Chapter 8

Application of Magnetic Core-Shell Nanostructures for Magnetic Fluid Hyperthermia

Non-aqueous to aqueous phase transfer of oleic acid coated iron oxide nanoparticles for hyperthermia application


Superparamagnetic iron oxide/chitosan core/shells for hyperthermia application: Improved colloidal stability and biocompatibility


IN VITRO HYPERTHERMIA WITH IMPROVED COLLOIDAL STABILITY AND ENHANCED SAR OF MAGNETIC CORE/SHELL NANOSTRUCTURES

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8.1. Introduction

Cancer is one of the most challenging diseases being faced by modern medicine. It is a major contributor of world’s high mortality rate. Current cancer treatments include radiotherapy, chemotherapy and tumor extirpation. Although these approaches have saved myriad lives, they are not always sufficient to eliminate the disease. In addition, radio and chemotherapy produce such unbearable side effects that the patient’s quality of life is so pitiable that they often refuse further treatment. There is a necessity for localized, efficient treatments that allow the patient a better quality of life. Efforts are being made in locally treating tumors with high temperatures. The idea behind this approach is that due to poor oxygenation, tumor cells are more vulnerable to damage from heat. Healthy cells, however, can survive temperatures of up to 46 °C. Hyperthermia treatment kills cancerous cells by elevating their temperatures to the therapeutic temperature range of 42-46 °C, according to the National Cancer Institute. This approach can destroy tumors with least damage to healthy tissues and hence, limit negative side effects [1]. In addition to eliminating many cancerous cells, hyperthermia can make resistant cells more susceptible to other treatments.

Hyperthermia can treat specific locations, larger regions or the entire body. Local hyperthermia may be applied externally to treat tumors near the skin’s surface or with probes to reach deep-seated cancerous tissues. For larger tumors or multiple tumor locations, regional hyperthermia focuses microwave, laser or ultrasound energy on diseased tissues. Whole-body hyperthermia treats cancers that have spread to several regions by using thermal chambers to elevate a patient’s body temperature to just below 42 °C.

MFH is a relatively modern method used for cancer therapy. MNPs are inserted into the blood stream and they are targeted to the cancerous cells or they are directly injected inside the tumor. When an AC magnetic field is applied to the MNPs, they generate heat due to the different heat loss mechanisms. The generated heat destroys cancer cells. Frequently, ferromagnetic MNPs and
SPIONs are used for cancer therapy, the latter is preferred because of the lower magnetic fields required to generate heat. In MFH, a colloidal fluid containing the MNPs is injected directly into the tumors. The MNPs should evenly disperse throughout the fluid and must be small enough to avoid precipitation due to gravitational forces [2].

8.2. Theory of MFH

When ferrofluids is infused into a tumor and exposed to an external AC magnetic field, it becomes a heat source capable of raising temperatures high enough to weaken or destroy the cancerous cells. This concept has been proven to be effective in a number of in vivo studies where ferrofluids were used to destroy or seriously weaken tumors in animals. The application of ferrofluids for MFH was investigated in the work of Chan et al. [3] and Jordan et al. [4] in 1993. These studies experimentally prove the high efficiency of a superparamagnetic crystal suspension to absorb energy of an AC magnetic field and convert it into heat. A tumor cell is more sensitive to a temperature increase than healthy cells [5, 6]. This property can be used in vivo to destroy pathological cells by MFH. It is notable that the results of current/ongoing clinical trials show significant reduction in side effects [7, 8].

8.3. Generation of heat by MNPs for hyperthermia application

The mechanisms for heating MNPs with an AC magnetic field include three types of losses viz. hysteresis loss, Néel and Brownian relaxation. The relative contribution of each process depends strongly on the crystal size and composition of the particles. NPs with core diameters of less than 20 nm or so are used in most MFH applications. In such small NPs, magnetic relaxation is governed by a combination of the external rotation (Brownian) and internal (Néel) diffusion of the particle’s magnetic moment with negligible contribution of hysteresis loss [9].
8.3.1. Hysteresis loss

Ferromagnetic materials when placed in an AC magnetic field tend to lose heat due to hysteresis loss. When exposed to an external field, the magnetic moments start to align in the direction of the field. This usually happens only at high field magnitudes. Those domains whose magnetic moments are along the external field axis grow while those that are aligned oppositely shrink. This ‘domain wall displacement’ carries on until the point of saturation when the growing domain encompasses the entire volume.

