A Thesis on

MAGNETIC NANOPARTICLE HPERTHERMIA FOR THE TREATMENT OF CANCER

Submitted in the partial fulfilment of requirement of the award of degree of

MASTERS OF SCIENCE

2019 – 2021

Submitted By

ASHIMA (301904003)

Under the supervision of

Dr. Bhupendrakumar Chudasama

(Professor)

SCHOOL OF PHYSICS AND MATERIAL SCIENCE

THAPAR INSTITUTE OF ENGINEERING AND TECHNOLOGY

PATIALA- 147004, INDIA

JULY-2021

Dedicated To

My Parents & Brother

CERTIFICATE

I hereby certify that the work is being presented in the thesis, titled **“Magnetic Nanoparticle Hyperthermia For The Treatment Of Cancer”** in the partial fulfilment of the requirements for the award of degree of **Masters of Science** in **Physics** and submitted to the school of Physics and Material Science, Thapar Institute of Engineering and Technology, Patiala, is an authentic record of her own work, carried out under my supervision. The matter presented in this thesis has not been submitted elsewhere for the award of any other degree or diploma for any institution.

Dr. Bhupendrakumar Chudasama

Professor

School of physics and material sciences

Thapar Institute of Engineering and Technology, Patiala

DECLARATION

I hereby declare that this thesis titled **“Magnetic Nanoparticle Hyperthermia For The Treatment Of Cancer”,** by **Ashima,** is submitted in the partial fulfilment of the requirements for the **Degree of Masters of Science in Physics** of Thapar institute of engineering and technology, Patiala during the academic year of 2019-2021, is bonafide record of my original work carried under the supervision of Dr. bhupendrakumar chudasama, Professor, School of Physics and Materials Science (SPMS) Thapar University, Patiala, Punjab and is not submitted anywhere else for the award of such kind of degree.

Ashima.

ASHIMA

301904003

School of Physics and Material Science

Thapar Institute of Engineering and Technology, Patiala

ACKNOWLEDGEMENT

This thesis is done in School of Physics and Material Science, Thapar Institute of Engineering and Technology, Patiala, under the guidance of Dr. Bhupendrakumar Chudasama, Associate Professor. It is my privilege to owe my gratitude to each and every one who supported me and encouraged me.

First and foremost, I want to express my sincere appreciation and gratitude to my supervisor Dr. Bhupendrakumar Chudasama, for his profound support, guidance, patience and motivating me time to time during this research project. His immense knowledge and suggestions made difficult and challenging concepts seems interesting to me. I could not have imagined a better advisor and mentor for my master's thesis.

I want to thank Dr, O.P. Pandey, Senior Professor and Head, School of Physics and Material Science, for giving me necessary infrastructure to carry out my research work.

I would like to express my sincere regards to Dr. Chandni Khurana, for her patience, encouragement and insightful comments and the staff of School of Physics and Material Science for aiding me in this research work.

I want to convey my special thanks to Mrs. Yashpreet Kaur, for her timely guidance, help and motivation. I am sure the knowledge gained through my association with her shall go a long way. I am also grateful to Dr. Purnima Sharma and Ms. Sonal Singh, research scholars, for the help provided by them.

I want to thank my parents and my brother for their constant encouragement and for believing in me. This thesis would not been possible without their support. Above all, I pay me regards to almighty god for giving me power to follow the right direction in achievement of my goal.

Ashima

Ashima

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Treatment of cancer remains a major challenge in modern medicine. Magnetic Hyperthermia is a non-invasive cancer therapy that uses heat generated by magnetic nanoparticles to kill or damage the cancer cells without effecting the healthy cells. Specific absorption rate (SAR) is one of the most important parameters used in designing nanoparticles for the magnetic hyperthermia. In this work we have review physics of magnetic hyperthermia with a focus on effects of nanoparticle size, shape and their aggregation; amplitude and frequency of applied alternating magnetic field on SAR. Some work on synthesis of water based oleic acid coated Fe_3O_4 nanoparticles have also been reported as it is one of the most important magnetic nanoparticle system which is approved for in-vivo use by food and drug administration of USA.

1. INTRODUCTION

Cancer is a dreadful disease, which immensely affects civilization. It is a disease in which some genetic changes within the cell leads to uncontrolled cell division, which then spread across the body [1]. Other triggers of cancer include but not limited to carcinogens, radiations and life-style related issues [1]. According to a report by World Health Organization (WHO), in 2018, 18.2 million cancer cases have been reported globally, which is expected rise soar to 23.6 million by 2030 [2].

Treatment of cancer remains a major challenge in modern medicine. Generally, early symptoms of cancer are unnoticeable and thus, difficult to detect [2]. Chemotherapy, surgery and radiotherapy are the most common cancer treatments, out of which chemotherapy is most popular [3]. In chemotherapy, drugs are highly cytotoxic in nature and since they cannot discriminate between a healthy and cancerous cell, substantial side-effects of the therapy has been observed[2]. These side effects include but not limited to loss of immunity, damages to nervous system and digestive system, etc. [4]. Surgery can be successful only in such cases wherein the exact location of tumor is known. Further, it is not an option in case of deep-seated tumors[4].

Despite discoveries of new chemotherapeutic drugs and combinational therapies, mortality due to cancer remains high and unchanged in past six decades. With the advances in nanotechnology, it is expected to treat cancer at very early stage. Recently, use of nanoparticles in medicine has gained significant attention. Biol5ogical processes leading to cancer occur at nanoscale, thus use of nanomaterials provides researchers unique advantage in diagnosing and treating cancer [3]. Nanomaterials owing to their small size can easily internalize into cancer cells and thus provides an opportunity to treat cancer at cellular level with very high precision.

Cancer treatments can be divided into following two groups:

- Conventional treatment (classical treatments)
- Non-conventional treatment (modern treatments)

Classical treatments include surgery, chemotherapy and radiation therapy. Depending on the medical conditions, these methods are used standalone or in combination. Hyperthermia is classified as non-conventional means of cancer treatment wherein heat is used for killing the cancer cells. Hyperthermia can also be combined with other classical therapies of cancer for effective treatment with minimal side effects.

2. HYPERTHERMIA

Hyperthermia is a non-invasive cancer therapy that uses heat to kill or damage the cancer cells without effecting the healthy cells. The main objective of this therapy is to raise the temperature of the target tissue to $41^{\circ} - 44\,^{\circ}C$. This increase in cell temperature can affect performance of various cellular structures like cell membrane, proteins, nucleic acid repair enzymes, protein denaturisation, etc. leading to the cell apoptosis or necrosis. Based on stage of cancer, target area or organ, hyperthermia can be classified into three sub categories:

(i) **Whole body hyperthermia**: In this approach, heat is applied to whole body to raise the temperature to at least 41°C. In this treatment, heat is applied superficially to whole body using inductive loops, hot water blankets or hot wax.

(ii) **Regional hyperthermia:** In this approach, heat is applied to a large part of the body or specifically to whole organ using external arrays of applicators. Heat is distributed in progressive way. This method is generally used in the treatment of tumors in advance stages. In a technique called "regional perfusion hyperthermia", part of blood is removed from the patient's body, heated and pumped back into the body.

(iii) **Local hyperthermia:** Local hyperthermia technique is used on relatively small tumors, which are located close to the surface of the body. In this approach, heat is distributed in a localized manner. Localized heating is achieved by using electromagnetic waves such as radio, microwaves or ultrasound, which are by applicators beneath the surface or under the skin of superficial cancer or implanted inside the targeted region [5].

Local and regional hyperthermia provides greater advantages over whole body hyperthermia. Effectiveness of hyperthermia treatment depends on the temperature to which the tumor is exposed, duration of exposure and properties of the tumor cells. Initially, hyperthermia treatment was performed with external devices which transfer thermal energy to the targeted tissues either by irradiation with light or electromagnetic waves, ultrasound, microwaves, infrared irradiation, and tubes with hot water. Advantages and disadvantages of different hyperthermia sources are listed in Table 1 [6].

Table 1 Advantages and disadvantages of various modes of heat delivery in hyperthermia [6]

Hyperthermia type	Delivery mode	Advantages	Disadvantages
Thermal conduction	Heated water	None	Heat penetration up to 3-5 mm
Radiation hyperthermia	Low frequency waves	Moderate penetration	Hyperthermia of healthy tissues
Magnetic induction hyperthermia	Magnetic field	Deep penetration	Toroidal heating pattern and low magnetic energy absorption
Ultrasound power deposition	Pressure waves	Optimization of penetration by modulating frequency	Limited application in anatomic body locations

Other limitations of EM wave based hyperthermia are: excessive heating in healthy tissues, low penetration of heat in tumor cells, dissipation of heat by blood, etc. To overcome these limitations, use of magnetic nanoparticles was first proposed in 1957[7].

