A

Thesis

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"SiO_2 Functionalized Fe_3O_4 Nano Drug Delivery Vehicle for Active Targeting of Cancer Tumors"

Submitted in the fulfilment of the partial requirements for the award of degree of

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Submitted by

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July 2017

DEDICATED TO MY LOVING GRANDPARENTS AND PARENTS

CERTIFICATE

This is to certify that this Thesis entitled **"SiO_2 functionalized Fe_3O_4 nano drug delivery vehicle for active targeting of Cancer tumors"** is submitted by **Ms. Rubal Gupta** (Roll. No. 301504029) in the fulfilment of the partial requirement for the award of degree of Master in Science in Physics from School of Physics and Materials Science, Thapar University, Patiala (Punjab), India. It is an exclusive record of candidate's own research under the supervision of **Dr. Bhupendra Chudasama**. This Thesis in part or full has not been submitted in any other institute for award of such kind of degree.

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And above all, I pay my regards to the Almighty for his blessings.

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Abstract

Chemotherapy by magnetic nano particles is a new and minimally invasive cancer treatment. For magnetic targeting, a drug or therapeutic radionuclide is bound to a magnetic compound, introduced in the body, and then concentrated in the target area by means of a magnetic field (using an internally implanted permanent magnet or an externally applied field). Depending on the application, the particles then release. Core-shell nanostructures have emerged as an important class of functional materials with potential applications in diverse fields, especially in health sciences. Magnetic nanostructures have potential applications in many biological and medical fields such as drug delivery, hyperthermia treatment, magnetic resonance contrast enhancement and cell separation. In the present work, we report here synthesis and properties of a unique drug delivery system, which could be used for diagnostic and therapy purposes. Magnetite nanoparticles were synthesized by traditional co-precipitation route. These single domain magnetic nanoparticles were loaded into the surface modifiable shell of silica and then grafted by folic acid. Drug methylene blue was co-loaded into the FA@silica shell by demethylation reaction. Hydrolysis and condensation kinetics have been established to control the shell size and then released at in-vitro environment. Fabricated drug delivery system was characterized by XRD, FTIR, TGA, DLS, UV-visible spectroscopy and VSM measurements. X-ray study confirms the formation of single phase magnetite nanoparticles with average size of 8.4 nm. Formation of silica shell was confirmed from the FTIR spectroscopy. This is in good agreement with the X-ray analysis. .The loading of methylene blue was also confirmed from UV- visible spectra.

Chapter1

Introduction

1.1 Cancer

The term cancer refers to the class of disease in which abnormal cells divide in an uncontrolled manner. Cancer is of more than 200 distinct types. At beginning stage of cancer tumor is called primary tumor and when Cancer spread to other parts of the body then it is known as a secondary tumor or a metastasis by travelling through blood, lymph system [1]. Due to cell's gene mutations cells are not able to correct DNA damage and not able to commit suicide therefore cancer starts. The genetic changes that contribute to cancer tend to affect three main types of genes. These genes are proto-oncogenes, tumor suppressor genes, and DNA repair genes. Proto-oncogenes are included in typical cell development and division. When these genes are adjusted in certain ways or are more dynamic than ordinary, they may progress toward becoming cancer causing genes (or oncogenes), enabling cells to develop and survive when they ought not. Tumor suppress genes are additionally required in controlling cell development and division. Cells with specific adjustments in tumor suppress genes may partition in an uncontrolled way. DNA repair qualities are included in settling harmed DNA. Cells with transformations have a tendency to build up extra changes in different genes. Together these transformations may make the cell cancerous [2].

