Chapter 1

Introduction and literature review

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1.1. Introduction

In the last few decades, medical sciences and technologies have achieved significant advancement. Nevertheless, ‘cancer’ still remained a global and significant public health concern. It is a condition developed by the uncontrolled growth of the abnormal cells with a high morbidity rate and ranks as a leading cause of death [1]. A recent data provided by International Agency for Research on Cancer (IARC) reported 19.3 million new cancer cases and almost 10.0 million cancer deaths in 2020 alone [2] up from 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 [3]. These figures are expected to increase further to 30.2 million new cancer cases and almost 16.3 million cancer deaths in 2040, which is a matter of grim concern for the humanity. Thus, the treatment of cancer or malignant tumors is the foremost priority of the World Health Organization (WHO). The traditional approaches for the treatment of these malignant tumors include surgical treatment, radiation therapy and chemotherapy. These treatment approaches, however, are less efficient in targeting the tumors precisely which can cause severe collateral damage to healthy body tissues deteriorating the condition of the patients [4]–[6]. Yet another approach utilizes temperature for the destruction of malignant tumors. Conferring to a famous aphorism by Hippocrates (460–370 BC)- “those diseases which medicines do not cure, the knife cures; those which the knife cannot cure, fire cures; and those which fire cannot cure, are to be reckoned wholly incurable”. The heating of certain organs or tissues to temperatures between 42 to 46 °C, preferentially for cancer therapy is called “Hyperthermia” [7], [8]. The malignant tumors are more sensitive to heat than that of the normal tissues due to its tortuous, disorganized vasculature which overflows with blind ends and abnormal bulges, these can be preferentially destroyed in 42 to 46 °C temperature range [8], [9]. Conventionally, hyperthermia treatment was administered through electromagnetic waves, for e.g., ultrasound, radiofrequency, microwaves and infrared radiation, generated by some external devices. Hot water tubes and baths were also in tradition for hyper-thermic treatment [10]. A major drawback of these conventional modalities is the exertion in heating the local tumor region and the deep seeded tumors to the therapeutic temperature range without damaging the normal cells. Also these devices are designed to heat the tissue to approximately 42 to 46°C. However, introducing higher temperatures can kill a greater number of tumor cells through thermal ablations and necrosis which would prove favorable during tumor-specific hyperthermia [11]. Thus, effective treatment methodologies which can selectively kill cancer cells or malignant tumors are in demand all the time in global arena. Among several strategies, nanoparticles (NPs) mediated localized hyperthermia treatment modalities have shown most promising domino effect during clinical and various *in-vivo* and *in-vitro* studies [12]–[15]. These localized treatment approach utilizes the heat generated by NPs in selective destruction of cancer cells under the application of a distant energy source and offers several advantages. For example, NPs can easily pass into several tumour region whose pore sizes are in 200–780 nm range, increasing the effectiveness by delivering therapeutic heat directly to them [16]. In addition, these NPs can be functionalized with cancer-specific binding agents and the possibility of NPs to form stable colloidal fluids allow for the intravenous or local hypodermal injection to the tumor tissues, resulting in obvious advantages such as less invasion, high targeting and low side effects [17], [18]. In the present-day of nanoscale era the NPs assisted localized treatment based on magnetic- and photo-induced thermal therapy has gained much popularity in which heat is generated using either magnetic nanoparticles (MNPs) or photo-sensitive NPs, respectively [19]. However, the use of NPs in biomedical applications is conditioned to their associated toxicity in biological systems. The magnetic-induced thermal therapy employing MNPs is known as magnetic fluid hyperthermia (MFH) while photo-induced thermal therapy with photo-sensitive NPs is known as photo-thermal therapy (PTT).

1.2. Magnetic properties at nanoscale

The magnetic properties of NPs behave unusually from their bulk counter-parts. The surface effects become substantial in NPs because the ratio of the number of surface atoms to the core atoms increases. In such small particles the magnetism is subjugated by finite-size and surface effects, such as reduced coordination and symmetry of surface atoms, oxidation, and surface strain [20]. And thus the magnetic properties, such as, magnetic moment per atom (μ), magnetic anisotropy (K), Curie temperature (T_C), and the coercive field (H_C) modify when the size of the particles is reduced to nano-dimension, i.e. NPs behave differently than those of a bulk material. When the bulk magnetic grain are reduced to a critical diameter, D_C, it becomes energetically unfavorable to sustain the multi domain structure and the material behaves as a single magnetic domain where all the magnetic moments are rigidly aligned to produce a single giant spin [20], [21]. The critical size for single domain typically depends on the type of materials, for example, it is around 100 nm for $CoFe_2O_4$ while 80 nm for Fe_3O_4 [22]. In general, NPs of size around 10-50 nm are preferred in bio-medical applications due to advantages such as increased blood circulation time and accumulation in the tumor tissue through enhanced permeability and retention (EPR) effect [23]. Reducing the dimensions of the particle to this size scale (10-50 nm) results in a superparamagnetic state which is characterized by random orientation of the magnetic moments in the absence of an external magnetic field [20]–[22]. The measured magnetization against field ($M-H$) curves of the superparamagnetic MNPs display negligible coercivity (H_C) and remanence (M_R), values analogous to that of a paramagnet.

However, it possesses a large magnetic moment like a ferromagnet which separates it from a paramagnet. Yet again at room temperature there exists a critical size for superparamagnetic transition (D_{sp}) and depends on the type of the material and the temperature of the system [22]. The MNPs display superparamagnetic behavior above a certain temperature called the blocking temperature (T_b), which can be defined as, $T_b = KV/k_B \ln(\tau_{exp}/\tau_0)$, where K and V are the anisotropy energy constant and volume of the material respectively, k_B is Boltzmann constant, τ_0 is material-specific relaxation time and τ_{exp} is the experimental time scale . Above the blocking temperature the thermal energy ($k_B T$) is sufficient to overcome the anisotropy energy barrier (E_a). This causes a random magnetic orientation, due to magnetic relaxation (discussed in later section), resulting in a net zero magnetization in the absence of an external magnetic field. For instance, spherical Fe_3O_4 nanoparticles of diameter ~ 26 nm ($K \approx 1.1 \times 10^4$ Jm^{-1}) exhibit T_b around 300 K and, thus, particles larger than ~26 nm would have ferromagnetic behavior at room temperature, while smaller particles should exhibit superparamagnetism [24]. It will be discussed in subsequent sections that the anisotropy energy constant, K, plays an decisive role in determining the critical size for superparamagnetic behavior and can be modulated by altering the composition of the material with specific volume and structure. For example, T_b of Fe_3O_4 NPs is found to decrease with Zn^{2+} substitution [25]. Usually the saturation magnetization (M_S) of small particles is lower than the bulk magnetization caused by the formation of magnetically dead layer over their surface. The magnetically dead layer is made of the disordered magnetic dipoles having direction different from the core magnetic moment and acts as a negative force to reduce the magnetization. The effect of dead layer is more profound in smaller particles and increases with reducing the particle size due to greater surface to volume ratio.