3. MAGNETIC INDUCTION HYPERTHERMIA

In magnetic hyperthermia localized heating of tumor region is achieved by exposing magnetic nanoparticles to alternate magnetic field which are localized into the cancerous cells within the tumor region (Figure 1).

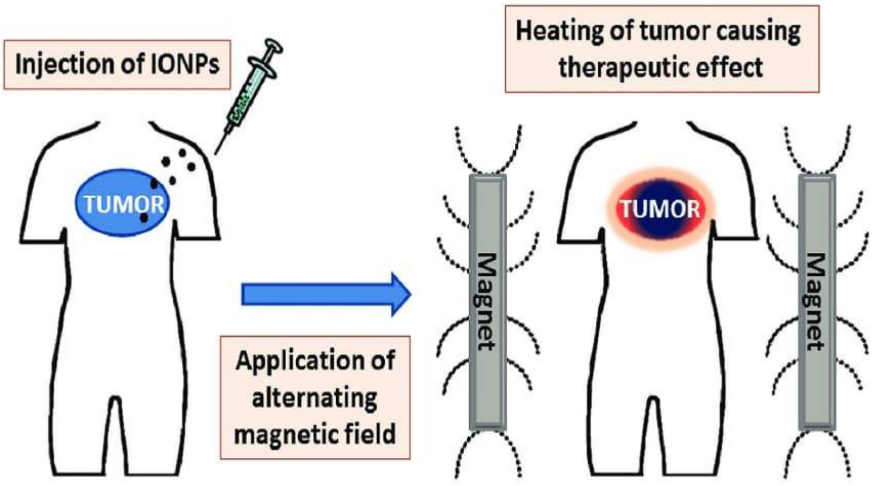


Figure 1 Concept of magnetic hyperthermia[7]

3.1 PHYSICAL PHENOMENON OF MAGNETIC HYPERTHERMIA

In 1950s, for the very first time, magnetic hyperthermia was investigated by heating few tissue samples with magnetic nanoparticles (MNPs) by exposing them to an alternating magnetic field. When the cancer cells with MNPs are exposed to alternating magnetic field, it produces enough energy that disintegrates the cancer cells causing their death [2]. In magnetic hyperthermia, MNPs are injected into the body and then subjected to alternating magnetic field to generate heat. This heat spreads into the surrounding infected area and damages the cancer cells when the temperature is maintained between 40-44 °C for requisite time.

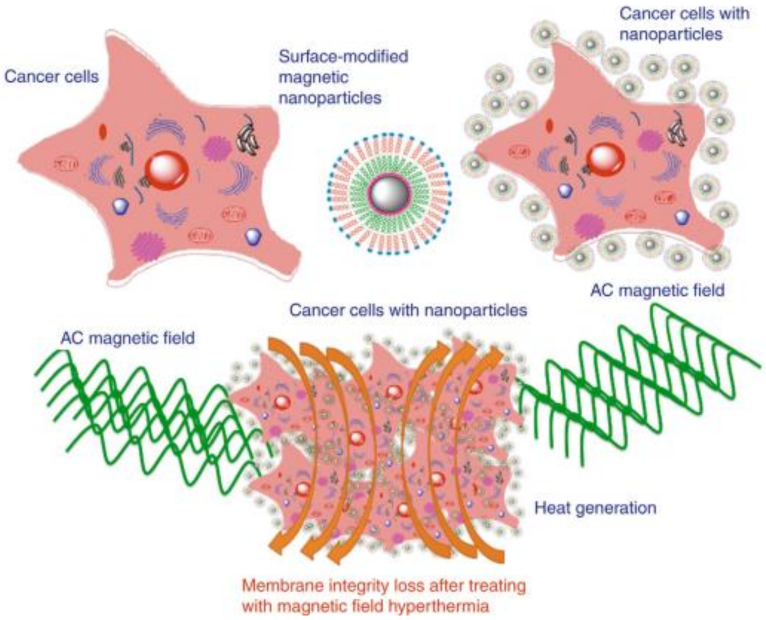


Figure 2 Schematic diagram representing mechanism of magnetic hyperthermia leading to the death of a cancerous cell [2].

3.1.1 HEAT DISSIPATION BY MAGNETIC NANOPARTICLES

When magnetic nanoparticles are exposed to an alternating magnetic field, magnetic moments will try to align along the direction of the field. As soon as the direction of external field changes, direction of magnetic moment will follow the field. This process leads to dissipation of stored magnetic energy in the form of heat. Three different mechanisms are responsible for this energy conversion:

1. Hysteresis losses
2. Relaxation losses
3. Eddy current losses

In general, heats generated by eddy currents are relatively very small as compared to hysteresis losses or the relaxation losses and hence, in magnetic hyperthermia, we neglect the contribution of eddy current losses.

HYSTERSIS LOSSES

Hysteresis loss is the measure of dissipation of magnetic energy in the form of heat per cycle of magnetization (Figure 3). Hysteresis loss can be calculated by measuring the area under the hysteresis loops. This mechanism is predominant in ferromagnetic materials. Amount of heat dissipated by ferromagnetic materials is given by

$$A = \int_{-H_{max}}^{+H_{max}} \mu_0 B(H) dH \tag{1}$$

Here, A represents the area under the hysteresis loop, H_{max} represents the highest magnetic field to which MNPs were exposed at which magnetization of particles saturates, M(H) is the magnetization of the particle at applied field H and μ_0 ($4\pi \times 10^{-7}$ H/m) is the permeability of the free space.

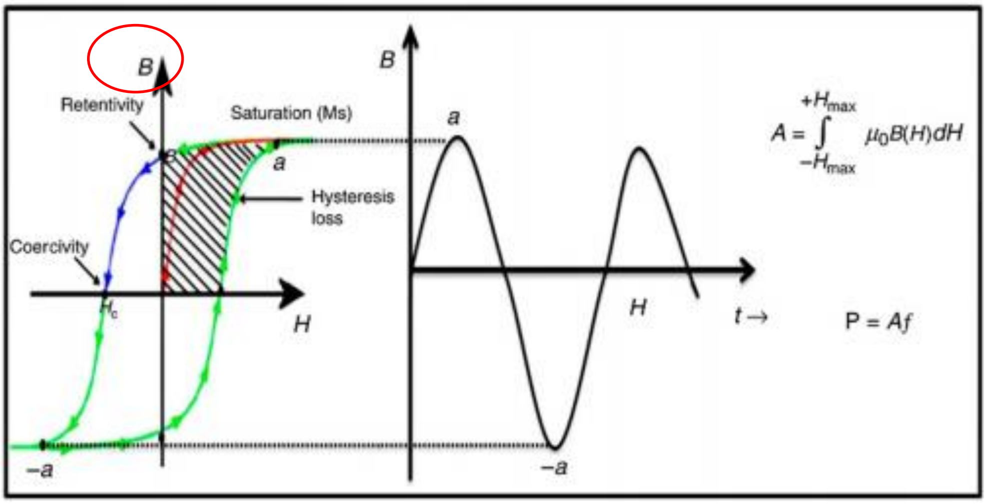


Figure 3 Hysteresis in ferromagnetic material when an AC magnetic field is applied [8].

Size and shape of hysteresis loops depends on the size of the magnetic particles. When the size of ferro or ferrimagnetic materials decreases, multi domain state of particles transform to single domain and on further decreasing the size below critical limit it transform to superparamagnetic state. In single domain state, nanoparticles have highest heat generating efficiency [8].

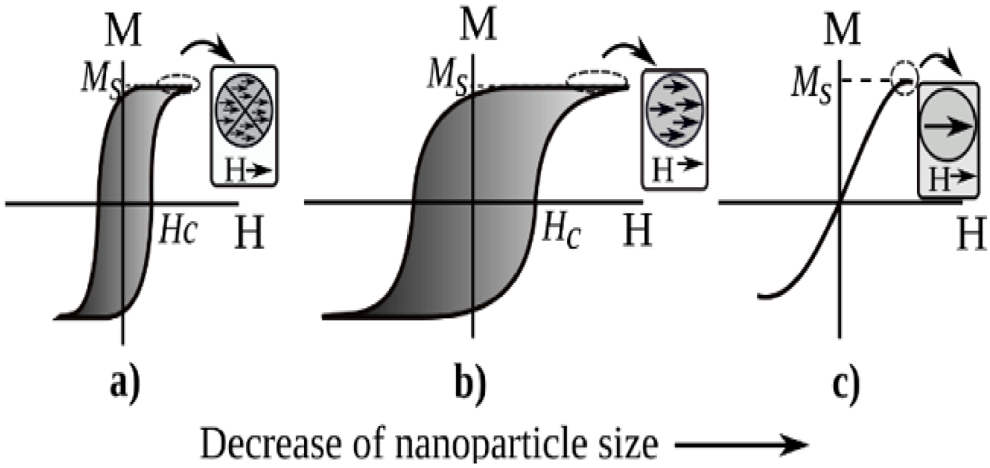


Figure 4 Effect of particle size on hysteresis loops of magnetic particles (a) multi-domain, (b) single-domain, (c) superparamagnetic [8].

