Chapter 1

Introduction

Alternating Magnetic Field (AMF)

- Heat
- Drug
- Apoptosis
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- Drug release

Magnetic nanoparticle
Anticancer drug

ON
Self-heating

Cancer

OFF
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1.1 Introduction

Nanoscience and nanotechnology involve the synthesis, characterization, exploitation and utilization of nanostructured materials which are characterized by at least one dimension in the nanometer (1 nm = 10^{-9} m) range. These nanostructured systems constitute a bridge between single molecules and infinite bulk systems. The nanostructures involve clusters, nanoparticles (NPs), nanocrystals, quantum dots, nanowires and nanotubes, whereas collections of nanostructures involve arrays, assemblies and super lattices of individual nanostructures [1, 2]. The dimensional range of 1 to 100 nm is generally considered the nanoscale and materials at this scale are called nanocrystals or nanomaterials.

The physical and chemical properties of the nanomaterials can significantly differ from those of bulk materials of same chemical composition. The distinctiveness of the structural characteristics, energy response, dynamics and chemistry of nanostructures constitutes the experimental and conceptual background for the field of nanoscience. Suitable control over the properties and response of nanostructures can lead to new devices and technologies. The basic themes of nanoscience and nanotechnology are twofold: first, the bottom-up approach of the self assembly of molecular components where each molecular or nanostructured component plugs itself into a superstructure [3]; second, the top-down approach of miniaturization of the components [4].

The differences in the properties of nanosized materials from bulk materials are due to surface effects which mainly depend upon the ratio of surface area to volume and size of the particles along with the chemical composition and interaction between particles. As the particle size decreases, the ratio of surface area to volume increases which lead to an increase in the dominance of the behavior of atoms on the surface of a particle over that the interior of the particle. This affects both the properties of particles in isolation and its interaction with the other materials. The large surface area of NPs results in a lot of interactions
between the intermixed materials in nanomaterials, leading to special properties such as increased strength and/or increased chemical/heat resistance.

Once material falls into a nanoscale, a lot of changes corresponding to the size occurs to the properties such as melting point, ionization potential, hardness, catalytic activity or magnetic properties such as coercivity, saturation magnetization and permeability. The drastic change in the properties of the materials motivates the use of NPs in various applications. Among the different types of NPs, magnetic nanoparticles (MNPs) are of great importance due to their various unusual properties that make them useful in biomedical applications. Introductory aspects of MNPs are discussed in the following section.

1.2 Magnetic nanoparticles
Magnetic nanoparticle is a class of nanoparticle which can be manipulated by using magnetic field. Nanoscaled magnetic materials have different magnetic properties as compared to the bulk material. In particular, it is shown that magnetization and the magnetic anisotropy of NPs could be much greater than those of bulk specimen, while differences in the Curie or Neel temperatures between the nanoparticle and corresponding microscopic phases reach hundreds of degrees. The magnetic properties of NPs are dependent on many factors, the key of these including chemical composition, type and degree of defectiveness of the crystal lattice, particle size and shape, morphology (for structurally in-homogeneous particles), interaction of the particles with the surrounding matrix, and neighboring particles. One can control the magnetic properties of the material by changing particle size, shape, composition and structure. However, these factors cannot always be controlled during the synthesis of MNPs nearly equal in size and chemical composition; therefore the properties of nanomaterials of the same type can be markedly different. In addition, magnetic nanomaterials are found to possess a number of unusual properties such as giant magneto-resistance, abnormally high magneto-caloric effect and so on.
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The use of MNPs in the field of biology and medicine has led to tremendous breakthroughs in the past decades. They have the potential to be used in many different biological and medical applications as in diagnostic tests assays for early detection of diseases, to serve as tools for non-invasive imaging and drug development, and to be used as drug delivery systems to minimize secondary systemic negative effects. The use of MNPs in the biomedical application is introduced in the next section.

1.3 Biomedical applications of magnetic nanoparticles

Over the time, the focus of nanotechnology research not only belongs to the nanomaterials, but also in the investigation of their physico-chemical properties in order to use them for applications in the future. One of the fields that can be enormously benefited from the advancement in nanotechnology is biomedical research. In particular, highly specific medical interventions at the nanoscale for curing disease and repairing damaged tissues such as bones, muscles and nerves are emerging as nanomedicine area [5].