In addition, a deviance from the normal cation distribution, site disorder and the presence of adsorbed water on particles' surface add to the reduction of magnetic properties of NPs.

1.3. Magnetic fluid hyperthermia

The use of temperature as a potential tool for the treatment of some diseases, including cancer, was already mentioned in the advanced cultures of the ancient India, Egypt and China [26], [27]. Recently, hyperthermia treatment utilizing NPs has received renewed attention due to the promising advances in clinical trials suggesting a potential application in cancer therapy. In 1957, *Gilchrist et al.* [28] first proposed the application of magnetic materials for hyperthermia treatment of malignant tumors which further promoted by the pioneering work of *Gordon et al.* [29] in 1979 documenting the intracellular application using dextran coated MNPs under high-frequency alternating current magnetic field (AMF). In mid 90s, *Chan et al.* [30] and *Jordan et al.* [31] experimentally prove the high efficiency of a superparamagnetic MNPs colloidal dispersion of converting the absorbed energy of the applied AMF into heat for hyperthermia application. In last three decades much efforts have been dedicated to improve the performance of magnetic hyperthermia through the optimization of the material and the field parameters to successfully employ it in clinical applications. Several metals, oxides and carbides holding magnetic properties have been gauged for their suitability for hyperthermia application. In fact, clinical trials of MFH have already been conducted on specific cancers such as glioblastoma [15], [32], prostate carcinoma [14], breast tumors [33], and so on. Following the positive outcomes of clinical trial, the European Union, in 2011, has approved the clinical practice of the MFH for the treatment of glioblastoma multiforme [34].

1.4 Magnetic fluids

The MNPs are introduced to specific tumor sites by means of intravenous or local hypodermal injection in the form of a stable colloidal suspension known as magnetic fluids (MFs) or ferrofluids [35]. The MFs are formed by dispersing surface modified MNPs in some carrier fluid which could be polar or non-polar. However, the choice of carrier fluids is restricted by cytotoxic associated with biological systems and thus the hydrophilic MNPs which could be readily dispersed in water (aqueous) based fluid preferred for various biomedical application such as drug delivery, cell sorting and cell labelling, DNA hybridization, MRI contrast agent, including MFH [36], [37]. The stability of fluid in biological system is critical for its successful implication. A mutual influence of various elements, such as the high surface energy of MNPs, inter-particle collisions as a result of rapid Brownian motion and adhesion due to attractive van der Waals forces and dipole–dipole interactions between larger particles contribute to the instability of a MF colloidal solution [38]. The small NPs have a tendency to aggregate and form large particles intending to reduce the surface energy. A stable fluid can be obtained by modifying the surface of MNPs with some stabilizing agents such as surfactants or polymers, during the synthesis process itself (one step method) or post synthesis surface modification of NPs obtained by various synthesis technique (two-step method). These stabilizing agents prevent the agglomeration and coagulation of colloidal particles by facilitating steric, electrostatic or electro-steric repulsion between them and also protect the colloidal particles from oxidation. For steric stabilization, non-ionic surfactants and polymers are always involved into the suspension system. They adsorb onto the particles surface which provide an additional steric repulsive force. Electrostatic stabilizers are usually anionic surfactants (salts of organic acids) which have a negative charge on their hydrophilic end and provide colloidal stability by electrostatic repulsion of charges on the particle surfaces and their associated double layers [36]–[39].

Surfactants used in MFs, called dispersants, consist of a hydrophobic tail portion, usually a long-chain hydrocarbon, and a hydrophilic polar head group. A polar or charged surfactants provide solubility in polar or aqueous solvents and particular dispersants must be chosen to match the dielectric properties of the carrier liquid [40]. Surfactants having carboxyl functional groups are widely used in the synthesis of aqueous MFs. These functional groups are known to bind to particles surface and induce hydrophilicity to the MNPs rendering them highly dispersible in aqueous media. For example, citric acid adsorb on the surface of the MNPs by coordinating via one or two carboxylate functionalities which leaves at least one carboxylic acid group exposed to the solvent, responsible for making the surface negatively charged and hydrophilic. *Răcuciu et al.* utilized citric acid to stabilize the iron oxide MNPs aqueous suspension [41]. *Li et al.* synthesized citric-acid coated iron oxide nanoparticles based on the co-precipitation method through both one-step and two-step process, respectively. They evaluated the effect of citric acid addition at different stages and varying coating temperatures in the two-step process. They observed that the phase dependent addition of citric acid can alter the nanoparticle core size, while the coating temperature affects the hydrodynamic size by regulating the citric acid adsorption around nanoparticles surface [42]. Other molecules, such as gluconic acid, phosphorylcholine and dimercaptosuccinic acid have been also utilized for the stabilization of iron oxide in aqueous medium. Various biological compatible polymers, such as dextran, chitosan, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyacrylic acid and so on have been used for stabilizing the MNPs surface and provide effective colloidal stability [43].

Dispersants can markedly affect the surface characteristics of a material. The organic material layers can impart MNPs with more functionality, providing functional terminal groups for further engineering or modification, such as further coating by other inorganic or organic materials, the encapsulation or attachment of therapeutic agents, targeting agents, and permeation enhancers. The MNPs can be functionalized with cancer-specific binding agents and assist in the 'biologically targeted magnetic hyperthermia' further minimizing the side effects linked to the destruction of surrounding healthy tissues [44]. Nevertheless, the surfactants bound to the MNPs' surface control the growth of NPs during synthesis and thus assist in obtaining size and shape controlled monodisperse MNPs, which significantly affect their MFH efficacy [36], [45]. Obtaining monodisperse MNPs is primary to comprehend efficient heating in MFH. Furthermore, the formation of dipolar chains and self-assembly of magnetic or nonmagnetic nanoparticles (NPs) in MFs are also highly intriguing research topics as it significantly alters the heating behavior of MNPs during MFH treatment [46].