RELAXATION LOSSES

Superparamagnetic materials do not generate heat through hysteresis losses. When superparamagnetic particles are kept in an external alternating magnetic field, they acquire sufficient energy to overcome the rotational energy barrier. When the magnetic field flips its direction, particle moment follows the field. In this process, stored magnetic energy will be released by the particle in the form of heat. This process of heat generation in nanoparticles is referred as relaxation losses. Magnetic nanoparticles can relax its magnetic moment via two distinct mechanisms:

I. Brownian Relaxation
II. Neel Relaxation

I. Brownian Relaxation

When a magnetic nanoparticle is kept in alternating magnetic field, the magnetic dipole moment will align in the direction of the external magnetic field. When the field direction flips, the particle moment follows the field direction by physical rotation of the particle (Figure 5). This process by which magnetic moment within the particle follows the external field is called the Brownian rotation. In this process stored magnetic energy will be released by the particle in the form of heat. The delay time between the magnetic field reversal and the magnetic moment is called as Brownian relaxation [2].

Brownian relaxation time at temperature T can be expressed As

$$\tau_B = \frac{3\eta V_H}{K_B T} \tag{2}$$

where η is the dynamic viscosity of the medium, V_H is hydrodynamic diameter of the suspended MNPs. As the viscosity of the suspension media increases, Brownian relaxation time also increases.

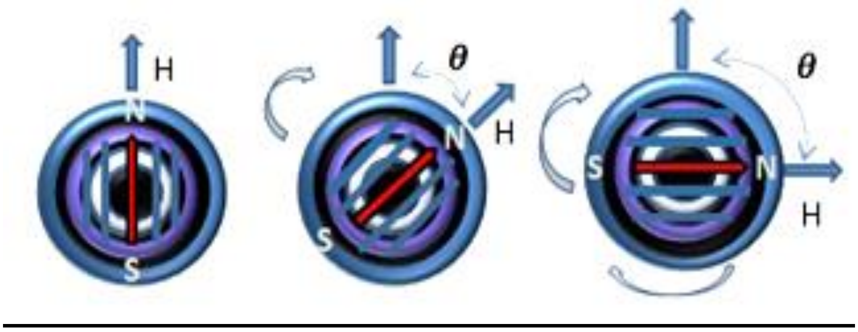


Figure 5 Brownian relaxation of magnetic nanoparticle in the presence of external magnetic field [2].

II. Neel relaxation

In Neel relaxation, when the field direction flips, the particle moment follows the field direction by internal rotation of the moment within the particle without the physical rotation of the particle (Figure 6). In this process stored magnetic energy will be released by the particle in the form of heat. The delay time between the magnetic field reversal and the magnetic moment is called as Neel relaxation [2].

Neel relaxation time at temperature T can be expressed as

$$\tau_N = \tau_0 \, exp \, (\frac{\Delta E}{K_B T}) \tag{3}$$

Here ΔE is the energy barrier for the rotation of magnetic moment; $K_B T$ is the thermal energy and τ_0 is called "attempt Time'' which is a characteristic of the material ($\tau_0 = 10^{-9}$ - 10^{-10} s). The energy barrier (ΔE) depends of magnetic anisotropy energy density of the particle. For small nanoparticles, the barrier energy (ΔE) is comparable to the room temperature thermal energy.

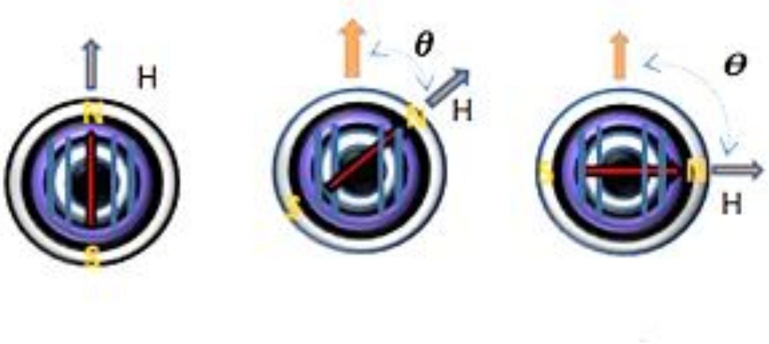


Figure 6 Neel relaxation of magnetic nanoparticle in the presence of external magnetic field [2].

Effective relaxation time or total relaxation of a magnetic nanoparticle can be expressed As

$$\tau = \frac{\tau_N \tau_B}{\tau_N + \tau_B} \tag{4}$$

EDDY CURRENT LOSSES

When a conducting sample is placed in alternating magnetic field, as per Faraday-Lenz law of electromagnetic induction, it induces the eddy currents in the direction that opposes the change in the magnetic field. In this process, part of the magnetic energy will be released by the material in the form of thermal energy which is referred as Eddy current losses[9]. Eddy current losses are very small as compared to hysteresis losses or relaxation losses and hence, ignored in magnetic hyperthermia.

In magnetic nanoparticles, depends on their size one or more mechanisms are responsible for relaxation losses, which are summarized in Figure 7.

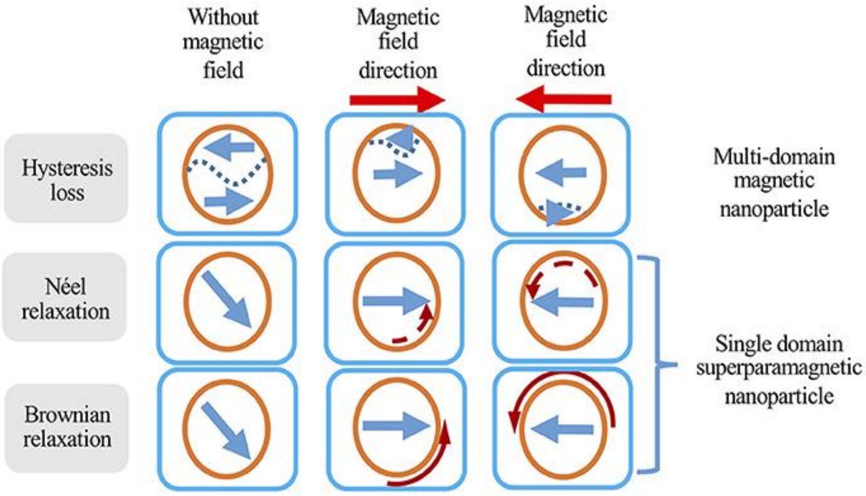


Figure 7 Relaxation losses in magnetic nanoparticles exposed to an alternate magnetic field [4].

3.2 SPECIFIC ABSORPTION RATE (SAR)

Specific absorption rate (SAR) is an important physical parameter represents the heating efficiency of magnetic nanoparticles. It is one of the most important parameter to design MNPs for particular magnetic hyperthermia application. SAR is defined as the ratio of absorbed power to the mass of MNPs [10].

$$\text{SAR} = \frac{absorbed\ power}{mass\ of\ MNPs} \tag{5}$$

SAR of MNPs depends on various factors like amplitude and frequency of applied magnetic field; physical, chemical and magnetic properties of the MNPs, dispersion media, agglomeration of nanoparticles, etc[10].

MNPs used in hyperthermia should posses high SAR. Low values of SAR can result in requirement of large quantity of MNPs to reach to threshold temperature for hyperthermia treatment. Use of large quantities may result into enhanced viscosity, toxicity and aggregation.

There are various factors that affect the SAR values such as size, shape of MNPs, agglomeration degree, magnetic properties of MNPs, amplitude of applied alternating magnetic field, its frequency.

3.2.1 Effect of nanoparticle size on SAR

For determining the SAR value, size is a crucial factor, however researchers are still working to find the optimum size for magnetic hyperthermia. Magnetic susceptibility of MNPs depends on the size of particles, as the size of particle decreases, magnetic susceptibility also decreases. MNPs with large size have higher magnetic susceptibility as compared to smaller sized MNPs. Hence, as the size of MNPs increases, their SAR value increases up to certain size, above which it starts falling again. For bulk particles, both susceptibility and SAR values are independent of the particle size [11].

