Chapter 3

Experimental Techniques
3.1 Introduction

MNPs are of great interest in the area of MFH. The magnetic properties such as high magnetic susceptibility and saturation magnetization make them suitable for MFH. The required properties of NPs can be tailored by choosing proper synthesis method. In the present work, Ni$_{1-x}$Zn$_x$Fe$_2$O$_4$ NPs are synthesized by combustion method and further suspended in biocompatible media such as water and Acrypol 934 solution to prepare nanofluid. In this chapter, different analytical techniques used for chemical analysis or to characterize the prepared samples are described with principle and working.

3.2 Synthesis of Ni$_{1-x}$Zn$_x$Fe$_2$O$_4$ nanoparticles and preparation of their nanofluids

The novel properties and the numerous applications of nanomaterials, especially ceramic powders, have encouraged many researchers to invent and explore the methods, like chemical and physical, by which such materials can be prepared. Out of these, the chemical methods have many advantages. The major advantage of the chemical synthesis is its versatility in designing and synthesizing new materials that can be refined into final product. Another advantage of the chemical synthesis over physical synthesis is that it offers mixing of components at the molecular level, which leads to an excellent chemical homogeneity. There are various chemical methods proposed to synthesize nanocrystalline Ni$_{1-x}$Zn$_x$Fe$_2$O$_4$ such as aerosol route [1], citrate precursor [3], co-precipitation [4], combustion [5, 6], hydrothermal [7], microwave [8], reflux [10], reverse micelle [11], sol-gel [12] and solvothermal [13] methods, etc. However, combustion method is of great importance because of its potential advantages like fast production rate, low preparation cost, relatively simple preparation process [6]. In this work, combustion method is employed for the synthesis of Ni$_{1-x}$Zn$_x$Fe$_2$O$_4$ NPs.

In order to use the MNPs in MFH application, preparation of its suspension, i.e. nanofluid in a biocompatible media is necessary condition. It represents the
improved stability as compared to the conventional fluids prepared with millimeter-sized solid particles because of size effect and Brownian motion of the NPs in liquids. As nanofluid consists of the NPs, they can flow smoothly in a microchannel without clogging. Generally, two types of methods have been employed to prepare nanofluids, viz. one step method and two step method. The one step method represents the formation of NPs inside the base fluids while the two step method represents the formation of NPs and their subsequent dispersion. In the present work, two step method have been used for the preparation of nanofluid.

3.3 Characterization techniques
For the characterization of the NPs, various techniques have been used which are discussed in the present chapter.

3.3.1 Thermogravimetric and differential thermal analysis (TG-DTA)
Thermal analysis is carried out to determine physical or chemical changes in a system under investigation, as a function of temperature. It is a useful technique for the characterization of materials which includes determination of weight loss with increase in temperature, phase transition temperature and thermal stability, etc. In thermogravimetric analysis (TGA), the weight of a material or mixture is measured as a function of a temperature when the sample is subjected to a controlled temperature program. The weight changes can be monitored during heating, as a function of temperature in a specified atmosphere. The sample is placed in a small pan connected to a microbalance and heated in a controlled manner and/or held isothermally for a specified time. The atmosphere around the sample may consist of air or an inert gas, such as nitrogen, or reactive gas such as oxygen.
In Differential Thermal Analysis (DTA), the difference in temperature between the sample and a standard reference material is measured as a function of sample temperature. This differential temperature is then plotted against sample temperature. Changes in the sample which lead to the absorption or evolution of heat can be detected relative to the inert reference. A DTA may be defined formally as a technique for recording the difference in temperature between a substance and a reference material against either time or temperature as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate.

![Fig. 3.1: TG-DTA curve of precursor gel of NiFe$_2$O$_4$.](image)

In the present work, SDT-2960 Transanalytical instruments, USA is used for TG-DTA analysis. The experiment was performed in nitrogen atmosphere in temperature range from room temperature to 1000 °C with the heating rate 10 °C min$^{-1}$. For DSC, heat-flow accuracy is equal to 1%, whereas DTA sensitivity is
0.001 ºC and that of weight is 0.1 μgm. This SDT-2960 controller is interfaced to PC and ‘TA-Universal analysis 2000’ software makes the thermal analysis. It stores data as well as runs the analysis programs. An example of TG-DTA of precursor gel of NiFe\(_2\)O\(_4\) is shown in Fig. 3.1.