If MNPs are put into an AC magnetic field of frequency $f$ and amplitude $\mu_0H_{\text{max}}$, the amount of heat $A$ released by the MNPs during a cycle of the magnetic field equal the area of their hysteresis loop. The amount of heat released by ferromagnetic material through hysteresis loss is given by

$$\int_{-H_{\text{max}}}^{H_{\text{max}}} \mu_0(H) dH \quad \text{...................(8.1)}$$

Then the specific absorption rate (SAR) is

$$\text{SAR} = Af \quad \text{...........(8.2)}$$

where $f = \omega/2\pi$, $M = \text{magnetization}$ and $H = \text{applied magnetic field}$.

8.3.2. Eddy current loss

The generation of eddy currents (ED) is a consequence of law of induction. It is not constrained to magnetic materials; instead, it can be applied to all macroscopic conducting materials. Considerable eddy current is generated when these materials are subjected to an AC magnetic field, which then gives rise to a significant heating effect. An example of hyperthermia which employs this method is implantation of needle shaped copper followed by application of RF field [10]. The heat loss due to eddy currents occurs as a result of the interaction of a conductive material with an oscillating magnetic field according to Faraday’s and Lenz’s laws. The heat loss due to eddy currents is given by
\[ ED = \frac{(\mu \pi dfH)^2}{20 \rho} \]  

...............(8.3)

Where \( \rho \) = resistivity of the material, \( d \) = diameter of the particle and \( \mu \) = permeability of a material.

The SAR is calculated by using following relation.

\[ SAR = c \frac{\Delta T}{\Delta t} \frac{1}{m_{magn}} \]  

............... (8.4)

where \( c \) = the sample-specific heat capacity, which was calculated as a mass weighted mean value of MNPs and water. The heat-capacity of both samples is negligible because of its low concentration and thus a heat capacity for water (4.18 \( Jg^{-1} K^{-1} \)) is taken as the sample’s heat capacity. \( \Delta T/\Delta t \) is the initial slope of the time dependent temperature curve. Here, we considered time up 1–5 min to calculate the slope. The value of \( m_{magn} \) is considered as the amount of MNPs per total amount of MNPs and water.

**8.3.3. Heat dissipation mechanism by superparamagnetic nanoparticles**

The heat generations by SPIONs through different loss mechanisms are presented in Fig. 8.1. The well dispersed SPIONs fluid would show two types of relaxations. Brownian relaxation; particle rotation occur in all types of SPIONPs and Neel’s spin relaxation; magnetic moment rotation, occurs only in SPIONs. The heat loss from SPIONs is attributed to the relaxation phenomena of moments. In an external AC magnetic field, energy is provided to assist magnetic moments of the SPIONs which can rotate the SPIONs and results into overcoming the energy barrier \( E=KV \), where \( K \) is the anisotropy constant and \( V \) is the volume of the magnetic core. Heat dissipation from SPIONs is caused by the delay in the relaxation of the magnetic moment through either the rotation within the SPIONs (Néel) or the rotation of the SPIONs itself (Brownian), when they are exposed to
an AC magnetic field with magnetic field reversal times shorter than the magnetic relaxation times of the SPIONs.

(a) Brownian relaxation loss

In Brownian rotational relaxation ($\tau_b$), the magnetic moment aligns with the magnetic field ($H$) and the SPION rotates under an AC field. During rotation, collision with the surrounding medium generates heat. This is attributed to the rotation of the magnetic particle as a whole because of the torque exerted on the magnetic moment by the external AC magnetic field. Energy has to be provided to overcome the rotational friction offered by the adjacent liquid. This energy is released during relaxation is called Brown relaxation. Brownian relaxation at temperature ($T$) is given by

$$\tau_b = \frac{3\eta V_H}{K_B T} \quad \text{.................. (8.5)}$$

Where $V_H$ = the hydrodynamic radius of the particle and $\eta$ = the dynamic viscosity of carrier liquid, $k_B$ = Boltzmann constant, $\tau_0 = 10^{-9}$ s.

![Diagram](image)

**Fig. 8.1:** Represents the (a) Brownian rotation (the particle as a whole rotates) and (b) Neel rotation of magnetization in a MNPs (the particle does not rotate), which are responsible for heat generation.