3.2.2 Effect of particle shape and magnetic field frequency & amplitude of on SAR

SAR of nanoparticles are also depend on nanoparticle shape and amplitude & frequency of applied magnetic field. In an experiment, three samples of Fe_3O_4 having different size and shape have been tested under different values of amplitude and frequency of applied magnetic field to understand the effect of these parameters on SAR values of nanoparticles. Concentration and volume of samples under test were kept constant. The samples used were 22 nm spheres, polymorphous nanocrystals and 50×2 nm wires. Figure 8(a) shows temperature

- time graph at frequency 111.1 kHz with 25 mT magnetic field and figure 8(b) show temperature - time graph at frequency 629.2 kHz with 9 mT magnetic field [12]. When the frequency is 111.1 kHz and magnetic field is 25 mT, nanowires show maximum increase in temperature as compare to polymorphous and nanospheres. For nanowires, the total increase in temperature after 40 mins is 30 °C. For nanospheres, the increase is 6 °C and for polymorphous increase is just 1 °C [12].

In figure 8(b), when the frequency is 629.2 kHz and magnetic field is 9 mT, the trend is quite different as compared to figure 8(a). For nanowires, which shows the maximum increase in figure 8(a), here shows minimum increase, only 4 °C in 40 mins. Whereas nanospheres and polymorphous show maximum increase in temperature. For polymorphous crystals, the total increase in 15 °C where as for nanospheres, the total increase is temperature 16 °C in 40 min test run [12]. It was expected that SAR value and hence heating efficiency increase with increase in magnetic field frequency. In this experiment frequency is increased from 111.1 kHz to 629.2 kHz, so the expected increase is approximately 6-fold but in case of nanowires, it turned out to be opposite, whereas in case of polymorphous crystals and nanosphere, increase in heating power is not large [12]. Hence, dependence of heating efficiency or SAR of nanoparticles on their size, shape and magnetic field frequency and amplitude is quite complex. SAR values of different magnetic particle systems possessing different size and shape at different frequencies and field strength were reported in table 2.

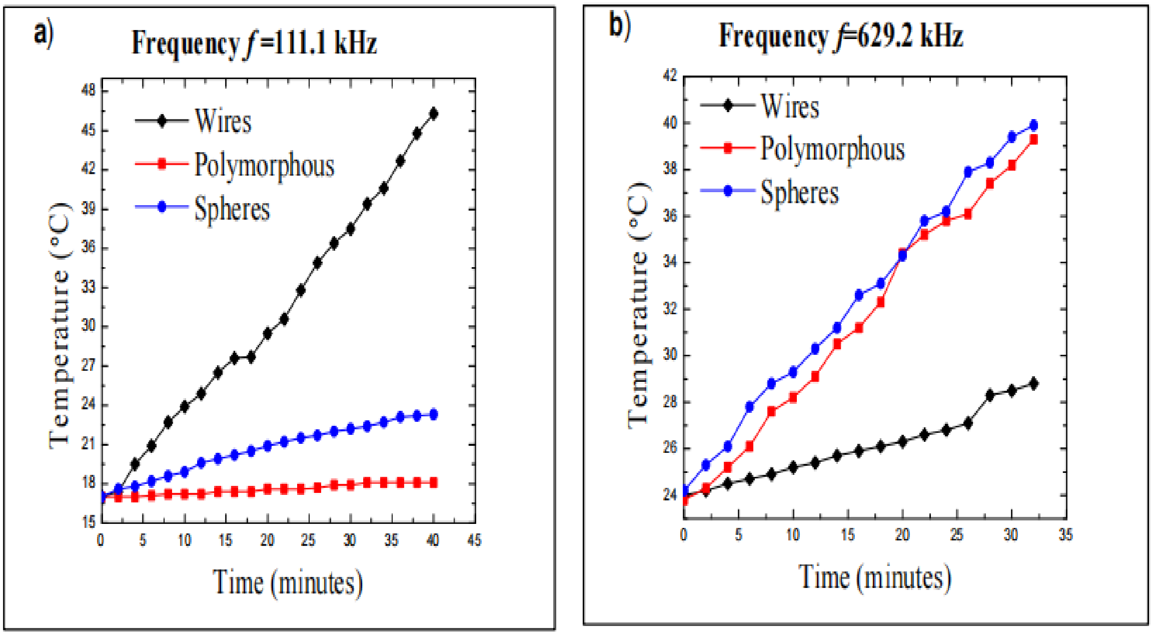


Figure 8 Dependence of heating efficiency / SAR of Fe_3O_4 nanoparticles having different shapes on .magnetic field amplitude and frequency (a) 11.1 KHz with 25 mT, (b) 629.2 KHz with 9 mT [12].

Table 2: SAR values of various magnetic nanoparticles of different shape and size

Sample	Size (nm)	Shape	Coating	H (KA/m)	f (KHz)	SAR (W/g)	Ref.
Co	6	Random	-	16	500	1600	[9]
$Co_{0.5}Zn_{0.5}Fe_2O_4$	13	Sphere	-	167	265	45	[10]
FeCo	13.3	Random	-	14	171	15	[13]
$MnFe_2O_4$	14	Sphere	GO	60	240	1588	[14]
$NiFe_2O_4$	15	Sphere	Oleic acid and TMAH	17.2	170	4	[15]
$CuFe_2O_4$	19.9	Sphere	TREG	19	120	44.9	[16]
$Mn_{0.5}Fe_{2.5}O_4$	20	Random	Citric acid	30	717	1661	[17]
$MnFe_2O_4$	20	Sphere	-	4	280	217.62	[18]
Fe_3O_4	22	Sphere	-	15.5	500	716	[11]
Fe_3O_4	33	Nanocluster	PMA	23.8	302	253	[14]
Fe_3O_4	45	Sphere	PVP	32.5	400	1100	[14]
Fe_3O_4	73	Rings	mPEG	35	400	2213	[14]
$La_{0.73}Sr_{0.27}MnO_3$	20-100	Random	Isopropyl alcohol	125	425	9.22	[19]
Fe_3O_4	225	Disc	CTAB	47.8	488	5000	[14]

3.2.3 Effect of Particle Size and Coating of MNPs on SAR

In this study Fe_3O_4 ferrofluids coated with oleic acid (OA) and PEG were used. Fe3O4 nanoparticles were prepared by co-precipitation method. In this study variation of SAR of nanoparticles as a function of there is reported [20]

Hyperthermia experiments were carried out at 265 kHz and magnetic field strength was 26.6 kA/m. Here, 10 mg each of MNPs sample was taken in 1 mL of water for the hyperthermia experiments [20]. The details are reported in table 3. The decrease in SAR values due to decrease in size can be explained with the decrease in magnetization. When the size of particle decreases, magnetic susceptibility decreases, which results in decrease in SAR values[11]. In the absence of magnetic field, the magnetization value for all the three samples is nearly zero that indicates the superparamagnetic behaviour of the particles. The heating ability of MNPs coated with oleic acid or PEG are observed even at low concentrations. It shows that these MNPs are suitable for magnetic hyperthermia applications [20]

Table 3 Effect of size of MNPs on their SAR values

Compound	Size (nm)	Coating Material	f (kHz)	H (kA/m)	SAR (W/g)
Fe_3O_4	20	-	265	26.6	38.45
	8	PEG			33.5
	6	Oleic acid			28.35

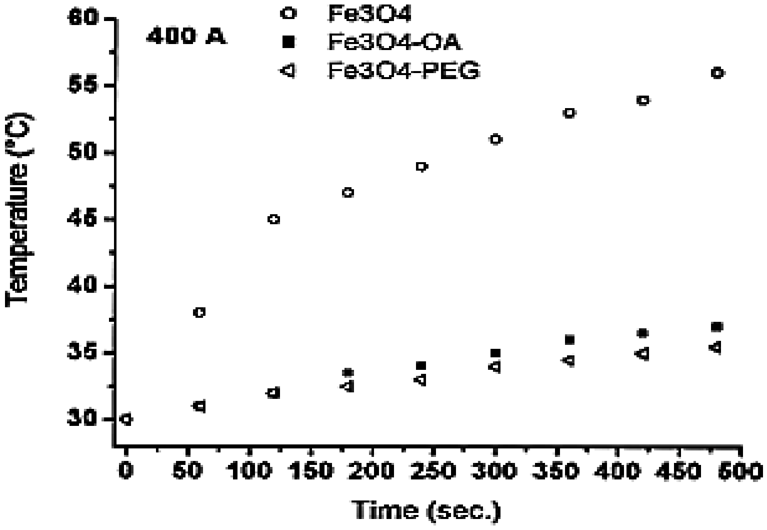


Figure 9 Temperature - time of Fe_3O_4- MNP; Fe_3O_4-OA-MNP and Fe_3O_4-PEG-MNP: at 265 kHz frequency, 26.6 kA/m alternating magnetic field [20].