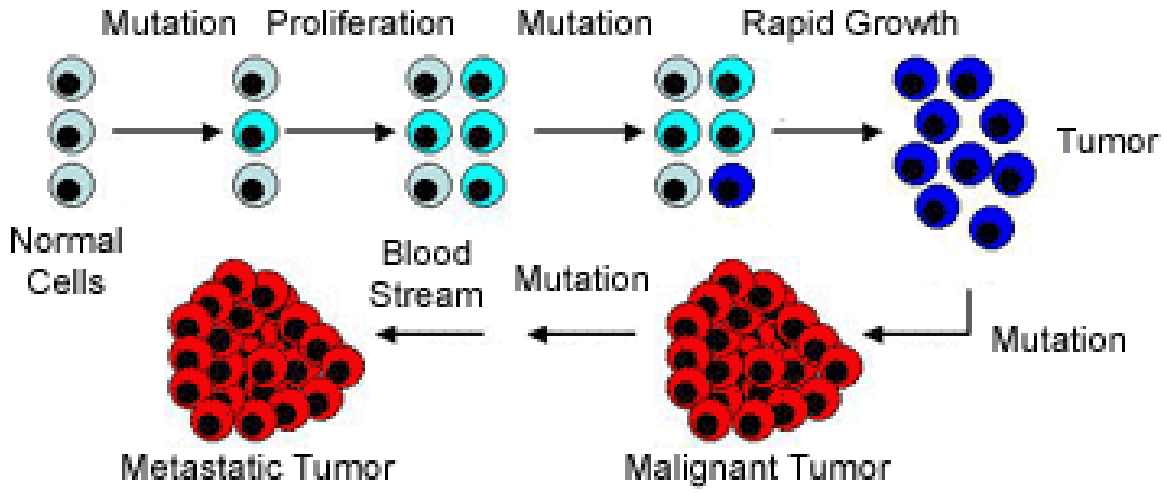

Fig.1.1: Normal cell to maetastatic tumor growth due to mutations

1.2: Types of cancer:-

Carcinoma:-When cancer starts from the skin or line from where the internal organ starts. The subtypes include transitional cell carcinoma, adenocarcinoma, and squamous cell carcinoma basal cell

Sarcoma:-cancer that starts in the supportive or connective tissue like fat, bone, blood vessels, muscle and cartilage.

Leukemia: - cancer that begin in the blood forming tissue like bone marrow

Lymphoma and myeloma: - cancer that starts from the cells of an immune system.

Brain and spinal cord cancers:-cancer which starts from the cells of spinal cord and brain [3].

1.3: Treatments of cancer:-

Surgery: - Cancer affected part removed or repair by medical treatment called surgery. It is also known as an operation or surgical resection. In surgery cuts are required through skin, muscles, and bones. Anesthesia is given during surgery for ignorance of pain. Anthesia may be any drug or substance. But this treatment is used only if cancer is in first stage or in the form of tumor [4].

Radiation therapy: - Radiation therapy (also called radiotherapy) is treatment by which cancer cells are destroyed and shrink the tumor by heavy doses of radiations. To shrink tumor and treat pain radiation therapy is used. Radiation therapy is of two types, external radiation therapy and internal radiation therapy. In external beam radiation therapy cancer cells are destroyed by exposing them with radiations externally and in the internal therapy source of radiations are put inside in body. This treatment is generally used with another treatment options to shrink the tumor.

Immunotherapy:-Immunotherapy is a treatment in which immune system is stimulated so that body can fight against cancer. Immunotherapy is a kind of biological therapy. Immunotherapy is also used with other treatment options. This therapy alone cannot eliminate cancer completely.

Hormone therapy: - Hormone therapy is also called hormonal therapy, hormone treatment, or endocrine therapy. Hormone treatment is a tumor treatment that moderates or stops the development of cancer that utilizes hormones to grow[6].

Chemotherapy: - In chemotherapy drugs are used to kill the cancer cells. The drugs which are used in chemotherapy are called cytotoxic that means toxic to cells. Many of drugs are created in laboratory and some of the drugs are made from natural sources such as plants. Almost all the drugs enter into the bloodstream then move all over the body and reach cancer cells in the organs and tissues. Also some chemotherapy drugs are reached at the tumor sites directly. In the Chemotherapy treatment drugs kill the cancer cells as they divide. In this way chemotherapeutic drug kill those cells which are divided more quickly. But some normal cells like bowels, blood cells and hair follicles divide quickly like cancer cells. So chemotherapeutic drug kills the normal cells along with cancer cells this is the main side effect of cancer but almost all the side effects are temporary because normal cells can recover but cancer cells cannot. There are different ways to give chemotherapeutic drug like cream, by mouth in form of tablet and injections. But most often method is via vein (intravenously) [7].

Targeted therapy:-It is a kind of cancer treatment that targets the changes in cancer cells that make them spread, grow and divide. It is difficult to design drugs for target because of the target's function in cells and structure of target drugs [5].

1.4:- Disadvantages of chemotherapeutic drug:-

Therapeutic drugs are administered intravenously leading to general systemic distribution. The non-specific nature of this technique results in the well-known side-effects of chemotherapy as the cytotoxic drug attacks normal, healthy cells in addition to its primary target, tumor cells.