(b) Neel’s spin relaxation loss

When the moment returns to its equilibrium orientation, energy is dissipated during the phenomena of Nèel Relaxation. In Neel’s spin relaxation
when SPIONs are exposed to an AC magnetic field with time of magnetic reversals less than the magnetic relaxation times of SPIONs, heat is dissipated due to the delay in the relaxation of the magnetic moment. Neel’s spin relaxation is given by

$$\tau_N = \tau_0 e^{\frac{KV_H}{K_B T}}$$  \hspace{1cm} (8.6)

The total relaxation is expressed as

$$\tau = \frac{T_B \tau_N}{T_B + \tau_N} \hspace{1cm} (8.7)$$

Where $V_H$ = the hydrodynamic radius of the particle and $\eta$ = the dynamic viscosity of carrier liquid, $k_B$ = Boltzmann constant, $\tau_0 = 10^9$ s.

Thus, the heat dissipation value is calculated using the harmonic average of both relaxations and their relative contributions depending on the SPION diameter. Degenerated heat can be given by

$$P = \mu_0 \chi'' f H^2$$  \hspace{1cm} (8.8)

Where $P$ = the heat dissipation value, $\chi''$ = the AC magnetic susceptibility (imaginary part), $\mu_0$ = permeability, $f$ = frequency and $H$ = the strength of applied AC magnetic field. The susceptibility ($\chi$) is defined as $M/H$ and can be expressed in terms of real ($\chi'$) and imaginary ($\chi''$) terms (i.e. complex function) in an AC field. The imaginary term is related to heat-dissipation.

8.4. Experimental

8.4.1. Induction heating system used for hyperthermia study

Induction heating of Fe$_3$O$_4$ MNPs for hyperthermia application was performed in plastic micro centrifuge tube (1.5 mL) using an induction heating unit (EasyHeat 8310, Ambrell; UK) with 6 cm diameter (4 turns) heating coil. To keep the temperature of the coil at ambient temperature, a provision of water circulation in coils was provided. The system consists with AC magnetic field
generator, induction coils and data acquisition unit. The mathematical formulation for conversion of AC electric current in to AC magnetic field is done by Cullity et al. [11]. The developed equation is as follows

\[
H = \frac{1.25ni}{L} \text{ Oe}
\]

\[\text{......................... (8.9)}\]

Where \(n\) = the number of turns and \(L\) = the length of the winding in centimeters.

### 8.4.2. Sample preparation and measurement

\(\text{Fe}_3\text{O}_4\) MNPs (2–10 mg) were suspended in 1mL of distilled water placed at the centre of the coil and the applied frequency was 265 kHz. MNPs were dispersed in water with concentration ranging from 2-10 mg/mL and ultrasonicated for 20 min for getting well dispersion of the MNPs in the carrier fluid. Samples were heated for 10 min with the desired current (200–400A). For the conducted experiments, the magnetic field was calculated from the relationship (Eq. 8.9). Calculated values of the magnetic field \(H\) at 200, 300 and 400A were, 167.6, 251.4 and 335.2 Oe (equivalent to 13.3, 20.0 and 26.7 kA m\(^{-1}\)), respectively. Temperature was measured using optical fiber probe with an accuracy 0.1 °C.

### 8.5. Results and discussion

#### 8.5.1. MFH Study of \(\text{Fe}_3\text{O}_4\)-CH/GLD core-shell nanostructures

Magnetic fluid hyperthermia involves applying an external AC magnetic field of suitable frequency which produces heat dissipation through the oscillation of the internal magnetic moment of MNPs. Fig. 8.2 (a-c) represents the temperature kinetic curves obtained after application of an alternating magnetic field on both samples which dispersed in water with concentration of 2 mg/mL and (d) SAR \(\text{vs}\) applied magnetic field for both samples. Temperature kinetic curves represents, rise in temperature is dependent on the applied magnetic field.
for both the samples. For SPIONs, the greatest relaxation losses are due to Brownian modes (heat due to friction arising from total particle oscillations) and Neel modes (heat due to rotation of the magnetic moment with each field oscillation). The estimated SAR values from Fig. 8.2 (a-c) and by using equation 8.4 for both the samples are graphically represented in Fig. 8.2 (d).

![Graphs of temperature versus time for different magnetic fields and SAR versus AC magnetic field for FeO, Fe3O4, and Fe3O4-CH/GLD](image)

**Fig. 8.2:** (a–c) Temperature kinetic curves obtained after application of an alternating magnetic field on both the samples dispersed in water with a concentration of 2 mg/mL, (d) SAR vs applied magnetic field for both samples.