3.2.4 Effect of Amplitude of Alternating Magnetic Field on SAR

In this experiment $MnFe_2O_4$ nanoparticles modified with chitosan were used for hyperthermia study [21]. The structural characterization of magnetic nanoparticles shows that the nanoparticle size is approximately 18 nm [21]. An aqueous solution of 1 mL at a constant concentration of 1.5 mg/mL of magnetic atoms were tested at various amplitudes of alternating magnetic field from 20 to 60 kA/m at a constant frequency of 307 kHz. It is observed that temperature increases with the increase in magnetic field amplitude and the heating time. Both the SAR and maximum temperature shows a linear relationship with the magnetic field amplitude as shown in the figure 10 [21]. Also from figure 10, it is concluded that rise in temperature saturates at higher magnetic field. The SAR values observed at different magnetic field amplitudes corresponding to maximum temperatures are given in the table 4.

Table 4 Effect of magnetic field strength on the SAR values and corresponding maximum temperature increase of MNPs [21].

Compound	Size (nm)	Frequency (kHz)	Magnetic field (kA/m)	SAR (W/g)	Max temperature increase (°C)
Chitosan-$MnFe_2O_4$	18	307	20	57.2	44.10
			30	97.5	48.24
			40	152.21	55.25
			50	209.41	62.81
			60	278.69	65.38

The chitosan coated nanoparticles shows superparamagnetic behavior. Heat dissipated during magnetic hyperthermia is associated with relaxation loses. This depends on various factors such as viscosity of the medium, magnetic properties of MNPs, size and shape of the nanoparticles, etc. Therefore, with the increase in amplitude of alternating magnetic field, both the SAR values and the maximum change in temperature both increases [21].

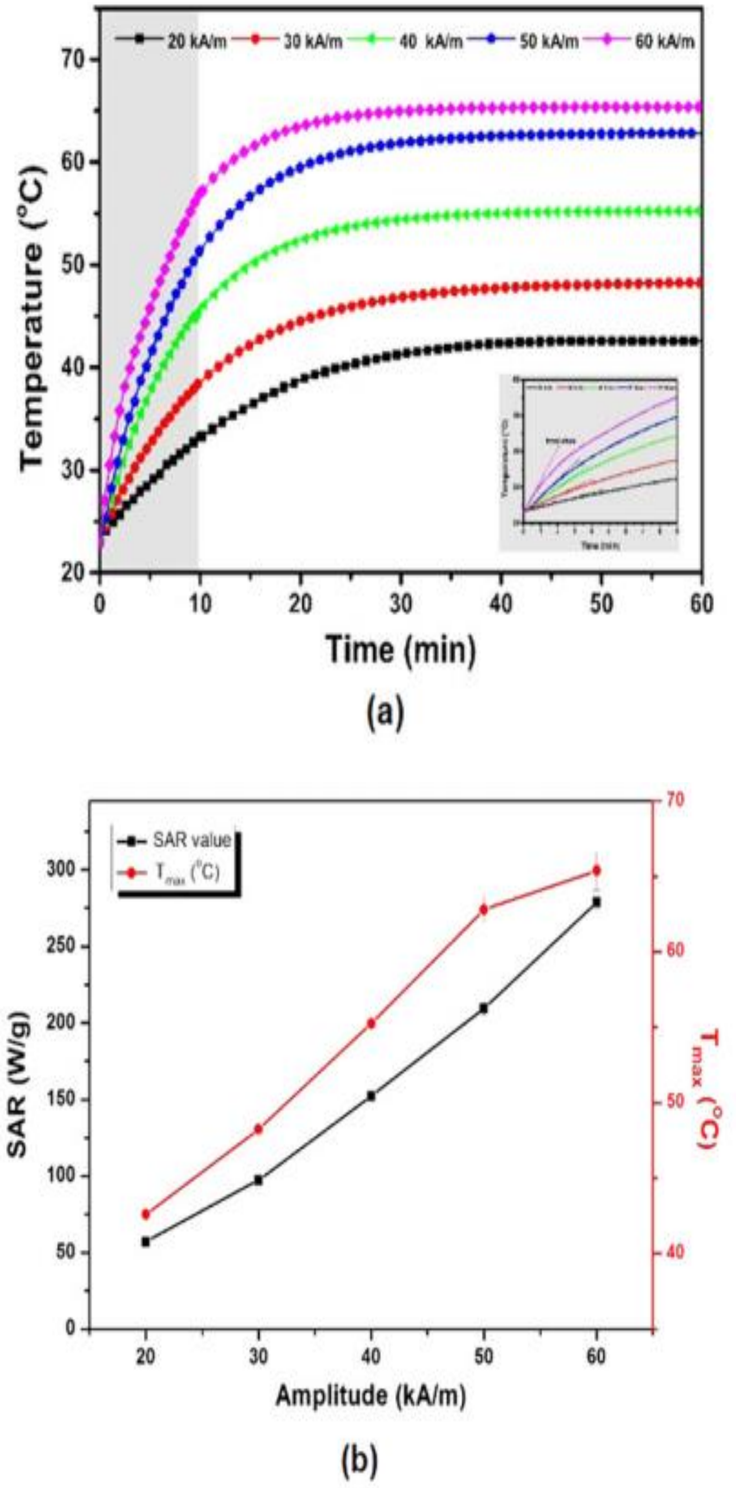


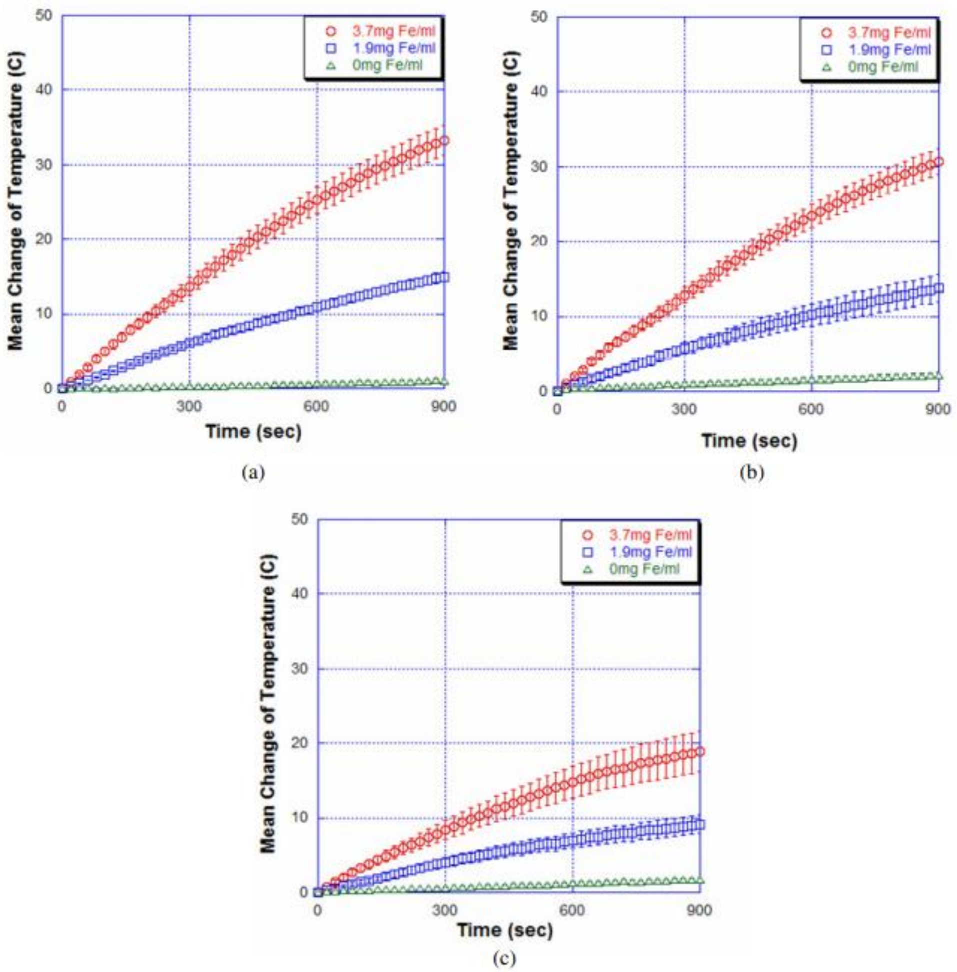
Figure 10 Effect of magnetic field strength on magnetic heating of a) 1mL of aqueous solution (1.5mg/mL) of chitosan-$MnFe_2O_4$ nanoparticles at 307 kHz; b) their SAR with corresponding maximum change in temperature [21].

