

3.4. Magnetic hyperthermia

Specific power absorption rate is defined as the power absorbed per mass of the sample and has units of watts per kilogram (W/Kg) [34]. From Fig. 6A it can be seen that the IOs-loaded micelles get...
heated up by increasing the duration of applied alternating magnetic field. From our experiments we found that the specific absorption rate (SAR) was 51.4 and 25.5 for the IOs-loaded TPGS and F127 micelles, respectively. The lower SAR value for the F127 micellar formulation of IOs can be explained due to the poor thermal conductivity of the F127 coating due to its higher molecular weight [35]. It is well known that the non-ionic surfactant of high molecular weight have poor thermal conductivity, thus the SAR value was low. Nevertheless the heating effect can be realized, and hyperthermia is dependent more on the amount of IOs reaching the region of interest, which will be discussed later.

Cell hyperthermia was performed on the MCF-7 cancer cell and the results are shown in Fig. 6B, from which it can be seen that a 1 h hyperthermia treatment showed a significant death of the cancer cells. The cells which received the magnetic field alone (without IOs) attached to the base and grew, whereas the cells which received the treatment were not viable and failed to attach. Thus, the IOs-loaded micelles can be useful for hyperthermia treatment.

![Graph showing temperature rise over time](image)

**Fig. 6.** (A) Hyperthermia study shows that the time-dependent temperature rise of 1 mg/ml of the IOs-loaded TPGS and F127 micelles on exposure to 89 kA/m alternating current field at 240 kHz frequency. The specific absorption rate value for the IOs-loaded TPGS and F127 micelles was found to be 51.4 and 25.5 Watt/g. (B) The MTT assay performed after hyperthermia treatment of MCF-7 cancer cells incubated with the IOs-loaded TPGS and F127 micelle and Resovist®. Hyperthermia treatment in all cases leads to significant cell death. Inset image (i) shows the cells incubated with the IOs-loaded TPGS micelles but no AC field applied and inset image (ii) shows the cells incubated with the IOs-loaded TPGS micelles and hyperthermia treatment using AC field. From (ii), it can be seen that after hyperthermia treatment, the cell loses viability and do not attach.
3.5. Cellular uptake

Fig. 7 shows the cellular uptake of the IOns-loaded micelles in terms of amount of Fe in pg (picogram) per cell. It was observed that cells cultured at the IOns concentration equivalent to 100 μg of Fe/well, Resovist®/C210 showed a significantly higher uptake than the IOns-loaded TPGS and F127 micelles. One can attribute this to the aggregation of the Resovist® particles, which were membrane bound and clumped (as observed under microscope, not shown). Cells cultured at lower IOns concentrations, however, the IOns-loaded TPGS and F127 micelles had a significantly higher uptake than Resovist®. This may be due to the multi-drug resistance (MDR) that exists in the cancer cells to prevent the cytotoxic effect of many drugs [36,37], and TPGS has shown to overcome such effect and improve the cytotoxicity of the drug [20]. Though IOns may not be affected by MDR, the increased cellular uptake of the IOns by MCF-7 cancer cells can be attributed to the surface coating of TPGS.

Fig. 8 shows the qualitative uptake of IOns-loaded TPGS micelles by means of TEM. The cells were viewed at different magnifications. Fig. 8A shows the MCF-7 cancer cell at lower magnification. The cell organelles are marked. It was observed that the IOns-loaded TPGS micelles were distributed within vesicles associated with lysosome inside the cell. These associations of phagocytic vesicles with a lysosome are called the phagolysosome. Formation of phagolysosome is part of the cells defense mechanism, as a result of which, the foreign substances are eaten up by the phagolysosome by a phenomenon known as xenophagy. Some IOns-loaded TPGS micelles were attached to the cell membrane and the membrane invagination could be observed, indicating the process of phagocytosis. Fig. 8B shows the TEM image of MCF-7 at higher magnification showing the phagolysosomes. One of the main observations was the spherically shaped IOns-loaded TPGS micelles being intact within the cells. Two important conclusions that can be drawn from the qualitative cell uptake TEM images are (i) for the cell uptake of the IOns-loaded micelles, surface chemistry plays a significant role and (ii) the IOns-loaded TPGS micelles were stable in physiological condition and do not aggregate or collapse.