It is demonstrated experimentally that the hyperthermia effect of Fe3O4 MNPs enhances dramatically after functionalized with CH/GLD. First possible reason for the enhanced hyperthermic effect after coating is that the ability of the
CH/GLD coating to retain the superparamagnetic fraction of the Fe₃O₄ much better as compared to Fe₃O₄ alone. The second is that coating layer prevents the formation of larger aggregates of Fe₃O₄ (also confirmed from DLS results) which make the better suspension of CH/GLD functionalized MNPs in the water as compared to naked Fe₃O₄. Thus the hyperthermia study also strongly supports the coating of CH/GLD on Fe₃O₄ MNPs and prevents particle agglomeration.

**Fig. 8.3:** Temperature rise of Fe₃O₄-CH/GLD MNPs in various media during induction heating experiment.

The effect of PBS, NaCl and glucose concentration on hyperthermia properties of CH/GLD coated MNPs (2 mg/mL) was evaluated at AC electric current of 400A i.e. 335.2 Oe. The main aim is to use these MNPs for *in vivo* biomedical application, hyperthermia was carried out in serum sample and in
combination with 0.85% concentration of NaCl, 1.2 mg/mL of glucose having pH 7.4 of PBS and obtained results are shown in Fig. 8.3.

From the obtained results it was found that the desired temperature for in vivo hyperthermia i.e. 42-46 °C was achieved within 3 min of applied external AC magnetic field in all the studied physiological media. No obvious effect of PBS, NaCl and glucose concentration was found on hyperthermia produces by the coated MNPs.

The more distinct picture of hyperthermia measurements are cleared after detailed study on SAR. The heating capacity of a magnetic material is quantified through the SAR, defined as the amount of energy converted into heat per time and mass. The effect of PBS, NaCl concentration, glucose concentration, combination and serum on SAR of functionalized MNPs is shown in Fig. 8.4. The SAR values for various pH of PBS were between 123 to 132 W/g, which showed high SAR value which is in comparison with water (SAR=133.71 W/g). These results showed that there was no effect of PBS on SAR i.e. on the coating material which was strongly bonded to the MNPs.

The SAR values for 0.75, 0.80, 0.85, 0.90 and 0.95% concentration of NaCl were 116.80, 116.31, 115.20, 113.87 and 110.93 W/g respectively, which show increase in concentration results in decrease in SAR value. The coated MNPs could even achieve an appreciable SAR of 110.93 W/g at high NaCl concentration of 0.95%. Similarly high SAR values with different glucose concentrations were also observed. However, SAR values decreases with increasing glucose concentration. The observed values were 122.39, 123.99, 120.16, 117.36 and 115.20 W/g for 0.6, 0.8, 1.0, 1.2 and 1.4 mg/mL respectively. This may be due to the effect of decrease in colloidal stability of functionalized MNPs and even decrease of MNPs concentration with addition of NaCl/glucose that is not susceptible to AC field.

The SAR values of coated MNPs (2 mg/mL) in water, serum and combination were 133.71, 110.23 and 105.83 W/g respectively. The coated MNPs
achieved a high value of SAR in all media owing to their high potential to be used for \textit{in vivo} hyperthermia therapy of cancer.

![Graphs showing SAR values in various media](image)

**Fig. 8.4:** SAR of Fe$_3$O$_4$-CH/GLD MNPs in various media during induction heating experiment: (a) PBS with pH range 7.0 – 7.8, (b) saline with NaCl concentrations 0.75 – 0.95 %, (c) glucose solutions with varying concentrations of glucose from 0.6 – 1.4 mg/mL and (d) comparison between SAR values in different media i.e. water, human serum and the medium with standard physiological values, i.e. pH 7.4, 0.85 % salinity and 1.2 mg/mL glucose concentration as base medium.

As the CH/GLD coated MNPs form a stable suspension and show high SAR in biological media and human serum, their effectiveness in killing human cancer cells was checked on MCF7 (human breast cancer) cell lines. The relative decrease in percentage cell viability was determined by trypan blue viability assay. The temperature for hyperthermia was maintained in between 44-45 °C. The
readings have been taken after 10, 20, 30, 45, 60 and 90 min of incubation in presence of CH/GLD coated MNPs (1 mg/ml). The post hyperthermia cell viability assay was performed after 2 h and 24 h of incubation. As earlier report for LSMO MNPs, the exposure of cells to the magnetic field does not show any significant cytotoxic effect on cells. From the results it was found that more than 30 % MCF7 cancer cells were killed within 20 min of hyperthermia exposure. Very interestingly, 90 min period of hyperthermia exposure is required to kill about 74% and 86% of MCF7 cells after 2 h and 24 h post hyperthermia incubation. The obtained results are shown in Fig. 8.5.