3.2.5 Effect of Concentration of MNPs and Suspension Media on SAR

Concentration of MNPs and suspension media plays a crucial role in determining the SAR values for magnetic nanoparticles. Higher is the concentration of MNPs, higher the SAR value. Also, the viscosity of suspension media affects the heat generation by magnetic nanoparticles, higher is the viscosity of suspension media, lesser is the heat generation and hence, lower would be the SAR values. In this hyperthermia experiment were performed on MNPs dispersed in three different suspension media, with varying nanoparticle concentration. Size of $\mathrm{Fe_3O_4}$ nanoparticles is estimate to be 10 nm. These MNPs are uncoated but to prevent agglomeration of MNPs, thin layer of anionic surfactants is added in the carrier liquid [22]. MNPs suspended in three different medium; a) water, b) glycerol, c) collagen are heated with 9 kA/m of alternating magnetic field having 175 kHz frequency. The thermal curves are recorded at three different concentrations; 3.7 mg Fe/mL, 1.9 mg Fe/mL and 0 mg Fe/mL as shown in the figure 11 [22].

When the thermal responses of MNPs suspended uniformly in water, glycerol and collagen were studied, it has been observed that in all the three cases, on increasing the concentration of MNPs, rise in temperature was observed. Specifically on doubling the concentration, from 1.9 mg Fe/mLl to 3.7 mg Fe/mL, the effective temperature rise also doubled. Maximum temperature rise for MNPs suspended in water is 15 °C when concentration is 1.9 mg Fe/mL and it increases to 33 °C when concentration changes to 3.7 mg Fe/mL. In case of glycerol and collagen, maximum rise in temperature observed is 13.5 ℃ and 9 ℃, respectively for concentration of 1.9 mg Fe/mL, which further increases to 30 ℃ and 19 ℃ when concentration increases to 3.7mg Fe/mL[22]. Average heating rate calculated were approximately, 0.048 and 0.02 ℃/s for water; 0.047 and 0.0195 ℃/s for glycerol; 0.0313 and 0.0139 ℃/s for collagen at 3.7 mg Fe/mL and 1.9 mg Fe/mL, respectively [22].

The temperature rise in water is higher than glycerol, although the difference is minor at all MNPs concentrations. Increase in temperature decreases significantly when observed in collagen as compared to water and glycerol [22]. This can be attributed to the resistance offered by suspension media for the relaxation process to generate heat due to its high viscosity. In addition to its high viscosity when compared to water and glycerol, there might be some interactions between the MNPs and the collagen fibers, that could also resist the relaxation of particles [22].



(a) (b)

(c)

Figure 11 Mean change in temperature as a function of time for Fe_3O_4-MNPs suspended in a) water, b) gylcerol, and c) collagen[22].

Table 5 Rise in temperature of Fe_3O_4 MNPs suspended in different media[22].

Compound	Size (nm)	Magnetic field (kA/m)	Frequency (kHz)	Suspension media	Concentration (mg/mL)	Temperature rise (℃)
Fe_3O_4	10	9	175	Water	1.9	15
					3.7	33
				Glycerol	1.9	13.5
					3.7	30
				collagen	1.9	9
					3.7	19

3.3 MAGNETIC NANOPARTICLES

Magnetic nanoparticles possess different properties as compared to their bulk counterparts and these properties depend on various factors such as particle size and shape, chemical composition, defects and surroundings. Therefore, properties of nanoparticles can be altered by changing the particle size, shape and structure [14]. Some these factors can be controlled during the synthesis process which we will discuss at a later stage in the synthesis section. Apart from this, magnetic nanoparticles meant for biomedical applications should also be biocompatible and non-toxic and possess high colloidal stability.

3.3.1 Nature of Magnetic Nanoparticles

Ferro, Ferri- and Superparamagnetic nanoparticles are suitable for magnetic hyperthermia. When these three different classes of magnetic materials are exposed to alternating magnetic field, they produce heat via different mechanisms which were discussed previously. Hence, nature of magnetic nanoparticles is vital for a successful magnetic hyperthermia therapy.

3.3.2 Biocompatibility / toxicity

Magnetic nanoparticles used in hyperthermia must be biocompatible. Nanoparticles may be toxic and can cause serious problems if they are not biocompatible. This is one of the most important requirements. To minimize the toxicity of nanoparticles they are coated with suitable surfactants.

3.3.3 Colloidal Stability

MNPs in hyperthermia are used in the form of nano fluids, i.e. a colloidal suspension of magnetic nanoparticles in bio-compatible media. MNPs tend to aggregate and form clusters. Aggregation of nanoparticles further increases in the presence of magnetic field. Consequently, such aggregation of particles decreases the efficiency of heating.

3.4 SYNTHESIS OF MAGNETIC NANOPARTICLES

Magnetic nanoparticles can be synthesized with various chemical methods like sol-gel, thermal decomposition, microemulsion solvothermal, co-precipitation, etc. Amongst these methods, co-precipitation method is most popular due to ease, large yield, and use of low toxic compounds. Synthesis of Fe_3O_4 nanoparticles by coprecipitation method is explained below.

$FeSo_4.7H_2O$ gives Fe^{2+} whereas $Fecl_3.6H_2O$ gives Fe^{3+} in aqueous solution. These are taken in the stoichiometric ratio of 1:2 in aqueous solution. Under continuous mechanical stirring, 25% of ammonia solution is added to the above mixture. Instantly, black precipitate of Fe_3O_4 are formed. pH between 10 to 11 is maintained for 20 mins (add excess ammonia solution if required). Now, the nanoparticles are obtained by magnetic decantation. These are washed with distilled water so as to remove water-soluble impurities. The wet slurry is obtained and used for the coating process. Following chemical reaction takes place:

$$2FeCl_3.6H_2O + FeSO_4.7H_2O + 8NaOH \longrightarrow Fe_3O_4 + 6NaCl + Na_2SO_4 + 23H_2O$$

3.4.1. SYNTHESIS OF WATER BASED MAGNETIC FLUID

In order to obtain water soluble Fe_3O_4 nanoparticles, they are coated with oleic acid twice. A clear solution of ammonium oleate is obtained by adding oleic acid into ammoniated water under continuous stirring at 40°C. Now, ammonium oleate solution is added to the wet slurry of nanoparticles under continuous stirring and is kept at room temperature for 1 hr. after that temperature is raised to 92 °C and maintained for 2 mins. Solution is cooled to room temperature; oleic is chemically absorbed on the surface of nanoparticles [23]. Now, the solution is cooled down to room temperature and flocculated with dilute HCl followed by washing with water and acetone.

For second coating, ammonium oleate solution was prepared as explained previously. It was then added dropwise into the slurry of oleic acid coated Fe_3O_4 nanoparticles. Dropwise addition of ammonium oleate under mild heating and stirring will be continued until the all the nanoparticles come in suspension state. Second coating of oleic acid is physisorbed [23]. Water based Fe_3O_4 magnetic fluid is thus obtained which is stored at room temperature. There is no control over size distribution and shape of nanoparticles, when synthesized with this method. The product yield is low, but this is fast, low cost and low temperature method.

3.5 MAGNETIC HYPERTHERMIA MEASUREMENT

3.5.1 MagneTherm Experimental setup

MagneTherm is used for magnetic hypothermia experiments. It is specifically designed by nanoTherics, UK for studying magnetic fluid hyperthermia. The main features of this system are:

- It has convenient size
- It can be used for *in-vitro* / *in-vivo* studies,
- It is specially designed for fluid MNPs for hyperthermia

The schematic diagram of the setup is shown in figure 12 and actual photographic view of laboratory setup is presented in figure 13.

MagneTherm hyperthermia setup is controlled by a software called ‘MagneSoft’. It provides control environment, wherein the conditions of the experiments like, magnetic field strength, frequency, exposure time, number of cycles of treatment, etc. can be set. In addition, the setup is designed in a way, one can perform experiments on cell lines in Petri dishes or on small animals in external coils. Temperature rise in the fluid can be monitored by three channel fiber optic temperature sensor.