The rationale for magnetic micro- and nanoparticles-based targeting lies in the potential to reduce or eliminate the side effects of chemotherapy drugs by reducing their systemic distribution but more accurately targeted doses of the cytotoxic compounds used in these treatments. The nanoparticles based drug-delivery systems have made a remarkable difference in site-specific release of drugs especially

chemotherapeutic agents, owing to their physical and chemical characteristics and biological attributes. Various researches in this exciting area have been conducted, several of the formulations are released in the market and are now routinely used in clinics by using nanoparticles these therapeutic agents can not only be delivered site specifically but also there is the possibility of loading nanoparticles with high concentration of drugs [8].

1.5: Use of magnetic nanoparticles in Chemotherapy:-

The use of magnetic nanoparticles for drug delivery was given by Widder, Senyi and colleagues. Therapeutic agents are attached to magnetic nanoparticles. Magnetic nanoparticles contain magnetic cores with a polymer or metal coating which can be functionalized or may consist of porous polymers that contain magnetic nanoparticles precipitated within the pores. They utilize following characteristics of nanoparticles based drug delivery system:-

- Nano scale dimension
- Capability of carrying active biomolecules for specific tasks
- Magnetic properties
- Super paramagnetic

For targeting by using nanoscale particle, nanoparticles pass from the narrowest blood vessel and when required also penetrate through the cell membrane. Due to superparamagnetic properties of nanoparticles this is very easy to target only to specific sites because magnetic nanoparticles can handle by external magnetic field. The active biomolecules bound to the surface of these nanoparticles are released. Magnetic nanoparticles consist of three components magnetic core, the protective coating and surface functionality. The common material used for magnetic nanoparticles are cobalt, chromium, iron based materials such as magnetite and maghemite. Iron based nano particles are used for drug delivery [9].

1.6: Surface functionalization:-

Iron based magnetic nanoparticles are used as magnetic core because of its properties

like non toxic, highly magnetic etc. There is need of non-permeable coating to prevent from leaching. Coating of nanoparticles is possible by natural polymer, synthetic organic polymer, silica, gold etc. Silica can be used for coating because of its properties like higher mechanical strength, non toxic and its bio-compatible nature. It is further surface modified for better release of drugs.

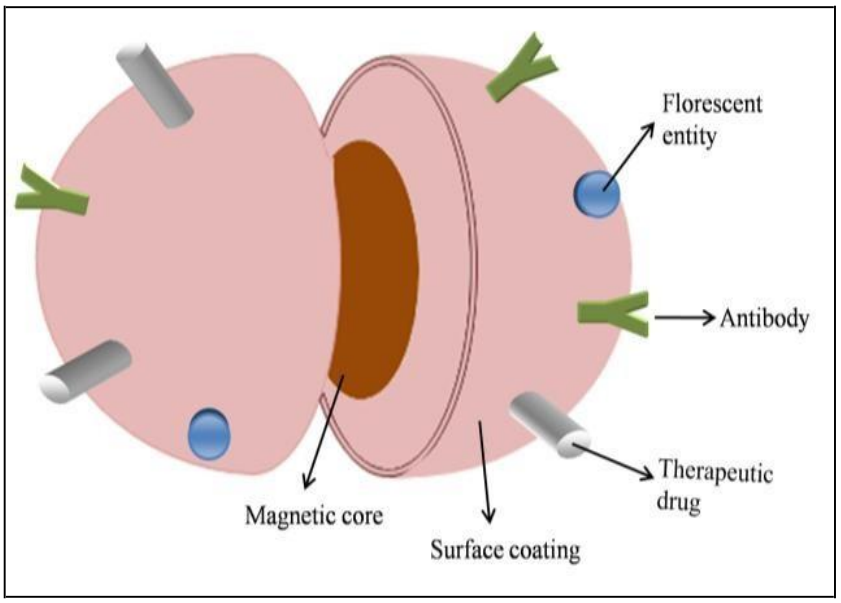

Fig.1.2: Surface modification on magnetic nanoparticles

Surface modification can be done by organic linkers. The organic linkers which create electrostatic interaction are widely used for drug delivery. The surface should be positively charge. Drug delivery system should have hydrophilic surface to escape from microphage capture [10]. Folic acid is a non-immunogenic receptor specific gland. Folic acid (FA) serves as an excellent marker. Folic acid change empowered the multifunctional nanoparticles to specifically expand the delivery of drugs to cancer cells that overexpresses folate receptor. Along these lines, folate conjugated mesoporous silica particles encourage the productivity of cell take-up by cancer cells because of receptor-mediate endocytosis [11].