1.5 Obtaining monodisperse MNPs

Researchers have extensively focused on the synthesis of monodispersed highly crystalline MNPs and reported numerous preparation methods to attain desired structural and magnetic properties. These include coprecipitation [47], thermal decomposition [48], solvothermal [49], sol-gel [50], microemulsion [51], sonochemical [52], electrochemical [53] and microwave assisted methods [54]. In particular, thermal decomposition of iron precursors (for e.g. $Fe(CO)_5$, $Fe(acac)_3$, iron oleate complex, ferrocene ($Fe(C_5H_5)_2$) and $Fe_3(CO)_{12}$) in high boiling point organic solvents (such as, phenyl or benzyl ether) has aided to obtain highly crystalline and size controlled MNPs with narrow size distributions. However, MNPs synthesized via thermal decomposition of the iron precursors at elevated temperature in the presence of high boiling-point non-polar solvents and organic surfactants (for e.g., oleic acid/oleylamine) can only be dispersed in the organic solvents (e.g., hexane) which are not straightway useful for biomedical applications. The obtained MNPs thus require surface modification via ligand exchange or polymeric coating to induce hydrophilic surface property. For example, *Palma et al.* [55] used either citric acid or 2,3-dimercaptosuccinnic acid (DMSA) for ligand-exchange phase transfer of hydrophobic iron oxide NPs obtained by the thermal decomposition of $Fe(acac)_3$ in benzyl ether. *Chen et al.* [56] coated iron oxide NPs prepared through the thermal decomposition of FeO(OH), oleic acid, and 1-octadecene with a polysiloxane-containing copolymer to create hydrophilic surface. *Guardia et al.* [57] used an amphiphilic polymer to transfer iron oxide NPs from the oil- to water-soluble phase. While *Xu et al.* [58] have cast-off several polymers such as polyacrylic acid, polyethylenimine, or glutathione to alter the hydrophobic coatings of the iron oxide NPs obtained via thermal decomposition. However, these post synthesis surface modification processes require costly and environmentally-hazardous chemicals, time-consuming as well as sophisticated in practice [59], [60].

Recently, thermal decomposition of iron precursors in polyol solvents has received remarkable attention, as a facile strategy, to obtain water-soluble magnetic nanoparticles through one step synthesis approach [61]–[64]. The choice of solvents includes various polyols such as ethylene glycol (EG), diethylene glycol (DEG), triethylene glycol (TREG), tetraethylene glycol (TEG), or short-chain polyethylene glycol [65]. Being polar in nature, polyols offer the ability to dissolve various inorganic salts and provide high reaction temperature owing to their higher boiling point. Interestingly, they can reduce several metal ions for the controlled synthesis of metal or metal oxide nanoparticles. Thus, they act as solvents, surfactants and reducing agents as well, bringing versatility to the synthesis process. *Cai et al.* [66] investigated different polyols solvents for the synthesis of iron oxide MNPs from the thermal decomposition of $Fe(acac)_3$ without introducing reducing agents and surfactant. They found that as-prepared MNPs in TREG are monodisperse and highly crystalline which can be easily dispersed in aqueous media and other polar solvents owing to the surface coated by a layer of hydrophilic polyol ligands in situ. *Maity et al.* [67] have synthesized water-soluble magnetite nanoparticles by the thermal decomposition of $Fe(acac)_3$ in TREG. The obtained nanoparticles were highly stable in water due to steric repulsion arising by the surface adsorbed TREG molecules and electrostatic interactions between the particles because of associated positive charges. *Qu et al.* [68] have demonstrated the use of polyamines (for e.g., triethylene tetraamine, TETA) in thermal decomposition to straightway obtain hydrophilic amine ($-NH_2$) functionalized MNPs. *Hachani et al.* [69] have adapted the polyol synthesis at high pressure and high temperature conditions using an autoclave which allowed better control of morphology of the obtain iron oxide NPs with enhanced magnetic properties. These reactions were carried out in various solvents of different boiling points which confirmed that solvents were critical to obtain high-quality nanoparticles. *Kandasamy et al.* [70] reported the synthesis of hydrophilic functionalized superparamagnetic iron oxide MNPs with one-step thermolysis of $Fe(acac)_3$ using the polyamines or mixture of polyamines and polyols. *Cheah et al.* [64] recently reported a continuous growth strategy to synthesize iron oxide nanoparticles in DEG. The strategy is proven to be facile to produce highly water-dispersible nanoparticles without any surface modification with nanometer-scale size increment. The only reactant is $Fe(acac)_3$

whose step by step addition facilitated nanometer-scale size increment. They further evaluated the role of the reaction temperature in the continuous growth of water-dispersible iron oxide nanoparticles in DEG solvent. Larger MNPs were obtained with the increase of the temperature as 190, 220, and 235 °C [65].

The solvothermal reduction of iron precursors in polyol solvents has also proved as a facile strategy to obtain water-soluble magnetic nanoparticles through one step synthesis approach. The reaction is carried out in a sealed vessel (Teflon lined autoclave) which provide reaction temperature higher than the boiling point of the solvent as well as significantly high pressure [71]. It enables precise control over the shape, size distribution, and crystallinity of NPs by manipulating the reaction time, temperature, iron precursors, and other additives (e.g., surfactants, anions, and metal ions). For example, *Deng et al.* [72] produced a series of monodisperse magnetite submicrospheres in a sealed teflon-lined stainless-steel autoclave at 200 °C. They used $FeCl_3 \cdot 6H_2O$ salt as iron source and ethylene glycol as solvent along with sodium acetate (NaOAc) and PEG surfactants to prevent agglomeration. The manipulation in precursor concentration and reaction time were responsible for tuning the microsphere diameter from 200 to 800 nm. *Zhu et al.* [73] have obtained uniform-sized, monodisperse, and single-crystal Fe_3O_4 hollow spheres through a simple one-step template free method. The reaction duration and the ethylenediamine amount are shown to play important roles in the formation of the magnetite hollow spheres. *Xuan et al.* [74] have established a novel binary solvent (EG and DEG) route to prepare a series of high-quality monodispersed ferrite nanospheres with tunable size and narrow size distribution utilizing PVP as surfactant. The solvothermal synthesis methods have been extensively developed to obtain highly crystalline magnetic clusters with controlled morphology. *Cheng et al.* [75] explored the morphological evolution of iron oxide MNPs, from clusters to mesoporous structures. The reduction of iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in the company of succinic acid and urea in ethylene glycol or 1,2-propylene glycol produce clusters or mesoporous structure, respectively. They examined and demonstrated the importance of the choice of solvent and reaction conditions in molding the structural and magnetic properties of the nanoparticles. *Kim et al.* [76] explored the solvothermal synthesis method for the up scaling of a close-packed superstructure of crystalline magnetic nanoclusters. The varying molar ratios of precursors and surfactants effectively tuned the morphology of clusters.