MNPs offer some attractive possibilities in medicine. Living organisms are built of cells that are typically 10 µm in diameter. Since, the cell parts are much smaller and in the sub-micron size. The major advantage of MNPs in medicine is that NPs have controllable sizes ranging from a few nanometers up to tens of nanometers, which place them at dimensions that are smaller than or comparable to those of a cell (10-100 µm), a virus (20-450 nm) or a protein (5-50 nm). This means that they are close to the biological entity of interest. This simple size comparison suggests that the idea of using NPs as very small probes that would allow us to spy at the cellular machinery without introducing too much interference. They can be coated with biological molecules to make them interact with or bind to a biological entity in order to provide a controllable means of “tagging” and addressing it.
One of the advantages of MNPs is that the NPs are magnetic; hence they can be manipulated by using an external magnetic field gradient. This action at a distance combined with the intrinsic penetrability of magnetic fields into human tissue opens up many new applications involving the transport and immobilization of MNPs and magnetically tagged biological entities. In this way they can be made to deliver a package, such as anticancer drug, to a targeted region of the body.

Another advantage is that the MNPs can be made to respond resonantly to a time-varying magnetic field, with advantageous results related to the transfer of energy from the existing field to the NPs. For example, the particles can be made to heat up, which leads to their use as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumors; or as chemotherapy and radiotherapy enhancement agents, where the moderate degree of tissue warming results in more effective malignant cell destruction. Along with these, many other potential applications, are made available in bio-medicine as a result of special physical properties of MNPs.

The properties of MNPs listed above make them useful in variety of in vitro and in vivo applications. Some current applications in biology and medicine include fluorescent biological labels [6-7], drug and gene delivery [9, 10], biodetection of pathogens [11], detection of proteins [12], probing of DNA structure [13], tissue engineering [14, 15], magnetic fluid hyperthermia (MFH) [16], separation and purification of biological molecules and cells [17], MRI contrast enhancement [18] and phagokinetic studies [19].

Some of the important biomedical applications of the MNPs are shown in Fig. 1.1. This include drug delivery, gene delivery, MRI, Cell labeling, multimodal imaging and MFH. Among these, MFH has emerged as one of the promising cancer modality either alone or in combination with existing cancer modalities [20, 21]. In MFH, MNPs are used for heating the specific region of body via absorption of radio frequency when subjected to an alternating current (AC) magnetic field [22]. Present work is focused on the MFH.
1.4 Magnetic Fluid Hyperthermia

Hyperthermia is a type of cancer treatment in which cancer cells are killed by exposing it to high temperatures (42-46°C). In this temperature range, tumor cells are generally found to be more heat-sensitive compared to normal cells because the tumor cells are hypoxic (poorly oxygenated) while normal cells are euoxic (well oxygenated) [23-25].

In MFH, for heating of body tissues, MNPs can be used. MNPs under AC magnetic field show heating effects due to the losses during the magnetization reversal process of the particles. The heat generation that occurs in MNPs under the influence of external AC magnetic field is used in hyperthermia treatment of cancer, which is schematically shown in Fig. 1.2.
The power of heating depends on the intrinsic and extrinsic properties such as size, composition, magnetic properties of NPs, the frequency and amplitude of the electromagnetic waves, etc. Further, the actual increase in temperature at a targeted site depends on magnetic losses, local blood circulation, the thermal conductivity and heat capacity of the surrounding medium which can diffuse the heat.

It is observed that when cells are exposed to MNPs, cells uptake them by a process of endocytosis. Further, cancerous tissue is known to engulf much larger amount of MNPs than normal tissue due to the leaky vasculature [26, 27]. Potential benefit of using MNPs is the use of localized magnetic field gradients to attract the particles to a selected site, to hold them there until the therapy is complete and then to remove them. Further these MNPs may be easily tagged to bio-molecules such as folate or antibodies and can be targeted to specific tissues. In addition, MNPs like iron oxides can be developed easily into multifunctional
agents taking advantage of its diagnostic imaging capabilities and magnetic properties in targeting drugs [28, 29]. Drugs can be immobilized on the surface of MNPs and can be targeted to a specific exposed tumor region with the help of magnetic field.

As compared to the traditional therapies of cancer like chemotherapy and radiotherapy, hyperthermia in general and hyperthermia using MNPs can reduce the severe side effects caused to normal tissue [30-35]. However, achieving controlled and uniform heating is the limitation of the existing heating methods such as capacitive or inductive coupling of radiofrequency fields, microwave, ultrasound and heat administered by the external contacts [34]. Further, it is suggested that regulated and uniform heating can be achieved by MFH [36, 37]. During MFH, heat can be applied locally with no systemic effects, and hence, the side effects become further reduced. In view of the above discussion, it is apparent that there are distinct advantages of MFH over the other hyperthermic processes.