There is a broad category of drugs used in chemotherapy which have important properties like cytoxicity, antineoplasticity, etc. But most of them do not have cell targeting properties and produce unwanted side effects. Successful chemotherapeutic drugs are paclitaxel, doxorubicin, and methotrexate (MTX). Doxorubicin drug is most common anti cancer drug used for cancer treatment. Doxorubicin is attached with the SiO_2-coated Fe_3O_4 nanoparticles via covalent bonding. This bonding makes the drug

carrier system stable and doxorubicin drug is cytotoxic to normal tissue [12]. The drug is then infused into the subject either by mean of intravenous or intra blood injection. The external magnetic fields generated by earth permanent magnets are utilized to guide and gather at the tumor location. Once the magnetic carrier is aggregated at the tumor site then the therapeutic agent is released from the magnetic carrier, either by mean of enzymatic activity or through changes in physiological conditions such as temperature/osmolality, pH governing to increased uptake of the drug by the tumor cells at the target locations. When nanoparticles based targeting system is designed then many parameters should be recognized such as physical and physiological parameters. Physical parameters like magnetic properties, size of the carrier particles, field strength, field geometry, and drug/gene binding capacity. Physiological parameters such as the depth to target, the rate of blood flow, vascular supply and body weight [9].

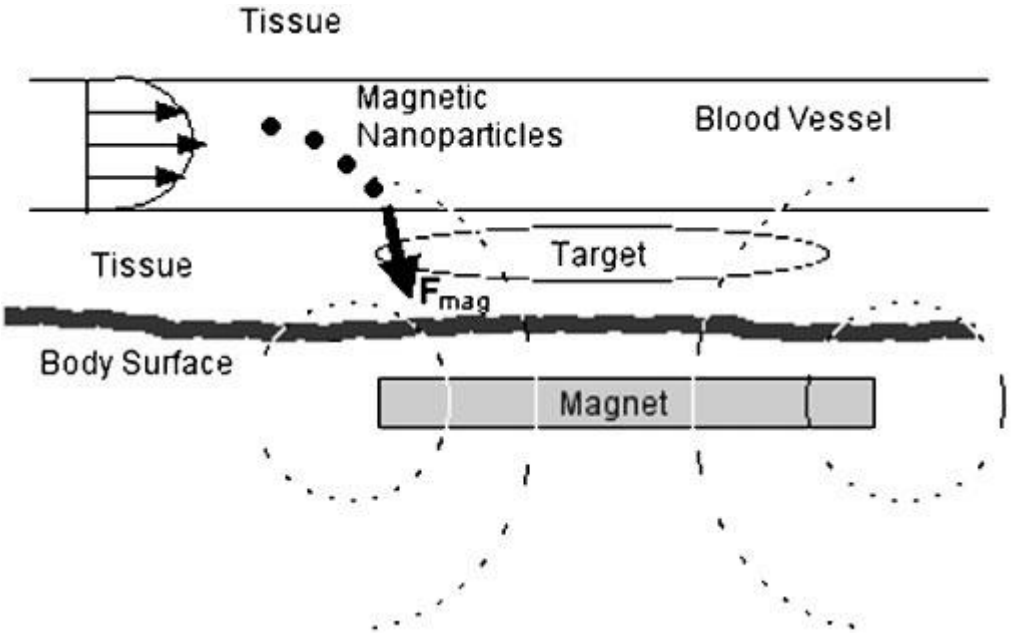

Fig.1.3: Targeting of magnetic nanoparticles inside the body with external applied field.

However in this approach, it is important to consider the following aspects:-

- The particles in the subject should not be aggregated at one point because if they are aggregated, then the distribution is hindered and rapid removal from the circulation is promoted.

- When the particles are infused into circulatory system they are quickly covered with the circulation components for example plasma, proteins. This adsorption of proteins at the particle surface is defined as opsonisation.

  The targeted delivery is a viable route to increase the effective use of drug and minimize toxicity and the side effects [13].