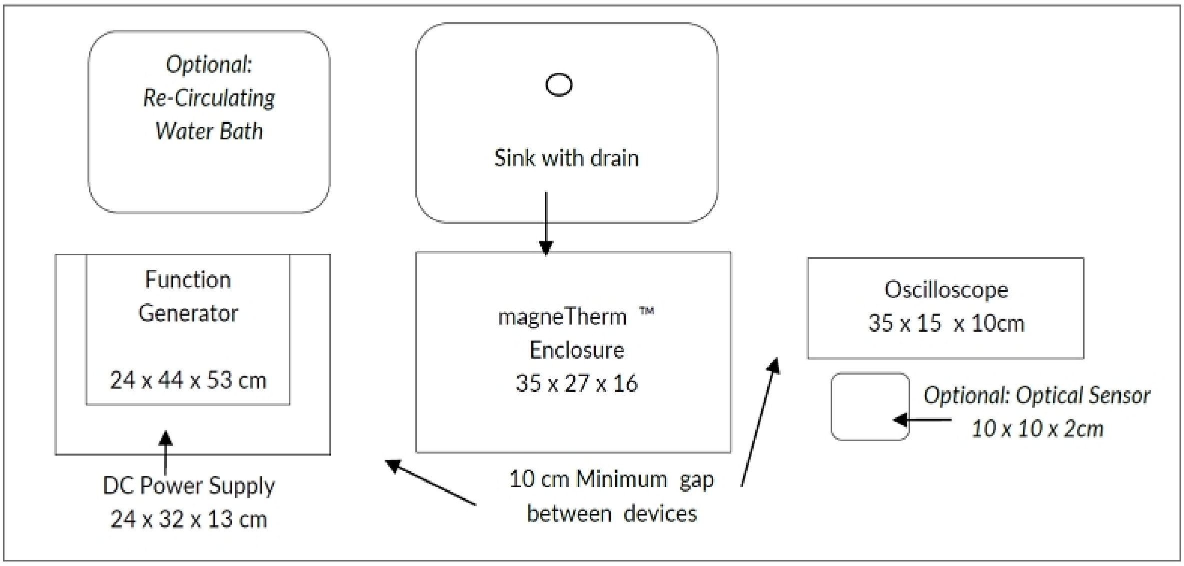


Figure 12 Schematic diagram of MagneTherm hyperthermia setup [24]

The AC coils (solenoids) were energized by high power radio frequency source. Resonance frequency of the solenoid is tunable and it can be tuned with the help of external capacitor bank. By appropriately choosing the inductance value of coil and capacitance of the capacitor, desired magnetic field frequency can be established for the safe hyperthermia application.

Generally, the amplitude of the AC field can also be altered by providing different input AC pulse from the power amplifier driving the solenoid. The typical field value varies between 0 – 50 mOe, while field frequency goes from 100 kHz to 935 kHz for most of the applications. The software works on resonant frequency according to the coil and the capacitor combination through which the particles are exposed to alternating magnetic field and the temperature data is recorded through Optical Sensors.

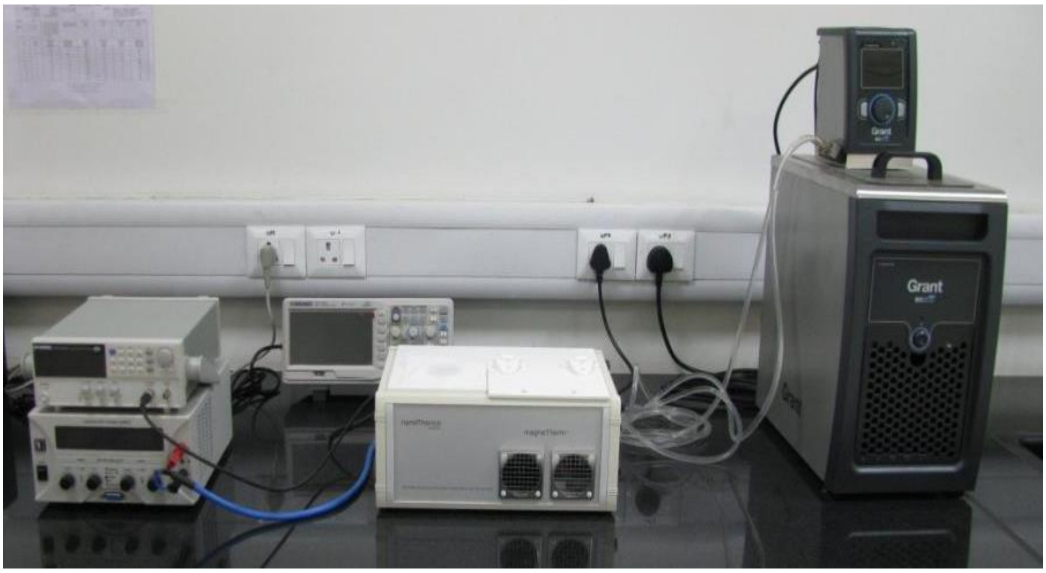


Figure 13 Photographic view of MagneTherm hyperthermia setup[24].

3.6 MAGNETIC HYPERTHERMIA COMBINED WITH OTHER TREATMENTS

Magnetic hyperthermia is currently under research for experimental therapeutic applications. It still demands research in the synthesis, stability and compatibility of MNPs for biomedical applications and clinical trials. So, magnetic hyperthermia treatment preceded into Phase II of clinical trials as a combination therapy with conventional treatment. Integrating hyperthermia treatment with other therapies gives potential advantages for the treatment of cancer [2]. In addition to its reducing toxicity and side-effects of the therapy therapies, it improves the condition of patients to a greater level [11].

When combining the magnetic hyperthermia with the radiotherapy for cancer treatment, magnetic hyperthermia plays a very important rule in radio-sensitization, which basically increase the damage to the tumor cells and the blood vessels which repair the tumor DNAs after injury. This combination of magnetic hyperthermia and radiotherapy prove to be less toxic to the normal tissues and more effective in killing the cancer cells, thus, resulting in the lower doses of radiation [11].

On combining the magnetic hyperthermia with chemotherapy, it has been observed that magnetic hyperthermia helps in increasing the permeability of cell membrane and increase the uptake of drugs by the cell. Magnetic iron oxide nanoparticles are used as nanocarriers to enhance the chemotherapy when combined with magnetic hyperthermia by controlling the the applied alternating magnetic field frequency, both the heat generation and drugs release can be controlled on demand. However, it has been observed that the cumulative drug release amount when exposed to alternating magnetic field is approximately two times higher than by water bath heating [11].

When magnetic hyperthermia is combined with immunotherapy, it has been observed that in addition to killing of the cancer cells by heat, it also triggers the antitumor immune response by releasing tumor antigens. In spite of all the therapies and progressions, chances of occurrence of tumor remain after the treatment [11]. Recent studies show that the immune therapies could help in preventing the tumor from reoccurrence by activating the antitumor immune system to attack the tumor cells. Specifically, extra heat shock proteins can activate antitumor mechanism. So, a model combining of magnetic hyperthermia and immunotherapy is capable of generating maximum number of heat shock proteins to stimulate the antitumor immunity and reducing its reoccurrence[2].

References

[1] J. Jose *et al.*, "Magnetic nanoparticles for hyperthermia in cancer treatment: an emerging tool," *Environmental Science and Pollution Research*, vol. 27, no. 16, pp. 19214–19225, (2020).

[2] S. Ahmed, B. L. Rajak, M. Gogoi, and H. D. Sarma, *Magnetic nanoparticles mediated cancer hyperthermia*. INC, (2020), DOI: https://doi.org/10.1016/B978-0-12-817913-0.00016-X

[3] "Benefits of Nanotechnology for Cancer - National Cancer Institute." https://www.cancer.gov/nano/cancer-nanotechnology/benefits.

[4] Suriyanto, E. Y. K. Ng, and S. D. Kumar, "Physical mechanism and modeling of heat generation and transfer in magnetic fluid hyperthermia through Néelian and Brownian relaxation: a review," *Biomedical engineering online*, vol. 16, no. 1, p. 36, (2017)

[5] Z. Behrouzkia, Z. Joveini, B. Keshavarzi, N. Eyvazzadeh, and R. Z. Aghdam, "Hyperthermia: How can it be used?," *Oman Medical Journal*, vol. 31, no. 2, pp. 89–97, (2016)

[6] J. Verma, S. Lal, and C. J. F. van Noorden, "Nanoparticles for hyperthermic therapy: Synthesis strategies and applications in glioblastoma," *International Journal of Nanomedicine*, vol. 9, no. 1, pp. 2863–2877, (2014)

[7] C. L. Dennis and R. Ivkov, "Physics of heat generation using magnetic nanoparticles for hyperthermia," *International Journal of Hyperthermia*, vol. 29, no. 8, pp. 715–729, (2013)

[8] C. M. Bedoya-Hincapié, E. Restrepo-Parra, and L. D. López-Carreño, "Applications of magnetic and multiferroic core/shell nanostructures and their physical properties ",*DYNA (Colombia)*, vol. 85, no. 207, pp. 29–35, (2018)

[9] D. Ortega and Q. A. Pankhurst, "Magnetic hyperthermia," vol. 1, pp. 60–88, (2012)

[10] D. S. Nikam, S. v. Jadhav, V. M. Khot, M. R. Phadatare, and S. H. Pawar, "Study of AC magnetic heating characteristics of Co0.5Zn 0.5Fe2O4 nanoparticles for magnetic hyperthermia therapy," *Journal of Magnetism and Magnetic Materials*, vol. 349, pp. 208–213, (2014).