### 3.3.2 X-ray diffraction technique

XRD technique is used to determine the crystal structure and analyze the phase of a subjected material. It is a non-destructive technique applied for the characterization of crystalline materials. It provides information about the structure, phase, preferred crystal orientation and structural parameters such as lattice parameter, crystallite size, strain and crystal defects.

The diffraction of X-rays occurs only when the wavelength of the wave motion is of the same order of magnitude as the repeat distance between scattering centers. This diffraction condition is nothing but Bragg’s law [17] and is given as:

\[
2d\sin\theta = n\lambda
\]

where, \(d\) is the interplaner spacing, \(\theta\) is the diffraction angle, \(\lambda\) is the wavelength of X-ray and \(n\) is the order of diffraction [17-19].

In this technique, material to be examined is ground to a fine powder. The fine grains of sample are generally spread uniformly over a rectangular area of a glass slide. The sample usually adhered to glass either using binders like collodion grease or wax. Several types of sample holders namely glass slide, circular disc or thin capillary, etc. are used for the different designs of the instrument.

Various kinds of sources for X-ray are available, but in the most common laboratory diffractometers, sealed X-ray tube source are used. The X-rays are produced from the sealed X-ray tube. The basic construction of the tube is similar to that Coolidge tube. The X-rays are produced by bombarding high speed electrons on a metal target. When the X-ray beam incidents on the sample, it gets scattered from the sample. The scattered rays from the sample constructively interfere according to Bragg’s law and produces a diffracted beam. The diffracted
beams are scanned by sweeping the detector from one angle to another and count or count rates of X-ray photon are measured at different angles. The output is obtained as a plot of the intensity of diffracted X-rays (counts) vs. angle (2θ). A diffraction pattern appears which can be analyzed to determine various structural properties of the material. A schematic of X-ray diffractometer is shown in Fig. 3.2.

When X-rays interact with a crystalline substance, phase one gets a diffraction pattern. Each crystalline substance gives a pattern; the same substance always gives the same pattern; and in a mixture of substances, each phase produces its pattern independently. The XRD pattern of a particular substance is, like a fingerprint of that substance.

![Fig. 3.2: Schematic of X-ray diffractometer.](image)

In the present work, the structure of samples were investigated by Philips automated X-ray diffractometer (model PW-3710) equipped with a crystal monochromator employing Cu-Kα radiation of wavelength 1.5406 Å in 2θ range from 20 to 80°. A typical XRD pattern of NiFe₂O₄ NPs is shown in Fig. 3.3.
Figure 3.3: A typical XRD pattern of NiFe$_2$O$_4$ NPs.

- **Identification of phase**

The powder XRD method is useful in the qualitative phase analysis because every crystalline material has its own characteristic powder pattern. Hence, this method is also called the powder fingerprint method. Powder pattern depends on two main factors namely (a) the size and shape of unit cell and (b) the atomic number and position of various atoms in the cell. Thus, two materials may have the same crystal structure, but almost certainly they have quite distinct powder patterns. The powder pattern has two characteristic features, viz. $d$-spacing of the lines and their intensity. Out of these two, the $d$-spacing is very useful and capable of precise measurement. The $d$-spacing should be reproducible from sample to sample unless impurities are present to form a solid solution or the material is in some stressed, disorder or meta-stable condition. While the intensities are more difficult to measure quantitatively and often vary from sample to sample. These can usually be measured only semi-quantitatively and may show variation.
1,50,000 unique powder diffraction data sets have been collected from organic, organo-metallic, inorganic and mineral samples. These have been compiled into a database known as the JCPDS (joint committee on powder diffraction standards). Identification of phase is made by matching the diffraction pattern with the standard JCPDS cards.

3.3.3 Scanning and transmission electron microscopy (SEM and TEM)

- **Scanning Electron Microscopy (SEM)**

  Scanning Electron Microscopy (SEM) technique is used to observe the morphology of a sample at higher magnification, higher resolution and depth of focus compared to an optical microscope. The SEM shows very detailed 2D image at much higher magnification than is possible with a light microscope.

  Qualitative and quantitative chemical analysis can also be done using an energy dispersive X-ray spectrometer (EDX) with the SEM. The SEM generates beam of incident electrons in an electron column above the sample chamber. Electrons are produced by a thermal emission source such as a heated tungsten filament or by a field emission cathode. Energy of the incident electrons can be as low as 100 eV or as high as 30 keV depending on the evaluation objectives. This beam travels downward through a series of magnetic lenses designed to focus the electrons to a very fine spot. Near the bottom, a set of scanning coils moves the focused beam back and forth across the specimen row by row.