1.6. Heating mechanism

In MFH, a substantial amount of heat is released when an assembly of MNPs is placed under an AMF which is used in the preferential destruction of the cancer cells. The magnetic losses arising during the process are responsible for the heat released which were grouped into 'relaxation losses' and 'hysteresis loss' [7]. The relaxation losses involve Néel and Brownian relaxations, rotation of magnetic moment and particle, respectively, under the influence of an AMF. Each relaxation mechanism is characterized with its own relaxation time. The Néel relaxation, $\tau_n = \tau_o \exp(E_a/k_B T)$ and the Brown relaxation, $\tau_B = 3\eta V_h H/k_B T$ are dependent on the anisotropy energy barrier, E_a the viscosity of the medium, η and the hydrodynamic volume, $V_h$of MNPs [77], [78]. Rosensweig suggested that the Néel and Brownian relaxations occur in parallel with an effective relaxation time, $\tau_{eff} = \tau_N \tau_B/(\tau_N + \tau_B)$ [79]. It is generally understood that superparamagnetic MNPs release heat through relaxation losses while hysteresis loss dominates in ferro-/ferri-magnetic MNPs exhibiting finite coercivity. However, a numerous recent investigation on the physics of heating mechanism designate the distinction between relaxation losses and hysteresis loss as improper and confusing [80], [81]. The Néel and Brownian relaxations occur when the atomic magnetic moments are enforced to align with an external filed, via rotation of magnetic moment and particle, respectively. Any lag in the magnetic response, through these relaxations, to shadow the applied field yields non-linearity in magnetization which lead/add to the hysteresis in single domain MNPs. In fact, MNPs, whether these are in superparamagnetic regime or ferromagnetic regime, release heat which is directly related to the arrival of hysteresis when MNPs are exposed under and is proportional to area enclosed by hysteresis loop defined by MNPs during the application of AMF. The observation is fairly straight for ferromagnetic MNPs but hard to digest for superparamagnetic MNPs which describe feeble or null hysteresis loop when a direct current magnetic field (DMF) is applied. However, noticeable hysteresis is witnessed when the same material is placed under an AC magnetic field due to the introduction of 'dynamic hysteresis' in the time-varying applied field [80], [82]. Thus, static hysteresis loop underestimates the heating potential of MNPs in MFH. Whether the superparamagnetic behaviour of a magnetic sample will be perceived during an experiment depends on characteristic measurement time (τ_m) associated to a particular experimental technique. The, τ_m, of the MFH devices typically range in 10^{-5} – 10^{-6} s, which is small enough to suppress the superparamagnetic character of a particle and an open hysteresis results on exposure to an AC magnetic field. Thus, a frequency parameter called the blocking frequency (f_b) is introduced above which hysteresis is observed and alike blocking temperature depends on several factors [83]. As a thumb rule one can perceive a better heating using MNPs which describe better dynamic hysteresis loop under the exposure of an optimal AMF for MFH which is essentially proportional to M_S and H_C of MNPs. Thus, enhancing the saturation magnetization of the MNPs is often treated as a straightforward tactic so as to achieve increased hysteresis losses and has been for quite a few times. It can be increased either by improving the crystallinity or reducing the surface disorders of MNPs, but still the maximum attainable M_S value remains less than that of bulk magnetization. Nevertheless in MFH, the accessible magnetic field amplitude (H_{AC}) and frequency (f) of AMF are restricted by the practical considerations of biological safety to avoid over-exposure and nonspecific heating of healthy cells by eddy currents and to elude nerve stimulation [7].

1.6.1. Dependence on field amplitude and frequency of AMF

The time varying magnetic flux associated with an AMF induces eddy currents in a conducting material as such to oppose to the applied field the cause of eddy current heating. These eddy currents are not exclusive of conducting materials and also generate in human tissues when exposed to an AMF of relatively higher magnitude and frequency leading to unintended heating at distant healthy tissue sites. Therefore considering the clinical biosafety H_{AC} and f of the applied AMF cannot be increased beyond a certain limit which is observed by *Hergt et al.* as $H_{AC}*f \leq 5*10^9$ $Am^{-1}s^{-1}$ [84]. Thus, the accessible field amplitude for MFH is far less than required to achieve magnetic saturation of MNPs and could produce only a minor hysteresis loop. As a result, the heat generated by MNPs depends on the area enclosed by the minor hysteresis loop formed under the applied AMF [81]. Any approach to improve the area enclosed by the minor hysteresis loop at an AMF of fixed amplitude and frequency would directly add to the heating performance of the MNPs and is strived by the researchers examining in this area.