1.7: Types of drug targeting:-

Active targeting: - Active targeting is ligand based targeting. Active targeting depends upon the selective expression of particular receptors in tumor and also on the specific physical quantities, so the vectors which are sensitive to the physical quantities (e.g. temperature, pH, electric charge, light, sound, magnetism) are attached to drugs. Active targeting may be based on over-expressed species such as low molecular weight ligands (folic acid, thiamine, sugars) peptides, proteins (antibodies, lectins) weight, polysaccharides (hyaluronic acid), polyunsaturated fatty acids, DNA, etc. For an active targeting system there are two qualities which are important, first is the specificity in which the ligand joints onto the receptors second is the ability to deliver the required dose of drug for the required period of time [14].

Passive targeting: - Passive targeting is generally using the anatomical differences between the normal and the tumor vasculature to allow a selective accumulation of drugs at the tumor site. Passive targeting means passive aggregation of drugs at the site of morphology due to the leaky vasculature. Size of the nanoparticles is the main factor affecting the passive targeting processes through the permeability of the capillary vessels [15].

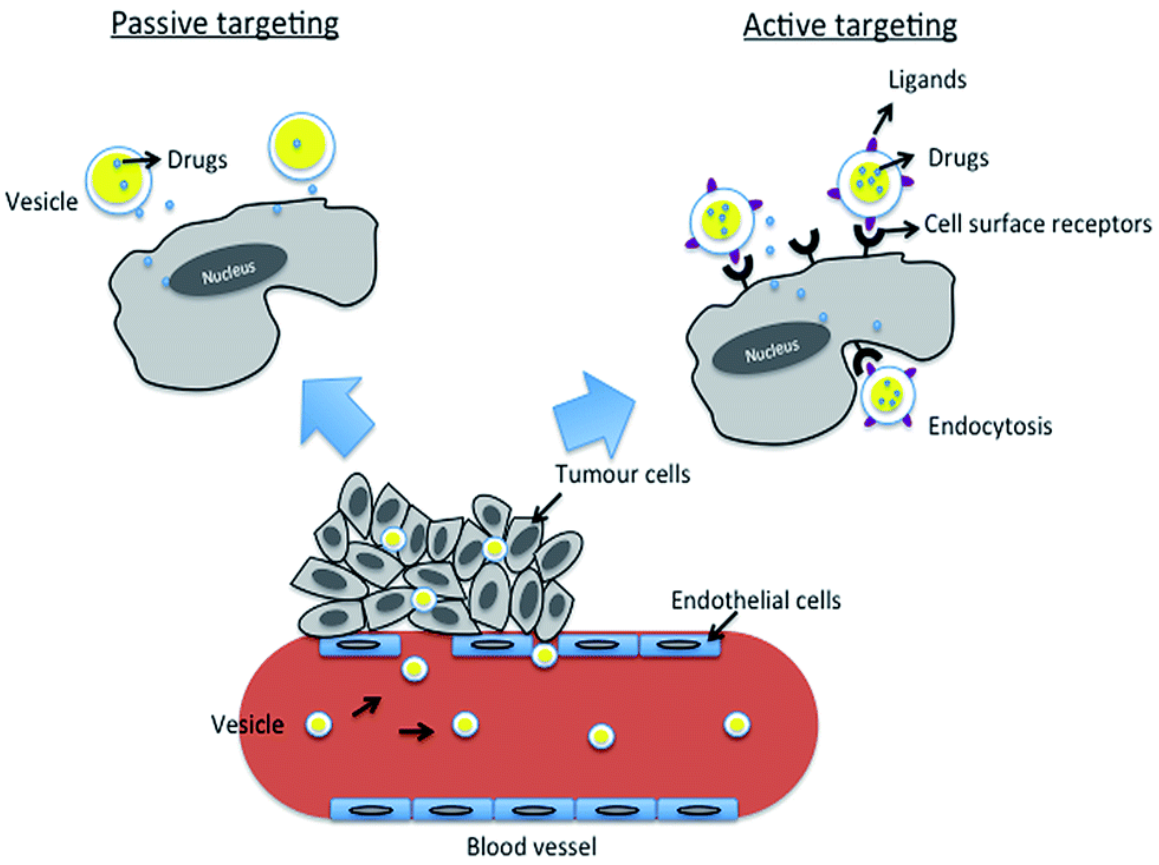

Fig.1.4: Passive and active targeting

Chapter2

Literature review

A nano particle used in the control release therapy for cancer treatment is a promising strategy. Different types of nano particles like polymeric nanoparticles and nanocapsules, liposomes, solid lipid nanoparticles, gold nanoparticles, carbon nanotubes have been used for drug delivery system. However magnetic nanoparticles have been used in the delivery system for the improvement of solubility, bioavailability, protection from toxicity, improvement of pharmacological activity and stability, enhancing tissue macrophages distribution, constant delivery. The priority gives to superparamagnetic nanoparticles iron oxide nano particles, maghemite, and magnetite are examples of superparamagnetic nanoparticles having size less than 10 nm diameter [9].