  When an electron beam incidents on sample surface, electrons are emitted from the sample’s surface due to elastic and inelastic scattering. High energy electrons, those are ejected by an elastic scattering are known as backscattered electrons. The energy of these electrons is comparable to that of incident electrons. Low energy electrons, those are emitted from the inelastic scattering are called secondary electrons. The energy of the secondary electrons is typically 50 eV or less. A detector counts these electrons and sends the signals to an amplifier. The
final image is built up from the number of electrons emitted from each spot on the sample. A schematic of scanning electron microscope is represented in Fig. 3.4.

Fig. 3.4: Schematic of scanning electron microscope.

In the present investigation scanning electron microscope (SEM, Model: JEOL JSM-6360A) was used with different magnifications. A typical SEM image of NiFe₂O₄ NPs is shown in Fig. 3.5.
Fig. 3.5: A SEM image of NiFe$_2$O$_4$ NPs.

- Transmission Electron Microscopy (TEM)

Fig. 3.6: Schematic of Transmission electron microscope.
Transmission electron microscopy (TEM) is the premier tool for understanding the structure of materials at the nanometer level. It operates on the same basic principle as the light microscope, but uses electrons instead of light. The TEM image gives more depth knowledge of morphology and direct estimation of its size determination.

Schematic of transmission electron microscope is shown in Fig. 3.6. In TEM, an electron gun at the top produces the stream of monochromatic electrons. This stream of electrons is focused to a small, thin, coherent beam by the use of coherent lenses 1 and 2. When a beam of electrons strikes on the specimen, part of it gets transmitted. This transmitted portion is focused by the objective lens into an image. There is a mandatory requirement of sample for TEM analysis is, it must be thin enough to allow the electrons to be transmitted. The recommended thickness of the sample for TEM is about 0.5 μm. The powder to be analyzed is dispersed in some dispersive media (inert to powder) to form colloidal solution, then a drop of solution is kept on a conducting grid of copper or silver (sq. size is ~1 μm) and dried. This dried grid is then act as specimen for analysis using TEM.

Fig. 3.7: A typical TEM image of Ni<sub>0.5</sub>Zn<sub>0.5</sub>Fe<sub>2</sub>O<sub>4</sub> NPs.
In the present investigation, Transmission electron microscope (TEM, Model: PHILIPS CM 200) with operating voltage 20-200 kV and resolution 2.4 Å was used. A typical TEM image of Ni$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ NPs is shown in Fig. 3.7.

### 3.3.4 Elemental analysis

Energy Dispersive X-Ray Spectroscopy (EDX), also referred to as EDS is an X-ray technique used to identify the elemental composition of a specimen. It is used in conjunction with SEM. The EDX technique detects X-rays emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume.

![Fig. 3.8: a) Scheme of X-Ray excitations and b) EDX spectrum of NiFe$_2$O$_4$ NPs.](image)

During EDX Analysis, the specimen is bombarded with an electron beam inside the SEM. The bombarding electrons collide with the specimen atom’s own electrons, knocks some of them off in the process. This results in the formation of the electron vacancies. Eventually the electron vacancies at the inner shell are occupied by the higher energy electron from outer shell. During this process, the
energy is emitted in the form of X-rays, which is equal to the difference between the energies of two electronic states [Fig. 3.8 (a)]. The X-ray energy is the characteristic of the element from which it was emitted.

X-ray detector measures the relative abundance of emitted X-rays versus their energy. When an incident X-ray strikes the detector, it creates a charge pulse, which then converted to a voltage (which is proportional to the X-ray energy) pulse by a charge-sensitive preamplifier. The energy, as determined from the voltage measurement, for each incident X-ray is sent to a computer. The computer is then used to store the data for its analysis. The spectrum of X-ray energy versus counts is evaluated to determine the elemental composition of the sampled volume [Fig. 3.8 (b)]. The sample’s X-ray energy values from the EDX spectrum are compared with known characteristic X-ray energy values to determine the presence of an element in the sample.

In the present investigation, the EDX analysis was done using JOEL JSM 6360 with accelerating voltage 20 kV and energy scan range 0-20 keV.