There are various requirements for MNPs in order to use them in MFH application, viz;

- Nature of MNPs
- Uniform and controlled heating
- Biocompatibility
- Site specificity
- Colloidal stability
- High specific absorption rate (SAR)
- Applied frequency and magnetic field

These are discussed below.

### 1.4.1 Nature of magnetic nanoparticles

In MFH, different MNPs such as ferromagnetic, ferrimagnetic or superparamagnetic NPs are introduced near the tumor in the form of bio-
compatible suspension. When these types of MNPs are subjected to an oscillating magnetic field, heat is generated due to different mechanisms such as Neel’s relaxation (magnetic moment rotation, occurs only in superparamagnetic NPs), Brownian motion (particle rotation, occurs in all types of NPs), hysteresis losses (occurs in ferrimagnetic and ferromagnetic NPs) and eddy current losses, etc [38].

1.4.2 Uniform and Controlled heating
For safe and effective hyperthermia therapy of cancer, it is important to maintain the temperature in the entire tumor within ±0.5ºC from the optimal value during the entire procedure. Low temperature reduces effectiveness of the treatment, while high temperature damages the surrounding normal tissue due to overheating. However, for most of the particles suggested so far for this application, the uniform and controlled heating is very challenging. Since, it is very difficult to achieve uniform distribution of the particles throughout the tissue. Nonuniform distribution of the particles creates zones with different levels of alternating magnetic field power absorption with different heating rates. Heat losses due to thermal conductivity and cooling by the blood flow are also difficult to account for, compounding challenges for achieving the uniform and controllable heating of the tissue. This requires introduction of heat probes into the tissue to control the temperature at different locations of the tumor, significantly increasing the complexity of the medical procedure [39]. However, a possible approach to control the temperature is to design the materials in such a way that it exhibits temperature sensitive magnetic properties. The use of a suitable Curie temperature ($T_C \sim 42$–$56^\circ$C) of a material is the smartest way to control in vivo temperature. Below the Curie temperature, these particles can be effectively heated by alternating magnetic field, but they stop absorbing the alternating magnetic field energy as their temperature approaches $T_C$ and the heating stops. This is because, the Curie temperature is the temperature at which ferromagnetic or ferrimagnetic
particles lose the magnetic properties and do not convert electromagnetic energy into heat. Thus, the material may act as \textit{in vivo} temperature controlling switch.

1.4.3 Biocompatibility

One of the requirements of MNPs for \textit{in vivo} hyperthermia application is that they should be biocompatible. If the non-biocompatible particles are injected into the body, there may be problems of toxicity. This is because these particles would be captured and stored by some of the organs from reticuloendothelial system (RES) after application, despite if these particles are coated with bio-degradable/non-biodegradable bio-compatible surfactants [40]. Acute side effects of these MNPs should be avoided by testing the cytotoxicity of the applied product \textit{in vitro} before the injection. Various \textit{in vivo} tests on animals have shown that with a large dosage of 3000 µmol Fe of iron based NPs per kg body weight the histology and serologic blood tests have indicated that no side effects occurred after 7 days treatment [41, 42]. In view of this \textit{in vivo} environment, the suspension of MNPs requires hydrophilic chemicals as solvent, such as MilliQ water or physiological brine, and be controlled at near neutral where pH value is about 7.4 [43]. However, toxicity of every novel product for \textit{in vivo} applications should be examined carefully.

1.4.4 Site specificity

Tumor targeted site-specific delivery is another major issue in order to use the MNPs for hyperthermia application. The conceptual representation of tumor-targeting of MNPs is shown in Fig. 1.3. Part (a) of the Fig. 1.3 shows the passive tumor targeting of the MNPs through the Enhanced Permeability and Retention (EPR) effect, part (b) shows the active targeting of tumor through molecular targeting while the part (c) shows the active targeting of the tumor through magnetic targeting.
Fig. 1.3: Conceptual representation tumor-targeting of MNPs. (a) Blood vessel in tumor cells and healthy cells showing the EPR effect in tumor cells, (b) Molecular targeting of MNPs and (c) Magnetic targeting of MNPs [44].