[11] X. Liu *et al.*, “Comprehensive understanding of magnetic hyperthermia for improving antitumor therapeutic efficacy,” *Theranostics*, vol. 10, no. 8, pp. 3793–3815, (2020)

[12] L. M. Armijo *et al.*, “Iron Oxide Nanocrystals for Magnetic Hyperthermia Applications,” *Nanomaterials*, vol. 2, no. 2, pp. 134–146, (2012)

[13] Ö. Çelik and T. Fırat, “Synthesis of FeCo magnetic nanoalloys and investigation of heating properties for magnetic fluid hyperthermia,” *Journal of Magnetism and Magnetic Materials*, vol. 456, pp. 11–16, (2018)

[14] I. M. Obaidat, V. Narayanaswamy, S. Alaabed, S. Sambasivam, and C. V. v. Muralee Gopi, “Principles of Magnetic Hyperthermia: A Focus on Using Multifunctional Hybrid Magnetic Nanoparticles,” *Magnetochemistry*, vol. 5, no. 4, p. 67, (2019)

[15] E. Umut, M. Coşkun, F. Pineider, D. Berti, and H. Güngüneş, “Nickel ferrite nanoparticles for simultaneous use in magnetic resonance imaging and magnetic fluid hyperthermia,” *Journal of Colloid and Interface Science*, vol. 550, pp. 199–209, (2019).

[16] S. M. Fotukian, A. Barati, M. Soleymani, and A. M. Alizadeh, “Solvothermal synthesis of CuFe2O4 and Fe3O4 nanoparticles with high heating efficiency for magnetic hyperthermia application,” *Journal of Alloys and Compounds*, vol. 816, p. 152548, (2020)

[17] E. Mazarío *et al.*, “High specific absorption rate and transverse relaxivity effects in manganese ferrite nanoparticles obtained by an electrochemical route,” *Journal of Physical Chemistry C*, vol. 119, no. 12, pp. 6828–6834, (2015).

[18] S. R. Patade, D. D. Andhare, S. B. Somvanshi, S. A. Jadhav, M. v. Khedkar, and K. M. Jadhav, “Self-heating evaluation of superparamagnetic MnFe2O4 nanoparticles for magnetic fluid hyperthermia application towards cancer treatment,” *Ceramics International*, vol. 46, no. 16, pp. 25576–25583, (2020).

[19] N. K. Prasad, K. Rathinasamy, D. Panda, and D. Bahadur, “TC-tuned biocompatible suspension of La0.73Sr 0.27MnO3 for magnetic hyperthermia,” *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, vol. 85, no. 2, pp. 409–416, (2008).

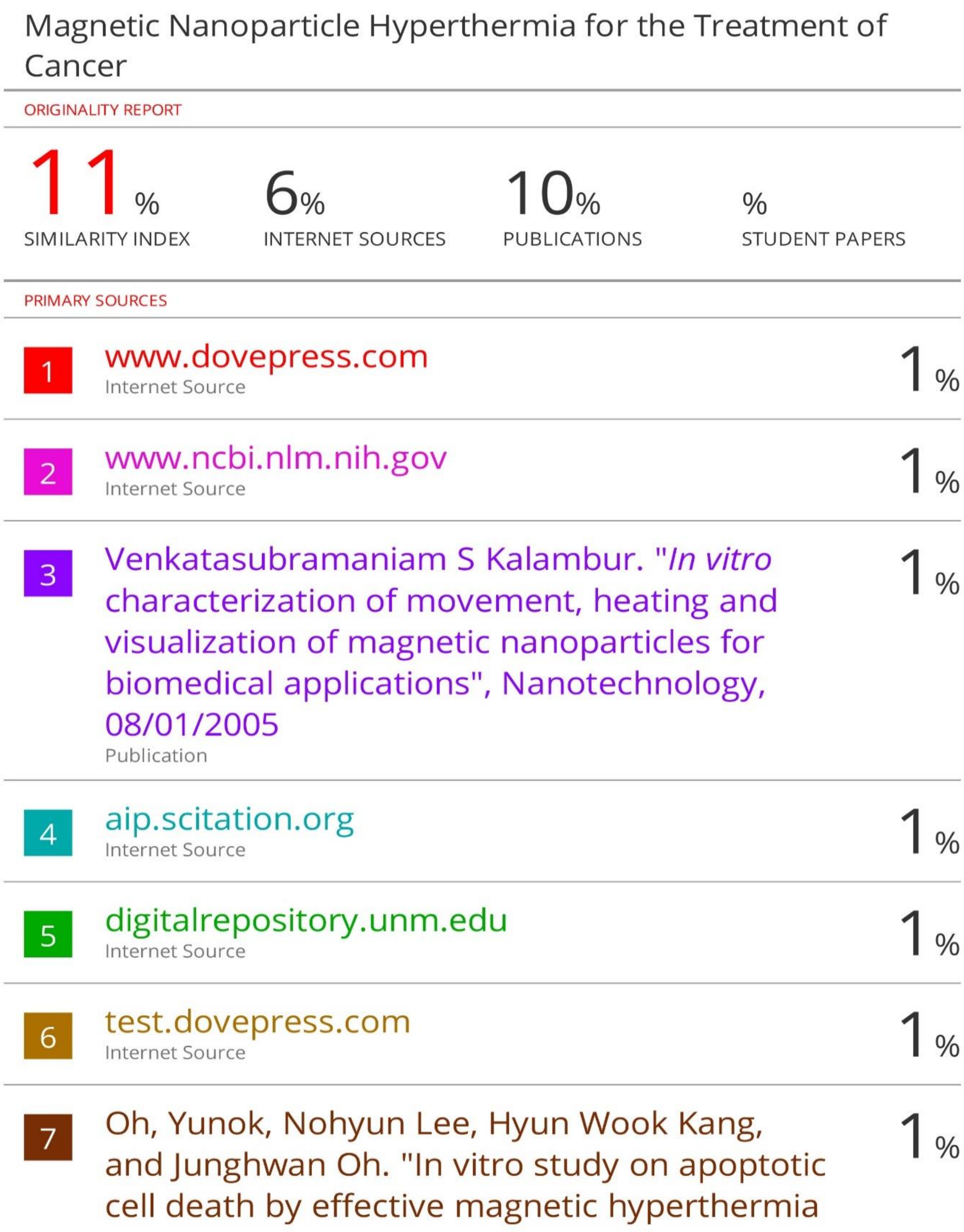
[20] R. Ghosh *et al.*, “Induction heating studies of Fe3O4 magnetic nanoparticles capped with oleic acid and polyethylene glycol for hyperthermia,” *Journal of Materials Chemistry*, vol. 21, no. 35, pp. 13388–13398, (2011).

[21] Y. Oh, N. Lee, H. W. Kang, and J. Oh, “In vitro study on apoptotic cell death by effective magnetic hyperthermia with chitosan-coated MnFe2O4,” *Nanotechnology*, vol. 27, no. 11, p. 0, (2016).

[22] V. S. Kalambur, B. Han, B. E. Hammer, T. W. Shield, and J. C. Bischof, “In vitro characterization of movement, heating and visualization of magnetic nanoparticles for biomedical applications,” *Nanotechnology*, vol. 16, no. 8, pp. 1221–1233, (2005).

[23] N. Andhariya, “Shodhganga@INFLIBNET: SYNTHESIS AND SIMULATION STUDY OF BIOCOMPATIBLE FERROFLUID FOR CERTAIN BIOMEDICAL APPLICATIONS.” https://shodhganga.inflibnet.ac.in/handle/10603/41784.

[24] “nanoTherics | MagneTherm ^{TM}.” http://www.nanotherics.com/magnetherm/



Magnetic Nanoparticle Hyperthermia for the Treatment of Cancer

ORIGINALITY REPORT

11%	6%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.dovepress.com Internet Source	1%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	Venkatasubramaniam S Kalambur. "*In vitro* characterization of movement, heating and visualization of magnetic nanoparticles for biomedical applications", Nanotechnology, 08/01/2005 Publication	1%
4	aip.scitation.org Internet Source	1%
5	digitalrepository.unm.edu Internet Source	1%
6	test.dovepress.com Internet Source	1%
7	Oh, Yunok, Nohyun Lee, Hyun Wook Kang, and Junghwan Oh. "In vitro study on apoptotic cell death by effective magnetic hyperthermia	1%

Ashima.

Dr. Bhupendrakumar Chudasama

SUPERVISOR

Ashima

STUDENT