![Graph showing cell death percentage over time](image)

**Fig. 8.5:** *In vitro* hyperthermia profile of Fe₃O₄-CH/GLD MNPs (1mg/mL) on MCF7 cell line.

The decrease in cell viability after irradiation by applying external magnetic field to coated MNPs can be attributed to good colloidal stability and high SAR value; due to which they could efficiently generate and distribute heat to cancer cells within short time span. From the results, coated MNPs are proved to be suitable candidates for *in vivo* hyperthermia application for cancer therapy.
Fig. 8.6: ROS measurement profile for CH/GLD functionalized Fe$_3$O$_4$ MNPs, 90 min after hyperthermia treatment to determine the role of ROS in inducing cell death.

It is well known that a regulated concentration of ROS is crucial for cancer cell proliferation, whereas the excessive production or reduction of ROS results in apoptosis and cell death. Therefore, disturbing ROS homeostasis can be a promising approach to kill the cancer cells [13]. Because cancer cells exhibit higher ROS levels than normal cells do, increasing these levels further offers an extraordinary opportunity for inducing damage to cancer cells. Hyperthermia can produce excess level of ROS, which can kill cancer cells by oxidative burst [14]. However, Conventional hyperthermia did not generate moderate ROS level in cancer cells [15]. To determine the role of ROS in inducing cell death, H2DCFDA fluorescent dye was used. The ROS-indicator dye is non-fluorescent in its reduced form. Removal of acetate groups of the dye by intracellular esterases yields the fluorescent form, which can be readily oxidised by ROS. Fig. 8.6 shows the increase in fluorescence intensity with increase in exposure time with magnetic
hyperthermia from 10 to 90 min of exposure. The results were taken in comparison with a control which was not exposed to magnetic field showing low fluorescence intensity. Hence hyperthermia temperature maintained in between 42-43 °C was sufficient to induce ROS production in MCF7 cell lines. The results are in good agreement with the literature which show hyperthermia at 43 °C causes a significant increase in ROS production [14].

Fig. 8.7: Confocal microscopy images of non-treated and treated MCF7 cells for CH/GLD functionalized Fe₃O₄ MNPs after 60 min MFH.

In *in vitro* MFH for 90 min using CH/GLD coated MNPs, about 86% cell death was observed. To determine the exact nature of cell death, MCF7 cells were stained with DAPI, FITC and PI dyes after MFH using CH/GLD coated MNPs. This type of multiple staining coupled with confocal microscopy observations identifies dead and live cells more accurately and qualitatively [16-18]. Fig. 8.7 shows confocal images of DAPI, FITC and PI stained control and post MFH (60 min) MCF7 cells. From the FITC staining images it was observed that the control cells uniformly and deeply fluoresce green while the post MFH cells were not stained uniformly owing to loss cell viability. As FITC enters live cells and emits green fluorescence only live cells can be seen green in colour. DAPI generally binds to the nucleus of live cells, in the post MFH cells the fluorescence was very
rarely observed than control cells. No any fluorescence was observed in PI stained cells in control because PI could only stain cells that had lost membrane integrity and was observed in the post MFH cells which loses their membrane integrity may be due to high ROS production by MFH. The results imply cell shrinkage and formation of apoptic bodies. The simultaneous staining of the nucleus with DAPI and PI is advantageous and represents the accurate nature of live and dead cells [12].

8.5.2. MFH Study of Fe3O4-OA-BTH core-shell nanostructures

In similar way, Fig. 8.8 (a-c) represents the temperature kinetic curves obtained after application of an alternating magnetic field on both samples which dispersed in water with concentration of 2 mg/ml and (d) SAR vs applied AC magnetic field for both samples. Temperature kinetic curves represents, rise in temperature is dependent on the applied magnetic field for both the samples. For superparamagnetic NPs the greatest relaxation losses are due to Brownian modes (heat due to friction arising from total particle oscillations) and Neel modes (heat due to rotation of the magnetic moment with each field oscillation) [12]. The estimated SAR values from Fig. 8.8 (a-c) and by using equation 8.4 for both the samples are graphically represented in Fig. 8.8 (d).