Choi et al. have reported the use of magnetic nanoparticles for drug delivery. Iron carbonyl ($Fe(CO)_5$) and cobalt carbonyl ($Co_2(CO)_8$ were used for the synthesis of Fe and Co by chemical vapor condensation method. Particle size of nanoparticles was found in the range from 5 to 13 nm which were in the superparamagnetic limit. Nano particles were found uniform in size. It was observed that with the increase in the decomposition temperature the particle size also increases [17].

Kandpal et al. reported the synthesis of magnetic nanoparticles by co-precipitation method. The size of particles was observed in the range of 20-22 nm. The aggregation of nanoparticles was also observed. The magnetization value of particles synthesis by co-precipitation method was higher than magnetic nanoparticles prepared by other methods [18].

Kaur et al**.** has synthesized silica-coated magnetic nanoparticles for bio-medical applications like cell separation, magnetic resonance imaging, drug delivery, tissue repair and hyperthermia. The nanoparticles were synthesized by co-precipitation method and coated with thin layer of silica by hydrolysis of (TEOS) tetraethylorthosilicate. The average size of magnetic nanoparticles was observed to

be 8.4 nm and structure of silica –coated nanoparticles is spinal. The binding of silica with (Fe_3O_4) magnetic nanoparticles was confirmed by FTIR. Silica –coated magnetic nanoparticles are superparamagnetic in nature and have good stability and have excellent magnetic properties. Also they had non –aggregated nanostructure which is perfect for drug targeting [19].

Bumb et al**.** have synthesized Fe_3O_4 nanoparticles by co-precipitation method. Nano-particles were cover by thin layer of silica of about 2 nm. Numbers of characterizations were performed for the magnetic properties, surface charge and size measurements. Diameter of silica-coated magnetic particles was about 18 nm [20].

He et al. reported that Fe_3O_4 nanoparticles were prepared by chemical co-precipitation method and thin layer of silica was coated on iron nanoparticles by condensation of tetraethylortho silicate (TEOS).Well dispersed Fe_3O_4 nanoparticles were synthesizes from this method. The diameter of these nanoparticles was found 6-7nm and having cubic spinel structure and in the superparamagnetic range [21].

Stella et al. reported that magnetic nanoparticles were coated with polyethylene glycol (PEG) attached with folic acid and then target to the folate –binding protein. The tumor cells were folic deficient and in the soluble form. The nanoparticles were synthesized by co-precipitation method. Number of characterizations was performed to investigate size and hydrophobicity. Folic acid was attached with nanoparticles by PEG terminal amino groups. In this manner folate conjugate nanoparticles shows a potential to new drug carrier for tumor cell selective targeting [23].

Venkatasubbu et al. reported that for many biomedical applications magnetic nanoparticles were used. PEG coated hydroxyapatite (HAp) nanoparticles attached with folic acid was synthesizes and characterized. Polclitaxel, the anticancer drug was attached with PEG coated hydroxyapatite nanoparticles. The drug release response was investigated by two steps, initial rapid release and then a sustained release. The release capacity of paclitaxel drug was found 50% for 3h stirring. Investigation of surface modification and linking of PEG coated nanoparticles with paclitaxel drug

was done by Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA) and UV spectroscopy [13].

Zhang et al. reported the response of (Fe_3O_4) nanoparticles coated with polyethylene glycol (PEG) which were activated with folic acid and linked with anticancer drug doxorubicin. The functionalization of magnetic nanoparticles coated with PEG and conjugated with folic acid, doxorubicin and its drug release response was characterized by two-steps that are active release followed by controlled release. Physical adsorption/ functionalization of PEG with nanoparticles were confirmed by FTIR technique. The drug release capacity of PEG coated nanoparticles was found 50% [24].