### 3.3.5 Fourier transform infrared (FTIR) Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful tool for identifying types of chemical bonds in a molecule by recording an infrared absorption spectrum that is like a molecular "fingerprint" [14-16]. It is based on the fundamental principles of molecular spectroscopy that specific molecules absorb light energy of specific wavelengths, called as their resonance frequencies. For example, the H$_2$O molecule resonates around the wavenumber at 3450 (given the symbol cm$^{-1}$), in the infrared region of the electromagnetic spectrum. In spinel ferrites, it is mostly used for phase confirmation on the basis of presence of octahedral and tetrahedral molecular vibrations. In order to use the MNPs in biomedical applications, they must be coated with organic, biocompatible materials. In view of this, FTIR spectroscopy helps to understand the successful attachment of coating agent on the surface of magnetic core.
Fig. 3.9: FTIR spectrometers are based upon Michelson interferometer.

The schematic of the FTIR spectrometer is shown in Fig. 3.9. It consists of infrared source, which is always Nernst filament (ZrO$_2$ + Y$_2$O$_3$) or Globar (SiC). The light originates from infrared source whose output is collimated; this light beam then impinges upon a beamsplitter. The beamsplitter transmits 50% of the light to one mirror, and reflects 50% of the light to a second mirror. The light reflected off from these two mirrors interferes on the other side of the beamsplitter, and are finally focused onto a sample. In the interferometer, one mirror is held in a fixed position relative to the beamsplitter during the measurement. A second mirror (the moving mirror) scans back and forth relative to the beamsplitter in a continuous fashion. When infrared radiation is incident on to the sample, some of it is absorbed by the sample and some of it is passed through (transmitted) which is then detected by the detector. This results in the absorption/ transmission spectrum as function of wave number (cm$^{-1}$) is obtained from the fourier transform of the interferogram, which is the function of the mirror.
movement. This instrument has the reference cell, which is generally used in the dispersive IR instrument, so a reference spectrum is recorded and stored in the memory to subtract from the sample spectrum. FTIR spectrum of a pure PEG is shown in Fig. 3.10.

![FTIR spectra of pure PEG](image)

**Fig. 3.10:** FTIR spectra of pure PEG.

This technique is used to analyze chemical composition of many organic chemicals, coatings, adhesives, polymers, lubricants, paints, coolants, biological samples, semiconductor materials, gases, inorganics and minerals. It is also used to analyze a wide range of materials in bulk or thin films, solids, liquids, powders, pastes, fibers, and other forms. In the present investigation, FTIR spectroscopy (Perkin Elmer-USA) in the range of 450–4000 cm$^{-1}$ with a sample in the form of KBr pellets.

### 3.3.6 Vibrating Sample Magnetometer (VSM)

Vibrating Sample Magnetometer (VSM) is used to characterize the magnetic properties of materials as a function of magnetic field, temperature and time. This technique was invented in 1956 by a scientist Simon Foner, at Lincoln Laboratory.
MIT. They are ideally suited for research and development, production, testing and process control.

VSM works on Faraday’s law of induction, according to which an e.m.f. is induced in a conductor by a time-varying magnetic flux. In VSM, the sample to be studied is placed in a constant magnetic field. If the sample is magnetic, this constant magnetic field will magnetize the sample by aligning the magnetic domains or the individual magnetic spins, with the field. The magnetic dipole moment of the sample will create the magnetic field around the sample. As the sample is moved up and down, this magnetic field around the sample is changing as a function of time and can be sensed by a set of pick-up coils [Fig. 3.11 (a)]. The resulting field change at a point inside the detection coils induces voltage. This voltage can be detected to a high resolution and accuracy by means of suitable associated electronics. For stationary pick-up coils with uniform and stable external field, the only effect measured by the coils is that due to the motion of the sample. Thus, voltage is measure of the magnetic moment of the sample [21].

![Diagram](image)

**Fig. 3.11:** a) Working principle of VSM and b) The typical Magnetic hysteresis loop of NiFe$_2$O$_4$ NPs.
In the present work, the magnetic characterization was carried out by vibrating sample magnetometer (model: Lakeshore 7307) under the applied magnetic field of ±9,000 Oe at room temperature. The typical Magnetic hysteresis loop of NiFe$_2$O$_4$ NPs is shown in Fig. 3.11 (b).

3.3.8 Magnetic Property Measurement System

Magnetic property measurement system (MPMS) is a highly integrated instrument system designed to study the magnetism in matter. This system is used for characterizing the magnetic properties of bulk, thin film, and superparamagnetic nanoparticle systems.