As mentioned above, there is an upper constraint on the magnitude of field and frequency of the AMF that can be employed in MFH. However, these field parameters directly impact the power dissipation which can be optimized by varying H_{AC} and f of the applied AMF [80], [81]. The choice of AMF parameters for maximum heat output strongly depends on magnetic properties of the MNPs, essentially the magnetic anisotropy energy, $E_a = KV$, where K is the anisotropy constant and V is volume of the material. The anisotropy constant, K arises due to the preferred crystallographic orientation of magnetic moment in a crystalline materials. The effective anisotropy constant, K_{eff} is a contribution from shape, size and volume of crystalline lattice and collective magnetic interactions such as exchange (coupling) and dipole interactions. An energy equivalent to K_{eff} is required to change the magnetization direction of the material with respect to some particular crystallographic axes. The field required to completely reverse the magnetization direction is the anisotropy field (H_K) which is related to effective anisotropy as $H_K = 2K_{eff}/M_s$ where μ_0 is the permeability of free space. The theoretical and experimental results showed that when the ratio, $H_{AC}/H_K > 0.5$, the hysteresis loss dominates and the maximum heat power is generated when the field strength equals the anisotropy field. According to the Stoner-Wohlfarth model, at T = 0 the coercive field of randomly oriented MNPs is related to the effective anisotropy, K_{eff}, and to the saturation magnetization, M_S as $H_C = K_{eff}/M_s$ ($\approx 0.48\, H_K$) [85]. The coercive field maximizes and becomes equal to anisotropy field when the easy axis of a thermally blocked MNP is aligned along the magnetic field direction. Such ideal situation results in a perfect square loop consequential for maximal hysteresis area. In other words, high anisotropy field causes high coercive field, and a higher coercive field is beneficial to heat output via hysteresis loss. Although high H_C could manifests in high heat production, but it is favorable up to a certain value as higher coercive filed would require a high field amplitude for effective heating at a given set of conditions, potentially limiting clinical application. Thus, at constant volume better heating could be obtained by enhancing the effective anisotropy of the MNPs which could be achieved by modifying the shape and structure of the particles. Further, it must be noted that there are two competing factors i.e. effective anisotropy constant, K_{eff} and volume of the particle, V contributing to the overall anisotropy energy, E_a. As has been discussed earlier surface plays dominant role in the small particles which introduces surface anisotropy (K_s) to the material and the effective anisotropy renovate as, $K_{eff} = K_\infty + 6K_s/D$, where D is the particle diameter and K_∞ is the anisotropy energy constant for $D \to \infty$ [86]. Usually, the surface anisotropy is higher than the bulk anisotropy which causes an enhancement in anisotropy field, H_K with a reduction in particle size. It should have led to higher coercive field and hence a better heating. However, a reduced coercive field is observed when the particles' size decreases in single domain region. As for such small MNP, the enhanced anisotropy effect competes with the Néel relaxation process due to volume influence, and the magnetization is thermally assisted to jump over the anisotropy energy barrier, E_a, reducing the coercive field and the squareness of the hysteresis loop. On further decreasing the size the anisotropy energy barrier, E_a shrinks due to volume impact and the thermal energy, k_BT causes the thermally activated magnetization reversal leading to a progressive decrease of the coercive field when the temperature is raised. It becomes zero when the thermal energy just exceeds the anisotropy energy, i.e. $k_BT \geq K_{eff}V$, and a state of superparamagnetism persist in the material [87]. The thermal agitation also facilitates randomly oriented MNPs exhibiting a reduced coercive field as conversed earlier. In such situation, obtained by *Garcia-Otero et al.* [88], the coercive field of randomly oriented MNPs modifies as, $H_C \approx 0.48H_K[(\frac{k_BT}{K_{eff}}(ln\frac{\tau_m}{\tau_0}))]$ and the Stoner-Wohlfarth equations are invalid when, $T \neq 0$. Thus, in spite of enhanced anisotropy energy, the heating effect diminishes due to reduce coercive field (hence a reduced hysteresis heat loss) for small MNPs.

The heat output of MNPs is modulated by the frequency of AMF which is closely associated with the anisotropy energy barrier and the heating. At higher frequencies the relaxation dynamics controls the magnetization behaviour of MNPs [81]. Both the Néel and Brownian relaxation mechanisms influence the magnetization behavior of thermally activated MNPs and ferrofluids when subjected to an external AMF. The system or MNPs under evaluation can be treated as being in equilibrium if the effective relaxation time of the MNPs is much less than the measurement time of AC magnetic field at each instant of time. However, for longer relaxation times the system does not attain equilibrium with the time-varying magnetic field and the phenomenon of relaxation panels the magnetization dynamics as a modification in magnetization and coercive field (hysteresis). In fact, the relaxation dynamics are central in determining the magnetization response of MNPs in MFH. At a given field strength, the achievable magnetization decreases while the coercive field increases with dynamic magnetization as the frequency of the applied AMF field increases. If the applied frequency is too high compared to the effective relaxation of the MNPs, then the time-scale of measurement is insufficient to allow for enough attempts to cross the energy barrier to actually result in a magnetization reversal. A partial restriction to the reversal, as conferred, will embark lessen magnetization while a complete restriction will generate a linear magnetization and no heat is generated. *Ota et al.* [89] observed that when a ferrofluid, consisting of MNPs of mean core diameter 11 nm, is subjected to a low-intensity pulse magnetic field the magnetization first increased by Néel rotation followed by Brownian rotation. The contribution of Néel rotation to the total magnetization was observed to be lower than the Brownian relaxation. An increased frequency depicted the dominance of Néel relaxation mechanism and the effective magnetization decreased due to suppression of Brownian relaxation. An intermediate frequency between those of Néel and Brownian relaxation time established an enhanced hysteresis due to the superposition of both mechanism and is preferable in MFH. A comparable decrease in magnetization was also observed by *Yamaminami et al.* [82] when the particles were immobilized to restrict the Brownian rotation. Both the liquid and immobilized samples showed a decrease in magnetization with increased frequency of the time-varying magnetic field due to progressive delay of the magnetization response to the applied AC field. Thus, conditional to effective anisotropy of the MNPs and the amplitude of the applied field an optimum frequency recites for maximizing the heat output. Thus, it is beneficial to determine the magnetic relaxation behavior of MNPs to optimize their performance for MFH application. AC magnetometers could assist in realizing the optimum frequency for MFH as the out of phase component of AC susceptibility, χ'', is related to power loss (as heat) of the MNPs. It is maximum when the angular frequency, $\omega(= 2\pi f)$ of the applied field conditioned to, $\omega\tau_{eff} = 1$, where, τ_{eff} is the effective relaxation time of the MNPs [90]. When, $\omega\tau_{eff} \rightarrow 0$, the magnetization follows a simple straight line with maximal magnetization and null hysteresis. But when, $\omega\tau_{eff} \rightarrow \infty$, the system does not have the time to respond to the magnetic field excitation generating minimal magnetization and null hysteresis [81].