(a) **EPR effect**: MNPs can accumulate in many tumor tissues passively through EPR effect [45]. Abnormal angiogenesis in cancer cells cause tumor vasculature leaky (shown in part (a) of Fig. 1.3) owing to fenestrations and gaps between the endothelial cells, unlike that found in normal tissue. This leaky vasculature of solid tumor facilitates passive extravasation of MNPs in circulation into the tumor.
interstitium where the MNPs can be retained because of poor lymphatic drainage found in some types of tumor tissues. As opposite to the tumor-tissue vessel, endothelial cells of normal-tissue vessels are closely packed and presented a greater barrier for penetration of MNPs. This difference in vascular permeability provides a means for tumor-selective penetration of MNPs.

**(b) Molecular targeting:** Concurrently along with the EPR mechanism, retention of MNPs can be achieved actively with the molecular targeting based on the use of tumor-selective ligands (represented in part (b) of Fig. 1.3). Specific Ligands (e.g. antibodies, peptides, small molecules, etc.) targeted toward moieties overexpressed or uniquely present on the plasma membrane of tumor cells can be used to actively enhance MNP accumulation at the tumor site and can also help to internalize particles into cells via endocytosis.

The increased metabolic function of cancer cells can also be exploited for targeting through attachment of molecules such as glucose or folic acid [46, 47]. Here, the specific interactions between the targeting ligand and cell surface receptors must outweigh the nonspecific interactions between the MNPs and cells. Thus, the MNPs core should be sufficiently coated to prevent nonspecific cell binding of the MNPs. Since, the coating must be near charge-neutral to prevent nonspecific electrostatic interactions between cells and the MNPs. Polyethylene glycol (PEG) is commonly used as the coating material of MNPs; since it improves circulation time in blood and displays targeting agents on its termini for cell recognition.

**(c) Magnetic targeting:** Active tumor targeting of MNPs can also be done by using their property to respond an external magnetic field. An external magnetic field can be applied to the tumor region produces a field gradient across the tumor (shown in part (c) of Fig. 1.3). Field gradient produces the magnetic force which attracts particles into the tumor space (through comprised vasculature) and helps in subsequent retention. MNPs are retained in tumors only when the magnetic
force is sufficient to overcome hydrodynamic drag forces exerted on the particles by blood flow [48].

Passive tumor targeting significantly enhances uptake of nanosized therapies while active targeting provides both better uptake and distribution in the tumor. Each form of targeting shown in Fig. 1.3 can be applied simultaneously to the others, depending on the particular strategy employed.

1.4.5 Colloidal Stability

In order to use the MNPs, it is necessary to make their colloidal suspension in bio-compatible media, i.e. nanofluid. The stability of MNPs in suspension is controlled by three principal forces: (a) hydrophobic–hydrophilic, (b) magnetic and (c) van der Waals. MNPs tend to aggregate to micron size clusters in suspension due to the hydrophobic interactions between the sub nm size particles (large surface area to volume ratio). Further these micron size clusters aggregate due to the magnetic dipole–dipole interactions and become magnetized by neighboring clusters. When an external magnetic field is applied, further magnetization of these clusters occurs which increases their aggregation [49]. Since, MNPs aggregate in suspension due to the attractive van der Waals forces in order to minimize the total surface or interfacial energy. Consequently, such aggregation can hamper the efficacy of MNPs in hyperthermia due to their low surface area and larger sizes.

Lee et al. reports the effect of dispersion of CoFe$_2$O$_4$ NPs in water on the heat generation for drug delivery and hyperthermia application [50]. They showed that the temperature increase for the non-dispersed NPs is lower than the dispersed NPs at the same experiment condition. The total surface area of MNPs plays an important role in generating the heat because the heat originates from the MNPs. If the MNPs are well dispersed in water, the surface area of MNPs will be increased and convective heat transfer rates to the fluid will be increased. Thus, a good dispersion of NPs may provide an equivalent energy transfer of a much larger
sample of bulk or microparticle-sized magnetic materials of the same composition. Thus, the stabilization of MNPs in suspension by modifying their surface is an important issue in the context of hyperthermia.

1.4.6 High Specific Absorption Rate

The application of MNPs in hyperthermia therapy requires the magnetic material having high heating ability (measured by specific absorption rate (SAR)). Low value of SAR causes the large quantity of MNPs to be inserted in order to increase the temperature rise, which will further cause the problem of toxicity. Thus, in order to increase the efficiency of MNPs and reduce the cytotoxicity of MNPs it is essential to increase the SAR of MNPs.