A Superconducting Quantum Interference Device (SQUID) is the most sensitive device available for measuring magnetic fields and although the SQUID in the MPMS is the source of the instrument’s remarkable sensitivity it does not detect directly the magnetic field from the sample. In the MPMS, the sample moves through a system of superconducting detection coils which are connected to the SQUID with superconducting wires allowing the current from the detection coils to inductively couple to the SQUID sensor. When the SQUID is properly configured, it produces an output voltage, which is directly proportional to the current flowing in the input coil of SQUID. Hence, SQUID device, which is placed approximately 11 cm below the magnet inside a superconducting shield, basically functions as an extremely sensitive current-to-voltage converter.

The schematic of MPMS is shown in Fig. 3.12. In the MPMS, measurement is performed by moving a sample through the superconducting detection coils, which are located outside the sample chamber and at the center of the magnet. When the sample moves through the coils, the magnetic moment of the sample induces an electric current in the detection coils. The detection coils, the connecting wires and the SQUID input coil form a closed superconducting loop, hence, any change of magnetic flux in the detection coils produces a change in the persistent current in the detection circuit, which is proportional to the change in
magnetic flux. Here, SQUID works as a highly linear current to voltage converter and the variations in the detection coils produce corresponding variations in the SQUID output voltage, which is proportional to magnetic moment of the sample. When the system is fully calibrated, measurements of the voltage variations from the SQUID detector as a sample is moved through the detection coils provide a highly accurate measurement of the sample’s magnetic moment. The calibration of the system can be done by using a small piece of material having a known mass and magnetic susceptibility.

Fig. 3.12: Schematic of Magnetic Property Measurement System.
3.3.8 Zeta potential Measurement

Zeta potential is the potential at the surface of shear between the charged surface and the dispersing media. The zeta potential value is used to study the stability of colloidal dispersion. When all the particles in the suspension have a large negative or positive zeta potential, then they will tend to repel each other and there is no tendency to flocculate and vice versa. The general dividing line between stable and unstable suspensions is generally taken at either +30 mV or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable.

There are various instruments based on different principles to measure the zeta potential. Zeta potentiometer is a device in which two coherent beams of LASER light, derived by splitting the output from a low powered He-Ne laser, is made to cross at the stationary level in the capillary cell as shown in Fig. 3.13. The resulting interference process causes bands of high and low intensity illumination and the particles are drawn across this pattern by the field. The scattered light shows a similar fluctuation and the frequency of the fluctuations is related to the speed of the particles. The scattered light is collected by a photomultiplier tube and analyzed by a digital correlator, which is able to extract the frequency component due the particle mobility, and hence can construct a distribution function of particle mobility. Zeta potential is calculated by applying Henry eq.
To determine the sign of the particle charge, one of the mirrors is set into oscillatory motion. When the particles are moving in the opposite direction to the mirror, they appear to move faster and this can be correlated with the field direction.

![Diagram of optical configurations](image)

**Fig. 3.13:** Optical configurations of the Zetasizer Nano series for zeta potential measurement.

In an alternative procedure, the motion of the particles in the field is subjected to standard optical image analysis in order to determine the distribution of particle mobilities and hence, the distribution of zeta potentials [22, 23]. Modern instrumentation allows the zeta potential ($\zeta$) to be measured automatically.
3.3.9 Dynamic Light Scattering

DLS is a technique, which can be used to determine the size distribution profile of small particles in the suspension [24]. Particles suspended in liquids are in Brownian motion due to random collisions with solvent molecules. It causes the particles to diffuse through the medium. The diffusion coefficient $D$ is inversely proportional to the particle size according to the Stokes-Einstein eq.:

$$D = \frac{k_B T}{3 \pi \eta d} \quad (3.2)$$

where $D$ is the diffusion coefficient, $T$ is the absolute temperature, $k_B$ is the Boltzmann constant, $\eta$ is the viscosity and $d$ is the hydrodynamic diameter.

![Block diagram of typical DLS instrument.](image)

Fig. 3.14: Block diagram of typical DLS instrument.

When laser light is directed onto the particles, light is scattered in all directions. The scattered light that is observed comes from a collection of scattering elements within a scattering volume that is defined by the scattering angle and detection apertures. The intensity of the scattered light observed at any instant will be a result of the interference of light scattered by each particle; and
thus, will depend on the relative positions of the particles. If the particles are in motion, the relative positions of particles will change in time; and hence, fluctuations in time of the scattered light intensity will be observed.