1.6.2. Dependence on size, shape and structure of MNPs

As introduced earlier variation in size, shape and structure affect the heating performance of MNPs as it alters the magnetic properties viz. saturation magnetization and anisotropy energy in addition to the possibility of thermal activation over the energy barrier. In fact, a precise control of the heat release at the nano-level is analytically related to the use MNPs with optimal and controlled size, shape and geometrical arrangement [46]. However, a huge discrepancy persists in literature regarding the optimal value of the size of MNPs for efficient MFH. For example, *Gonzalez-Fernandez et al.* [91] evaluated the heating behavior of Fe_3O_4 MNPs varying the size in 5 nm to 110 nm range and found 24 nm as an optimum size for achieving highest SLP (~130 Wg^{-1}) with AMF of amplitude 160 Oe and frequency 260 kHz. In work reported by *Khandhar et al.* [92] Fe_3O_4 MNPs ~ 16 nm illustrated maximum heating efficiency with a 373 kHz alternating magnetic field of 200 Oe amplitude. *Bakoglidis et al.* [93] studied the variation of SLP of iron oxide MNPs in the size range 5–18 nm under the excitation of 200 Oe, 765 kHz AC magnetic field. They observed a characteristic local maximum for 10 nm particles size which eventually improved for larger particles (18 nm) before a minimal fall in the SLP value. *Vreeland et al.*[94] employed extended LaMer mechanism to obtain size-tuned Fe_3O_4 MNPs in a close size range. They observed that for an AMF with excitation field and frequency of 460 Oe and 341 kHz, respectively, the optimum size showing maximum SLP was ~ 22 nm. The size dependent heating performance can be pictured from the thermally-activated Néel relaxation process for which the relaxation time (τ_n) follows Arrhenius kinetics ($\tau_n = \tau_0 exp(-KV/kT)$), where the activation energy is related to the particle volume. It means that the relaxation dynamics varies with particles' size (r) as, (r^3), and thus exhibit extraordinarily strong size dependence. Therefore, even minor variations in the size and size distribution can greatly influence the relaxation dynamics and hence the heating efficiency of MNPs during MFH. As the polydispersity in size of MNPs increases the dissipated energy i.e. heating efficiency during MFH decreases exponentially. Since a slight change in size influence the relaxation time, polydispersity will lessen the heat dissipation [95], [96]**.** Thus, monodisperse MNPs are sought for MFH to avoid distribution in activation energy and thus variation in relaxation dynamics to elude the detrimental heating drop during MFH. Interestingly, *Ovejero et al.* [97] used a combination of iron oxide magnetic nanoparticles of different sizes and geometries and demonstrated the possibility of creating both sequential and simultaneous multi-hot-spot conditions with different local temperature in a single pot using changed AMF strength and frequency. Such a control over local temperature distribution may create a new paradigm in the regulation of biological molecules such as the creation of one-pot multienzymatic cascades operating at multiple optimal temperatures or being sequentially activated with different magnetic fields. However, the existence of a differential heat dissipation spots may result in highly inefficient MFH cancer treatment with some areas being overheated and others remaining untreated [98].

Obviously the dissimilar magnitude of exciting field and frequency in above reports have affected the optimal size of MNPs for maximal heating. With time varying magnetic fields, prior studies performed at field conditions where, $H_{AC} \times f < 5 \times 10^{9}\, Am^{-1}s^{-1}$ suggested an optimal diameter of 14 nm for standard spherical Fe_3O_4 MNPs [99]. However, larger MNPs have shown better heating when the field amplitude is increased such that non-linear modification arises in the magnetization dynamics. For example, *Shaterabadi et al.* [100] showed that the optimal size for maximizing the heating efficiency of the dextran coated magnetite MNPs increases by enhancing the amplitude (H_{AC}) of the time varying magnetic field. A similar behavior was reported by *Mehdaoui et al*. [101] where the optimal size was not constant but increases with the amplitude of the applied AC magnetic field. *Nemati et al.* [102] studied the heating behavior for a series of 17–47 nm sized nano-octopods. At lower AC fields (< 300–400 Oe), the maximum heat dissipation was observed for the smallest size while the SLP increased linearly with size at higher fields (> 400 Oe). However, for even larger particles, in multi-domain region, the magnetization dynamics undergo a transition to non-coherent reversal process which is less costly energetically and hence less efficient for MFH.

The anisometric MNPs, furthermore, have accounted for better heating performance during MFH endorsed by their additional shape anisotropy. For example, cubic MNPs heat better than the spherical MNPs of equivalent volume [103]. *Guardia et al.* [57] reported a tremendous heating supremacy for superparamagnetic cubic iron oxide MNPs with a mean size of 19 nm at ~365 Oe and 520 kHz. *Nemati et al.* [103] showed larger cubic MNPs (~30-35 nm) can release better power at higher field amplitude of 800 Oe and was superior to spherical MNPs. The cubic-shaped MNPs display higher M_S values in comparison with regular spherical-shaped MNPs due to lower surface spin disorder and higher crystallinity and addition anisotropy due to shape effect which impart better heating performance during MFH. Frenetic research on the heat released by several other anisometric MNPs, including nano-cubes, nano-octopods, elongated nanoparticles (nano-spindels), nano-disks and nanoflowers have been executed. *Gavilán et al.* [104] compared the heating behavior of different shaped MNPs and the SLP values normalized by the volume of each particle followed the efficiency in order: flower > cube > spindle > disk.

Another strategy to improve the heating utilizes exchange coupled core-shell MNPs composed of magnetically hard and soft phases. The phenomenon of exchange coupling allows the tuning of magnetic properties by the interfacial exchange interaction between hard and soft magnetic phases, specifically the anisotropy energy constant (K) (without affecting the MNP volume), in order to maximize the SLP of the MNPs. For example, *Lee et al.* [105] have synthesized exchange coupled core-shell (CS) nanoparticles with magnetically hard $CoFe_2O_4$ as core and magnetically soft $MnFe_2O_4$ as the shell. The modified anisotropy, K value for the CS structure was 1.5×10^4 Jm^{-3}, while for $CoFe_2O_4$ and $MnFe_2O_4$ was ~2×10^5 and 3×10^3 Jm^{-3}, respectively. When exposed to an AMF of amplitude ~470 Oe and frequency 500 kHz, the SLP value for the exchange coupled CS nanoparticle was 2280 Wg^{-1} while the values for $CoFe_2O_4$ and $MnFe_2O_4$ were 433 and 411 Wg^{-1}, respectively for an equivalent volume. In another work, at similar AMF strength, *Noh et al.* [106] have reported a large SLP value for the cubic shaped exchange coupled CS nanoparticles which was almost an order higher than that for either component. Recently, *Nandwana et al.* [107] demonstrated exchange coupling in a CS structure with two magnetically soft phases, where $Zn_{0.2}Mn_{0.8}Fe_2O_4$ used as core and Fe_3O_4 or $MnFe_2O_4$ was used as the shell. The highest SLP value achieved for this exchange coupled CS nanostructures was 827 Wg^{-1} and was almost nine times higher than that for Fe_3O_4 nanoparticles with a time-varying magnetic field of amplitude 5 kA/m and frequency 300 kHz. Though, the highest SLP values reported was quite small compared to the values stated by *Lee et al.* and *Noh et al.*, it has certain advantages such as: lower coercive field, necessity of lower field strength and relatively higher field normalized heating efficiency i.e. higher ILP.