Chen et al. reported that anticancer agent DOX was effectively joined to the surface of the Fe_3O_4@SiO_2 core–shell nano particles by means of an amide bond. The DOX stacking proficiency controlled by UV–spectrometer was 86.5%. Drug release tests showed a pH-behaviour that DOX was cut from the nanoparticles effectively under low pH conditions within the sight of protease and that the majority of the conjugated doxorubincin was released inside the initial 12 h. The arranged DOX-joined Fe_3O_4@SiO_2 core–shell structure nanoparticles demonstrated a superparamagnetic property with saturation magnetization of 49.3 emu demonstrating an extraordinary potential application in the treatment of disease utilizing attractive focusing on drug delivery technology [25].

Arun et al**.** reported that previously in drug delivery the pharmaceuticals were not delivered to the target sites of the body is main problem but with magnetic nanoparticles it became possible in drug therapy *in-vivo*. Also *in-vitro* it made possible by doing some modification in drugs with ligands, antibodies, proteins. Magnetic nanoparticles Fe_3O_4 are synthesis by co-precipitation method and the average size of these particles were 60-70 nm. They are coupled with plasmid DNA and expressed with green fluorescent protein (EGFP) and then it was coated with chitosan for *in-vivo* experiment. In another model natriuretic peptide and carcinoembryonic antigen antibodies coupled to the chitosan-coated magnetic nanoparticles to target cells *in-vitro*. The magnetic targeting of functionalized nano-particles could prove more efficient for drug delivery [26].

Yufang et al. has reported that silica coated iron oxide nanoparticles were synthesized by solgel process. After that coating of folic acid was done by amide reaction. Then Doxorubicin hydrochloride (DOX), an anticancer drug was attached with the Fe_3O_4@SiO_2-FA spheres. The free DOX Fe_3O_4@SiO_2-FA spheres were less efficient and less cytotoxic then DOX-loaded Fe_3O_4@SiO_2-FA spheres because of increase in cell uptake of anticancer drug delivery vehicles mediated by the FA receptor. Thus they conclude that Fe_3O_4@SiO_2-FA are better for the targeted anticancer drug delivery of anti-cancer drugs [27].

Bhupendra et al. have reported that folic acid coating on nanoparticles was promising strategy for the drug targeting. They designed drug delivery system which can viably target cancer cells by methods of folate receptor-mediated endocytosis, have capacity to escape from opsonization and ability of magnetic targeting to withstand the drag constrain of the body fluid. PEG is attached with magnetic nanoparticles. An antitumor receptor i.e. folic acid was attached with PEG coated nanoparticles and then loaded with doxorubicin drug. 52 % of drug is loaded and doxorubicin is released over a period of 7 days. Thus there was control release of drug over several days which can use for chemotherapy [28].

Based on literature survey, it was decided to design a magnetic drug delivery system with active targeting encapsulation. Iron oxide nanoparticles are chosen as the core and it was covered with a thin porous layer of silica which will enhance the biocompatibility and prevent aggregation of magnetic nanoparticles. It is further decorated with folate receptors to improve its localization of tumor site and loaded with methylene blue as model drug. The loading efficiency and release kinetics study will also be conducted.

Chapter3

Experimental techniques

3.1 Synthesis of Fe_3O_4 magnetite nanoparticles

Magnetite (Fe_3O_4) nanoparticles have been synthesized by co-precipitation method. An aqueous solution of (0.001 M) Fe^{3+} and (0.002M) Fe^{2+} ions was made from anhydrous $FeCl_3$ and $FeSO_4.7H_2O$. pH of this mixture was maintained below 2 with addition of dilute HCl. After that (0.008M) NaOH was then drop-wise added in the aqueous Solution of Fe^{3+} and Fe^{2+} with continuous stirring. The pH of this solution was maintained at 10.5 with addition of extra NaOH solution. After 20 min of mechanical stirring, the particles were washed for three times with luke warm distilled water. During synthesis following chemical reaction take place

$$FeSO_4.7H_2O + 2FeCl_3 + 8NaOH \rightarrow Fe_3O_4 + 6NaCl + (Na)_2SO_4 + 11H_2O$$

3.2 Synthesis of silica coated- Fe_3O_4 magnetite nanoparticles:-

Silica coating on pre synthesize nanoparticles was performed by mixing of 80 mL of an ethanol, 20 mL distilled water and .5μL of TEOS then mixture was sonicated for 2-3 minutes. After that this solution was added into the presynthesize nanoparticles of Fe_3O_4 mixture was mechanically stirred for 1 h and washed 3 times.