It is demonstrated experimentally that the hyperthermia effect of Fe3O4 MNPs enhances dramatically after functionalized with OA-BTH. First possible reason for the enhanced hyperthermic effect after coating is that the ability of the OA-BTH coating to retain the superparamagnetic fraction of the Fe3O4 much better as compared to Fe3O4 alone. The second is that coating layer prevents the formation of larger aggregates of Fe3O4 (also confirmed from DLS results) which make the better suspension of OA-BTH functionalized MNPs in the water as compared to naked Fe3O4. Thus the hyperthermia study also strongly supports the coating of OA-BTH on Fe3O4 MNPs and prevents particle agglomeration.
Fig. 8.8: (a–c) Temperature kinetic curves obtained after application of an alternating magnetic field on both the samples dispersed in water with a concentration of 2 mg/ml, (d) SAR vs applied AC magnetic field for both samples.

The effect of PBS, NaCl and glucose concentration on hyperthermia properties of OA-BTH coated MNPs (2 mg/mL) is evaluated in same manner like CH/GLD. The main aim is to use these MNPs for in vivo biomedical application, hyperthermia was carried out in serum sample and in combination with 0.85% concentration of NaCl, 1.2 mg/mL of glucose having pH 7.4 of PBS and obtained results are shown in Fig. 8.9.

From the obtained results it was found that the desired temperature for in vivo hyperthermia i.e. 42-46 °C was achieved within 3 min of applied external AC magnetic field in all the studied physiological media. No obvious effect of PBS,
NaCl and glucose concentration was found on hyperthermia produces by the coated MNPs.

The effect of PBS, NaCl concentration, glucose concentration, combination and serum on SAR of functionalized MNPs is shown in Fig. 8.10. The SAR values for various pH of PBS were between 88 to 94 W/g, which showed high SAR value than water only (SAR= 91.02 W/g). These results showed that there was no effect of PBS on SAR i.e. on the coating material which was strongly bonded to the MNPs.

![Graphs showing temperature rise in PBS, NaCl, and Glucose](image)

**Fig. 8.9:** Temperature rise curves of Fe₃O₄-OA-BTH MNPs in PBS, NaCl, glucose and human serum during induction heating experiment.

The SAR values for 0.75, 0.80, 0.85, 0.90 and 0.95% concentration of NaCl were 87.88, 87.04, 84.10, 83.97 and 82.64 W/g respectively, which show increase in concentration results in decrease in SAR value. The coated MNPs could even
achieve an appreciable SAR of 82.64 W/g at high NaCl concentration of 0.95%. Similarly high SAR values with different glucose concentrations were also observed. The observed values were 91.93, 89.91, 87.88, 84.74 and 81.38 W/g for 0.6, 0.8, 1.0, 1.2 and 1.4 mg/mL respectively. However it was found that SAR values decreases with increasing glucose concentration. This may be due to the effect of decrease in colloidal stability of functionalized MNPs and even decrease of magnetic particle concentration with addition of NaCl/glucose that is not susceptible to AC field.

![Graphs showing SAR of MNPs in various media during induction heating experiment](image)

**Fig. 8.10:** SAR of MNPs in various media during induction heating experiment: (a) PBS with pH range 7.0 – 7.8, (b) saline with NaCl concentrations 0.75 – 0.95 %, (c) glucose solutions with varying concentrations of glucose from 0.6 – 1.4 mg/mL and (d) comparison between SAR values in different media i.e. water, human serum and the medium with standard physiological values, i.e. pH 7.4, 0.85 % salinity and 1.2 mg/mL glucose concentration as base medium.
The SAR values of coated MNPs (2 mg/mL) in water, serum and combination were 91.02, 78.45 and 75.38 W/g respectively. The coated MNPs achieved a high value of SAR in all media owing to their high potential to be used for hyperthermia therapy of cancer.