The SAR depends on composition, structural and magnetic properties as well as magnetic field amplitude. Among these, magnetic properties especially magnetization of NPs affects SAR. If the magnetization of NPs is higher, the rate conversion of magnetic energy into the heat energy, i.e. SAR will also be higher and vice versa. There are various reports in the literature which show that the magnetic properties of the substituted iron oxides can be further improved to that of parent oxide [51-55]. Another way for enhancing the SAR is to use the core-shell NPs. Lee et al. have reported the increased SAR for different types of magnetic core-shell nanoparticle [56]. In this way, by enhancing the SAR value, the amount required for the therapy can be reduced.

1.4.7 Applied frequency and magnetic field

For in vivo application of MFH, the applied frequency ($f$) and magnetic field ($H$) must be in the biological safety range and physiologically tolerable range. The usable field range is thought to be $0.05 < f < 1.2$ MHz and $H < 15$ kAm$^{-1}$. Although the limit has not yet been investigated exactly, an appropriate value has been reported for the fields with $H \cdot f < 4.85 \times 10^8$ Am$^{-1}$s$^{-1}$ acceptable [57, 58]. For a smaller diameter of the exposed body region and in dependence on the seriousness
of the illness this critical product may be exceeded. Accordingly, one can assume a weaker criterion $H \times f = 5 \times 10^9 \text{Am}^{-1} \text{s}^{-1}$ [59, 60]. However, some studies have reported that the use of a magnetic field amplitude higher than 80 kAm$^{-1}$ (1000 Oe) at 150 kHz seriously affected an animal body [61, 62]. Therefore, it is essential to select an appropriate frequency and amplitude of the alternating magnetic field to obtain an effective heating power.

Various MNPs have been used for the MFH application, however, ferrite NPs have received special attention due to its various properties. The biological applications impose some special requirements. For example, the well-known iron oxide NPs become undesirable because their iron atoms are poorly distinguishable from those of hemoglobin. A possible solution is to use mixed-ferrites ($\text{MFe}_2\text{O}_4$ where $\text{M}=\text{Co, Mn, Ni, Zn}$) to have a range of magnetic properties. These ferrites have attracted special attention because they save time and because of their ease of synthesis, low inherent toxicity, physical and chemical stabilities and suitable magnetic properties.

1.5 \text{NiFe}_2\text{O}_4 \text{ nanoparticles}

Out of the different spinel-type ferrites, Ni and Ni–Zn ferrites are the most useful materials due to their low cost, high electromagnetic performance, moderate saturation magnetization, low coercivity, high permeability, high resistivity, good chemical stability, low eddy current loss [63-68]. The above specifications make Ni-based ferrites a good potential for biomedicine applications [69-71]. Ni–Zn ferrite particles can be used as effective heating mediators for the hyperthermia application in cancer therapy [72]. The main reason for considering Ni-ferrite NPs as a hyperthermia agent is that these are expected to have urgently required magnetic properties for hyperthermia such as soft magnetism and small magnetic properties degradation at high frequency [73]. The magnetic properties of the substituted nickel ferrite ($\text{Ni}_{1-x}\text{M}_x\text{Fe}_2\text{O}_4$ where $\text{M} = \text{Cu, Zn}$) NPs can be further
improved as compared to that of parent NiFe$_2$O$_4$. Hence, the amount required for therapy can be reduced.

When Ni ferrite is substituted with the Zn ions then it results in frustrated magnetic structure. The frustration is a necessary condition for the appearance of a canted local state, which was obtained for the first time by Rossenweig [74]. This spin canting is responsible for the ferrite samples with varying magnetization. Ni-Zn ferrite is a mixed spinel in which tetrahedral sites are occupied by the Zn$^{2+}$ and Fe$^{3+}$ ions and the octahedral sites are occupied by Ni$^{2+}$ and Fe$^{3+}$ in the cubic spinel structure. The distribution of various ions in tetrahedral and octahedral sites is different when the ferrite is synthesized at low temperatures and the particle size is in nanometer region. An overview of the investigations done in the literature on this material for hyperthermia; their major interpretations and conclusions are reviewed below.