3.6. MRI

Relaxivity of the IOns-loaded TPGS and F127 micelles and Resovist® were found to be 152.7, 135.7 and 235 mM of Fe¹s⁻¹ respectively. The micelles are effective T2-contrast agent and produce negative-contrast in a T2-weighted imaging sequence.

In vivo imaging of tumor xenograft was performed using a Bruker 7T scanner. Pre-post T2 map was created for the IOns-loaded TPGS and F127 micelles and Resovist® samples. T2 map is essential for quantitative determination of the IOns at the region of interest (ROI) in vivo.

T2 image analysis showed that the liver seems to be the largest place for the IOns accumulation for all formulations with T2 values reaching almost zero at the end of the 4th hour of injection of the contrast agent. However the micelles persist to stay in the liver in the case of the TPGS micelle formulation and Resovist®. Instead, the F127 micelle formulation showed a gradual clearance from the liver at the end of the 24th hour. Thus it can be concluded that IOns are cleared from the liver and kidneys. The F127 formulation is cleared quicker.
from the liver and kidney, which can be due to the increased accumulation in the reticuloendothelial system (RES).

Fig. 9 shows the trend of T2 values measured from tumor before, 45 min, 4 h and 24 h post-injection of Resovist®️, TPGS IOs and F127 IOs respectively injected in xenograft mice bearing MCF-7 tumor, at a concentration of 5 mg of Fe/Kg of animal. It was seen that the T2 value decreases due to accumulation of IOs at tumor by ~4.3%, 7.3% and 6.9% for Resovist®, the IOs-loaded TPGS and F127 micelles respectively at 45 min post-injection.

Fig. 10 are the T2 mapped images of the xenograft mice bearing MCF-7 xenograft tumor at the various designated time before and 45 min, 4 h 24 h after administration of Resovists®, the TPGS and F127 micelle formulations respectively at an equivalent concentration of 5 mg of Fe/Kg of animal. The “Hot Metal” color scheme was chosen to represent the MRI results using MRIcro. The scale in the figure gives the value of the T2 as a gradient of the color from 0 to 160 ms. The T2 change can be seen in the figure. The arrow points the tumor in the axial image of the section of the SCID mice.

There are a great number of factors that needs to be taken into consideration when dealing with nano-system for biomedical imaging. For instance, size is of a concern, the nano size range of nanoparticles provides stealth from the reticuloendothelial system (RES) of the human body. It has been shown that nanoparticles of size less than 250 nm can efficiently prevent their phagocytosis by the kupffer cells using hydrophilic coating [38]. The enhanced permeation and retention (EPR) of the tumor vessels provide nanoparticles with the function to target the cancer cells [39]. Agglomeration is also of concern, wherein, the agglomerated nanoparticles are easily recognized by the RES system and are greatly cleared by the liver. Next important design criteria is the surface properties of nanoparticles. It is well known that TPGS inhibits p-gp activity and thereby enhances the cellular uptake of the chemotherapeutic drug [20,22,23], this property along with the stable nature of the micelles formed in saline, provide the TPGS micelle system to reach the tumor and accumulate.
4. Conclusion

We synthesized the I0s-loaded TPGS and F127 micelles and made close comparison with the commercial Resovist® for their thermal and superparamagnetic properties for thermotherapy and MRI. We intensively studied the stability of these I0s formulations in Milli-Q water as well as in saline. The TPGS micelle formulation was found to have greatest advantages in stability over the F127 micelle formulation and Resovist®. The I0s-loaded micelles showed potential to be applied for hyperthermia treatment. The micelle formulations showed reduced toxic effect and enhanced uptake by the cancer cells. In vivo MRI demonstrated that the TPGS micelle formulation of I0s is more promising than Resovist® and other micelle formulation such as the F127 micelles reported in the literature with an increased I0s uptake in the tumor region as shown in the T2 map.

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