![Graph showing cell death percentage over time for Fe₃O₄-OA-BTH](image)

**Fig. 8.11:** *In vitro* hyperthermia profile of OA-BTH coated MNPs (1mg/mL) on MCF7 cell line.

As the OA-BTH coated MNPs form a stable suspension and show high SAR in biological media and human serum, their effectiveness in killing human cancer cells was checked on MCF7 (human breast cancer) cell lines. The relative decrease in percentage cell viability was determined by trypan blue viability assay. The temperature for hyperthermia was maintained in between 44-45 °C. The readings have been taken after 10, 20, 30, 45, 60 and 90 min of incubation in presence of OA-BTH coated MNPs (1 mg/mL). The post hyperthermia cell viability assay was performed after 2 h and 24 h of incubation. From the results it was found that more than 60 % MCF7 cancer cells were killed within 20 min of hyperthermia exposure. Very interestingly, 90 min period of hyperthermia
exposure is required to kill about 86% and 97% of MCF7 cells after 2 h and 24 h post hyperthermia incubation. The obtained results are shown in Fig. 8.11.

![Graph showing fluorescence intensity over time](image)

**Fig. 8.12:** ROS measurement profile for OA-BTH functionalized Fe₃O₄ MNPs, 90 min after hyperthermia treatment to determine the role of ROS in inducing cell death.

The decrease in cell viability after irradiation by applying external magnetic field to coated MNPs can be attributed to good colloidal stability and high SAR value; due to which they could efficiently generate and distribute heat to cancer cells within short time span. From the results, coated MNPs are proved to be suitable candidates for *in vivo* hyperthermia application for cancer therapy.

To determine the role of ROS in inducing cell death, H₂DCFDA fluorescent dye was used. Fig. 8.12 shows the increase in fluorescence intensity with increase in exposure time with magnetic hyperthermia from 10 to 90 min of exposure. The results were taken in comparison with a control which was not exposed to magnetic field showing low fluorescence intensity. Hence hyperthermia temperature maintained in between 42-43 °C was sufficient to induce ROS
production in MCF7 cell lines. The results are in good agreement with the literature which show hyperthermia at 43 °C causes a significant increase in ROS production.

![Confocal microscopy images](image)

**Fig. 8.13:** Confocal microscopy images of non-treated and treated MCF7 cells for OA-BTH coated MNPs after 60 min MFH.

In *in vitro* MFH for 90 min using OA-BTH coated MNPs, about 97% cell death was observed. To determine the exact nature of cell death, MCF7 cells were stained with DAPI, FITC and PI dyes by MFH using OA-BTH coated MNPs. This type of multiple staining coupled with confocal microscopy observations identifies dead and live cells more accurately and qualitatively. Fig. 8.13 shows confocal images of DAPI, FITC and PI stained control and post MFH (60 min) MCF7 cells. From the FITC staining images it was observed that the control cells uniformly and deeply fluoresce green while the post MFH cells were not stained uniformly owing to loss cell viability. As FITC enters live cells and emits green fluorescence only live cells can be seen green in colour. DAPI generally binds to the nucleus of live cells, in the post MFH cells the fluorescence was very rarely observed than control cells. No any fluorescence was observed in PI stained cells in control because PI could only stain cells that had lost membrane integrity and was observed in the post MFH cells which loses their membrane integrity may be due to high ROS production by MFH. The results imply cell shrinkage and formation.
of apoptic bodies. The simultaneous staining of the nucleus with DAPI and PI is advantageous and represents the accurate nature of live and dead cells.

8.6. Conclusions

The brief introduction and physical basis of MFH is reviewed in this chapter. The chapter also contains the mechanism of heat generation by MNPs including ferromagnetic and superparamagnetic. The result and discussion section contains the experimental investigation of heat generation by Fe$_3$O$_4$ MNPs for magnetic fluid hyperthermia treatment. The result shows that the bare and core-shells of Fe$_3$O$_4$ MNPs were capable of generating the temperature required for hyperthermia (42-46 °C). The surface functionalized MNPs show increased SAR compared to bare MNPs which is due to the high colloidal stability of functionalized MNPs. High SAR values were obtained in various physiological media and in human serum at a concentration of as low as 2mg/mL. In vitro hyperthermia experiments of both coated MNPs on MCF7 cancer cell line with concentration of 1mg/mL showed high percentage of cell death within 90 min. The efficacy of killing cells was found to increase with increasing duration of exposure to magnetic hyperthermia. The increased ROS generation over a range of period after hyperthermia treatment might be the reason for induced cell death. Though CH/GLD coated MNPs have high SAR value than OA-BTH coated MNPs, the latter showed better cell death percentage; may be because of their high colloidal stability. The study concludes that the functionalized MNPs have a great potential to be used as a candidate for \textit{in vivo} cancer hyperthermia therapy.
REFERENCES