A quite exciting structure ‘magnetic nanoflowers’ (MNFs) have shown superior heating even at lower field strength and is being lauded as the most efficient candidate for MFH. The small constituent cores (single-crystals) which are typically magnetically ordered, aggregate together to form nanoflowers, where the cores are essentially in physical contact with each other [108], [109]. It has been reported that MFs are able to retain the soft magnetic nature even for the size greater than 50 nm [108], [110]. Others advocate for a superferromagnetic state in MNFs due to magnetic interaction between the cores which enhances the saturation magnetisation (M_S) and magnetic susceptibility of the material and hence improved heating performance in MFH [110]–[112]. One important aspect to consider in evaluating the heating performance is the tendency of MNPs to agglomerate in cellular environments. In fact, depending on the concentration and formulation (coating or surface treatment), MNPs can aggregate and form nano-assemblies as a consequence of magnetic dipolar interaction or under the influence of a magnetic field. This interaction directly affects the relaxation dynamics of MNPs and leads to a fluctuation in hyperthermia performance compared to well disperse non-interacting MNPs formulations. Several researchers testified a negative influence of disordered agglomeration on the heat generation efficiency of MNPs [46]. *Bender et al.* showed a predominance of antiferromagnetic-like moment correlations among superparamagnetic MNPs which verifies the negative impact of random clustering. While supraferromagnetic interaction witnessed in clusters of nanoflowers which was assigned to the reason for their improved MFH performance compared to superparamagnetic particles [112], [113].

However, in cases when the inter-particle dipolar interaction favors special arrangements, such as chain-like structure, the story turn around and display enhanced heating performance under the same time-varying magnetic field [46], [102], [104], [114]. The formation of chains introduces an additional uniaxial anisotropy along the length of the chain which is independent of the anisotropy of individual particles [115]. *Fu et al.* [116] supported this claim and showed that the dipole interactions improve the heating performance only when the clusters of MNPs are small enough to induce an enhancement in clusters' shape anisotropy through the formation of chains and cylinders. While *Serantes et al.* [117] supported for an optimum chain length to achieve high SLP values beyond which heating efficiency decreases with chain length.

1.7. Heat output measurements (SLP)

In calorimetric evaluation, heat quantification is executed through the direct measurement of the temperature evolution of the MF sample as a function of time when exposed to a radio frequency magnetic field. A fiber optic thermometer is generally used to measure the rise in temperature. The SLP value of the MF sample then can be calculated using the following equation [105]:

$$SLP\ (W/g) = C(V_s/m_{MNPs})(dT/dt)$$

Where, C is the volumetric specific heat capacity of the sample solution, V_s is the sample volume, m_{MNPs} is the mass of magnetic material in the sample, and dT/dt is the initial slope of the temperature evaluation curve as a function of time. Achieving an adiabatic condition, which is desirable, for the temperature evaluation of the sample is a challenge to this method and difficult to achieve. So most of the experiments are performed in non-adiabatic condition and is generally preferred which deliver simplicity to the experiment. The temperature evolution of the sample is then a result of the balance between the heat generated by the

MNPs and the heat exchanged with its surrounding, and thus depends on the thermal characteristics of both the MF sample and its surrounding.

As has been discussed earlier, the heat released by MNPs when exposed to an AC magnetic field is originated by the hysteretic dynamic response of these MNPs to the AMF excitation. Thus, a direct determination of hysteresis by AC magnetometry is a powerful technique in determining the heating effect, i.e, the SLP value. When a MF is exposed to an AMF of amplitude, H_0 and frequency, f the amount of heat released is proportional to the area, A enclosed by hysteresis loop times the frequency of the applied AMF. The area traced by the hysteresis loop during a cycle of applied AMF is expressed as $A = \int_{-H_a}^{+H_a} \mu_0 M(H) dH$, and the SLP is given as, $SLP = Af/c$, c being the weight fraction of MNPs in the sample [81].

Both the methods discussed above, in a sense, can be considered complementary to each other. As the calorimetric method can assist in comparing the temperature evaluation of different MNPs, AC magnetometry can review the query that why certain MNPs depict higher temperature rise than others. For example, *Nemati et al.* [103] evaluated the temperature evaluation of MNPs of varying shape and size via calorimetric measurement and the measurement of dynamic hysteresis loops of these MNPs could explain the high heating of some specific shape and size MNPs. The AC magnetometry, in addition, assisted *Garaio et al.* [118] in evaluating the temperature dependent specific loss power of MNPs. *Guibert et al.* [119] have measured SLP of MNPs through dynamic hysteresis loop measurement and found it in good agreement with the value obtained by calorimetric method. However different authors have measured the temperature evaluation of MNPs at diverse field amplitude and frequency which make difficult to compare these results. To overcome this issue *Kallumadil et al.* [120] propose an approach by introducing intrinsic loss power (ILP), as a standard alternative for SLP which normalizes SLP with respect to the field amplitude and frequency. It is given as, $ILP = SLP/{H_{AC}}^2 f$, where H_{AC} and f, are field amplitude and frequency.