- Bae et al. have investigated the self heating temperature rising characteristics, cytotoxicity and magnetic properties of NiFe$_2$O$_4$ NPs to use it as in vivo hyperthermia agent in biomedicine. They concluded that to generate maximum temperature of 44.2 °C, the product of frequency and applied magnetic field was $5.1 \times 10^8$ Am$^{-1}$s$^{-1}$, which is in the biological safety and physiological tolerable range [67].

- Tomitaka et al. have measured the self rising property of Fe$_3$O$_4$, ZnFe$_2$O$_4$ and NiFe$_2$O$_4$ in an alternating magnetic field at a frequency of 110 kHz and studied the bio-compatibility of these NPs by in vitro cytotoxicity assays using HeLa cells. ZnFe$_2$O$_4$ exhibited lower self-heating temperature. The three ferrite NPs show non-toxicity at concentrations lower than 10 μg mL$^{-1}$ and the cytotoxicity of NiFe$_2$O$_4$ at concentrations of 100 μg mL$^{-1}$ [68].

Though, Ni-Zn ferrite was found to be a material with high resistivity, low dielectric loss, high magnetization, high Curie temperature, high permeability, chemical stability and low eddy current loss when operating at high frequencies (10-500 MHz) which are suitable for hyperthermia, there is still very less
information available on the heat rising capacity of the NiFe$_2$O$_4$ NPs as seen above. So, it is essential to study deeply the heat rising capacity of NiFe$_2$O$_4$ NPs. One of the important factors that the Zn substitution in NiFe$_2$O$_4$ NPs varies magnetization, is important parameter affecting the heat rising capacity.

1.6 Statement of the problem
Ferrite NPs are of greater interest in hyperthermia therapy application. Out of different ferrite materials reviewed, Ni-Zn ferrite NPs is found to be having high resistivity, moderate saturation magnetization, low eddy current loss and higher biocompatibility. This material has looked forward to seeing potential candidate for the hyperthermia application. Eventually there is very less data available on the hyperthermia application of Ni-Zn ferrite NPs. In light of this problem, work was planned in three parts.

In first part, Ni-Zn ferrite NPs were prepared by combustion method. Combustion method is of great importance because of its various advantages like fast production rate, low preparation cost and relatively simple preparation process. Hence, a simple combustion method was employed to produce ferrite NPs.

Initially, preparation of Ni ferrite NPs were planned by using different fuels such as polyvinyl alcohol (PVA), glycine and urea. An effect of fuel on the thermodynamic, structural and magnetic properties of Ni ferrite NPs was planned for investigation. It was planned to prepare the Zn substituted Ni ferrite NPs by optimizing a proper fuel. Then, it was planned to study an effect of Zn substitution on the structural and magnetic properties of Ni ferrite NPs in order to optimize the proper composition for the hyperthermia therapy application.

In second part, it was planned to make the MNPs with high SAR and to prepare it’s biocompatible nanofluid. Firstly, NiFe$_2$O$_4$ NPs were planned to coat with the PEG in order to improve the biocompatibility. Further the nanofluid of
these NPs is prepared in water and induction heating ability of these nanofluids was planned to study.

In order to enhance the SAR value of NiFe$_2$O$_4$ NPs, Zn was substituted in their matrix for their potential use in hyperthermia therapy application. The biocompatible nanofluid of the NPs was prepared in DDW and Acrypol 934. This nanofluid was planned to characterize for the induction heating study. A study on effect of the concentration of NPs and applied magnetic field on temperature rise was planned by induction heating experiment.

The third part is to study the suitability of NPs in biomedical applications. In view of this, the size, toxicity of the NPs and preparation of their injectable nanofluid was planned for the study. For this purpose, hydrodynamic size measurement of the NPs was planned by using dynamic light scattering (DLS) technique while the suspension stability study was planned by using zeta potential measurement. *In vitro* cell cytotoxicity study of NPs on the HeLa (Human Negroid cervix Epitheloid Carcinoma) cell lines was planned by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay. The influence of concentration on the cell viability was investigated.

Present work includes the synthesis, characterization of structural and magnetic properties of Ni$_{1-x}$Zn$_x$Fe$_2$O$_4$ and core-shell CoFe$_2$O$_4@Ni_{0.5}Zn_{0.5}$Fe$_2$O$_4$ NPs for hyperthermia application. From this study it was expected to yield an appropriate material for hyperthermia therapy application.
References

72. X. Lu, G. Liang, Q. Sun, C. Yang, Mater. Lett. 65 (2011) 674.