As particles in Brownian motion move about randomly, the scattered intensity fluctuations are random. The smaller particles fluctuate rapidly while the larger particles fluctuate slowly. The fluctuations of the scattered light are recorded and then analyzed using the autocorrelation function to give the size (or distribution of size) of the particles in the sample.

Fig. 3.14 shows the block diagram of the typical DLS instrument. Typically it consists of the laser beam, cell containing suspension, lens and slit arrangements, photomultiplier tube, amplifier, correlator and computer assembly. For many particle systems, it is almost impossible to have all the particles having the same size. In this case, it is important to investigate the effect of the polydispersity.

3.3.10 Induction heating system

Induction heating systems are used in thermal therapy (thermotherapy) to generate alternating magnetic fields in the laboratory to elevate the temperature of a nanofluid \textit{in vitro} or \textit{in vivo} (in animal studies). Induction Heating system offers a controllable and localized method of production of heat without contact to the parts (components) being heated [25, 26].

The basic principle of working of induction heating system is based on the Faraday’s law, “The amount of voltage created is equal to the change in magnetic flux divided by the change in time”. Greater the change in the magnetic field, greater will be the amount of voltage. The source of high frequency electricity is used to drive a large alternating current through a coil. This coil is known as the work coil (Fig. 3.15). The passage of current through this coil generates a very intense and rapidly changing magnetic field in the space within the work coil. The work piece to be heated is placed within this intense alternating magnetic field.
The intense alternating magnetic field inside the work coil repeatedly magnetizes and de-magnetizes the magnetic particles. This rapid flipping of the magnetic domains of the particle causes considerable friction and heating inside the material. Heating due to this mechanism is known as Hysteresis loss, and is higher for materials that have a large area inside their B-H curve. This can be a large contributing factor to the heat generated during induction heating, but only occurs inside ferrous materials. For this reason, ferrous materials provide themselves more easily to heating by induction than non-ferrous materials.

![Fig. 3.15: a) A Schematic diagram of induction heating system and b) Work coil along with the work piece.](image)

In the present work, induction heating of MNPs for hyperthermia application was performed in a plastic centrifuge tube (1.5 ml) using an instrument (Easy heat 8310, Ambrell; UK shown in Fig. 3.15 (b)) with a 6 cm diameter (4 turns) heating coil. To keep the temperature of the coil at the ambient temperature, a provision of water circulation in coils was provided. MNPs suspended in 1 mL of distilled water were placed at the center of the coil and 265 kHz frequency was applied. The experiment was done for 10 min. with the desired current (200-400 A). The magnetic field for the corresponding current was calculated by using the following relation:
where, \( n, i \) and \( L \) denote the number of turns, applied current and the diameter of a turn in centimeters, respectively. The values of magnetic field were found to be 167.6, 251.4 and 335.2 Oe for the current 200, 300 and 400 A respectively.

### 3.3.11 Biocompatibility study: cytotoxicity study

Cytotoxicity is a vital factor that needs to be considered for in-vivo application of MNPs. This is because these particles would be captured and stored by some of the organs from RES after application, despite if these particles were coated with biodegradable/nonbiodegradable, biocompatible surfactants [28]. Acute side effect should be avoided by testing the cytotoxicity of the applied product *in vitro* before the injection. Several *in-vivo* tests [29, 30] on animals have revealed that, with a large dosage of 3,000 μmol Fe of the iron based NPs per kg body weight, the histology and serologic blood tests have indicated that no side effects occurred after 7 days treatment. In view of the *in-vivo* environment, the suspension of MNPs requires hydrophilic chemicals as solvent, such as water or physiological brine, and be controlled at near neutral where pH value is about 7.4 [31]. However, cytotoxicity of every novel product for in vivo applications should be examined carefully.

In the present work, *in vitro* cell viability study of \( \text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_{2}\text{O}_{4} \) NPs were done using HeLa (Human Negroid cervix Epitheloid Carcinoma) cell line by MTT \([3\text{-}(4,5\text{-dimethylthiazol-2-yl})\text{-2,5-diphenyl-tetrazolium bromide}] \) assay. Cell line was purchased from National Centre for Cell Science (NCCS), Pune, India and the detailed toxicity study was done in the National Toxicology Centre Pune, India (ISO 10993/USP 32 NF 27).

This chapter described the synthesis method of NPs, preparation of their nanofluid and various instruments used for characterization of the MNPs and nanofluid in this study.
References