1.8. MNPs as photo-thermal agents

In PTT, the NPs are utilized as localized source of heat, which efficiently translate the electromagnetic radiation of near-infrared (NIR) wavelength into heat [19]. The use of NPs in PTT makes the process more precise and also enhance the efficiency of treatment process. The photo-thermal (PT) agents can be used at four wavelengths range, which are expected as biological windows: NIR I (700–950 nm), NIR-II (1000–1350 nm), NIR III (1600–1870 nm), and NIR IV (2100–2300 nm) [121]. A wide range of materials, such as gold (Au) nanoarchitectures, carbon based nanomaterials, organic polymers and NIR-dyes have been investigated as PTT agents, till date. Au NPs being the most explored and popular photo-thermal (PT) agent for PTT, efficiently translate applied electromagnetic radiation through surface plasmon resonance (SPR) effect [122]. They demonstrated promising heating effect upon NIR exposure which found to be depending on the particles' size and shape. Still most studies evaluating Au NPs as potential PT agents have been conducted at in-vitro and in-vivo animal models whereas only a few nonrandomized and retrospective studies have demonstrated its efficacy in clinical trials [123]. The bio-persistent nature of Au is primary hurdle to its wide spread clinical applications, which could produce potential long term in-vivo toxicity. In addition, Au NPs and nano-rods lack photo stability due to the "melting effect" upon NIR-laser irradiation [124]. The recent reports on the photo-assisted heating effect of iron oxides (Fe_3O_4 and γ-Fe_2O_3 based) MNPs have attracted the attention of researchers. In addition to the easing the potential in-vivo toxicity, these oxides based MNPs deliver the benefits associated with their intrinsic magnetic properties, viz. magnetic targeting, in-vivo MRI and simultaneous MFH. *Espinosa et al.* [125] demonstrated enhanced heating of iron oxide cubic MNPs under simultaneous exposure of AMF and NIR-laser radiation as compared to either one alone.

The heating effect of iron oxide based MNPs under NIR irradiation is due to the non-radiative recombination of the electron-hole pair during the relaxation process associated with the generation of phonons [19]. This upsurge the vibrational state of the system which subsequently lead to temperature rise. Thus, introducing a more intermediate defect states in the optical band gap of MNPs, which act as sites of electron trap, might help in improving the heating efficiency by increasing non-radiative electron-hole recombination [126], [127] Nonetheless, higher heating by NIR radiations of IONPs could be achieved with a lesser quantity than used in MFH.

The photo-thermal conversion efficiency (η) is the parameter that determines the photon to heat conversion efficacy of a PT agent. A higher η value, in general, is desirable to realize efficient heating. However, η, is governed by the absorption intensity of the PT agent in the NIR region, which is a key factor to determine the photo-thermal heating capability (i.e. conversion fraction of absorbed photon energy to heat). Thus, a higher η value, does not always imply a higher heating. A material with low heating and absorbance in NIR region may show higher conversion efficiency than the materials having higher heating and absorbance [128]. Thus, higher absorbance at NIR region and non-radiative relaxation are the pre-requisites for observing higher heating under NIR radiation.

It has been observed that a controlled clustering of magnetic iron oxide NPs could facilitate a higher NIR absorption and thus photo-thermal response through non-radiative relaxation [129]. The organization of MNPs into certain super-structures has appeared as an effective pathway to enhance their absorption in NIR region [130]. Recently reported magnetic nanoflowers are also explored to be as efficient heat generators as the single crystals when irradiated with NIR lasers, making it possible to use radiations of low power density and low material concentration during PTT [131], [132]. Yet another approach to enhance the absorption and subsequent heating is to coat MNPs with NIR absorbing polymers (e.g., polypyrrole) [133] and dyes (e.g., IR806) [134]. Even, the particle size of MNPs has been found to play a critical role in shaping the heating behaviour during PTT. For e.g., after laser irradiation for same duration, *Zhang et al.* [135] observed higher heating as well as photo-thermal conversion efficiency for larger Fe_3O_4 nanoparticles.

In summary, MNPs can be employed for dual mode of heating, i.e., combined AMF and NIR-laser exposure enhancing the effectiveness of treatment. Such synergetic modalities will not only reduce the dosages but also the duration for the treatment. The unique magnetic properties, in addition to highly biocompatible nature, permit magnetically guided conjugate treatments, such as, drug delivery.

1.9. Motivation

Nanoparticles mediated localized hyperthermia treatment modalities have shown most promising effect during clinical and various in-vivo and in-vitro studies. These localized treatment approach utilizes the heat generated by NPs in selective destruction of cancer cells under the application of a distant energy source and offers several advantages. For example, NPs can easily pass into several tumour region whose pore sizes are in 200–

780 nm range, increasing the effectiveness by delivering therapeutic heat directly to them. In addition, these NPs can be functionalized with cancer-specific binding agents and the possibility of NPs to form stable colloidal fluids allow for the intravenous or local hypodermal injection to the tumor tissues, resulting in obvious advantages such as less invasion, high targeting and low side effects. In the present-day of nanoscale era the NPs assisted localized treatment based on magnetic- and photo-induced thermal therapy has gained much popularity in which heat is generated using either MNPs or photo-sensitive NPs, respectively. MNPs have shown great potential, as they generate heat not only under the exposure of AMF but also with NIR laser irradiation. The current emphasis is on the design of materials with high specific loss power (SLP), a parameter defining the efficiency of MNPs to convert electromagnetic radiations into heat. A specific morphology, i.e., magnetic nanoflowers have shown effective heating under both AMF as well as NIR laser. However, only Fe_3O_4 with this particular morphology has been explored in literature, which motivated us to explore the heating behaviour of other magnetic materials with this morphology. In addition, Fe_3O_4 nanoparticles with mesoporous morphology has also been explored as a potential candidate for MFH and PTT. Such morphology enables the high drug loading for the targeted drug delivery and can be further used in the synergistic hyperthermia along with chemotherapy.

1.10. Scope of Work

In the present work, the heating behaviour of γ-Fe_2O_3 nanoflowers, $MnFe_2O_4$ nanoflower as well as Fe_3O_4 mesoporous nanoparticles are evaluated when exposed to either an AC magnetic field or NIR laser of wavelength 808 nm. The formation of single phases is confirmed from X-ray as well as electron diffraction patterns. The Mössbauer spectroscopy helped in determining the ionic state of Fe-ions in respective samples which assisted the X-ray and electron diffraction patterns analysis. A through structural and magnetic characterizations of these magnetic materials are carried out which helped in answering the heating behaviour under AMF. The photoluminescence spectroscopy of the samples helped in corroborating their high heating performance with NIR irradiation.

1.11. Objective of Work.

The objective of the present work is the evaluation of the heating behaviour γ-Fe_2O_3 and $MnFe_2O_4$ nanoflowers as well as Fe_3O_4 mesoporous nanoparticles under an applied AMF and NIR laser.