3.3 Synthesis of folic coated $Fe_3O_4@SiO_2$ core–shell structure nanoparticles:-

Folic acid coating on $Fe_3O_4@SiO_2$ nanoparticles was done by adding distilled water into the freshly prepared $Fe_3O_4@SiO_2$ nanoparticles and centrifuged this mixture at 4000 rpm then decanted the supernatant liquid and left out solution was preserved. Then 5mL DMSO, 0.0090g folic acid and 0.0042 g DCC was mixed together in dark and after that solution was shake for 1.5 h at room temperature. The reaction mixture was further stir for ½ h and silica coated nanoparticles solution was added into this mixture. It was kept under shaking for 2 h. After that this reaction mixture was centrifuged at 4000rpm $Fe_3O_4@SiO_2$ –FA spheres.

3.4 Synthesis of Methylene Blue (MB) loaded silica encapsulated Fe_3O_4 nanoparticles:-

1 mM methylene blue (methylthioninium chloride) was dissolved in 50 mL distilled

water. For this synthesis 5 mL of this solution was mixed with 5ml solution of pre-synthesized Fe_3O_4@SiO_2-FA spheres and kept for shaking for 24 h. After that the reaction mixture was centrifuged at 4000 rpm. Isolated particles are washed and preserved for further characterizations.

3.5 Characterization Techniques:-

As-synthesized sample after each step was characterized by powder X-ray diffraction (XRD), UV-visible absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), Vibrational Sample Magnetometer (VSM) and dynamic light scattering (DLS)

3.5.1. X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is a flexible method that uncovers detailed data about the crystallographic structure material. When the X-rays of fixed wavelength are incident on the crystal at certain angle and if the wavelength of different X-rays are interfere constructively then reflected rays are formed and for the waves to be interfere constructively the differences in the travel path must be equivalent to integral multiple of wavelength.

Fig.3.5.1: X-Ray diffractometer by PANalytical X'Pert PRO with CuKα (λ = 1.5418 Å) radiation.

$$2dSin\theta=n\lambda$$

where n is an integer denotes the order of diffraction, λ is the wavelength of the incident X- rays, d is the interplanar spacing of the crystal and θ is the angle of incidence. Monochromatic X-rays are utilized to decide the interplanar spacing of the obscure materials. At the point when the Bragg conditions for constructive interference are acquired, a "reflection" is formed, and the relative peak height is directly proportional to the quantity of grains in a favored orientation.

3.5.2. Ultraviolet–Visible spectroscopy

The Ultraviolet-Visible (UV-Vis) spectroscopy is an expository method that utilizes

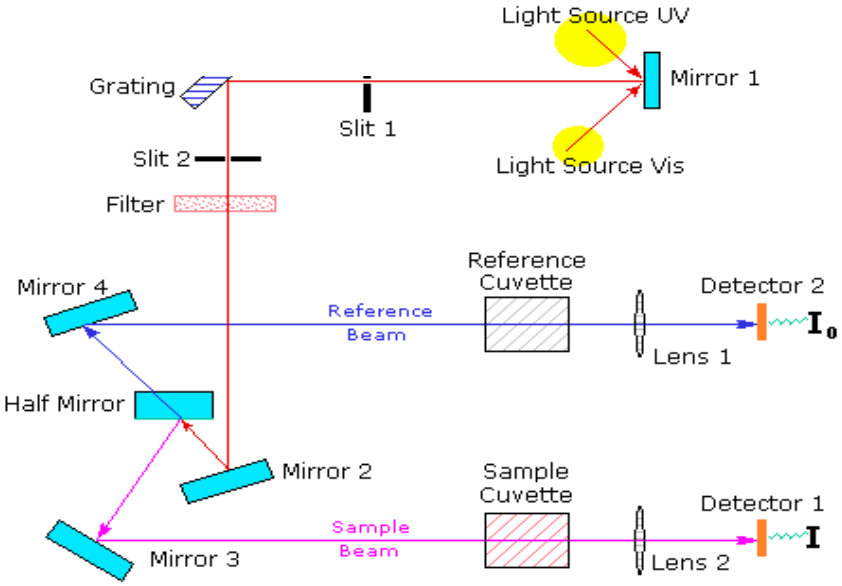

Fig.3.5.2: Schematic representation of principle of UV-Vis Spectrometer

the light in the UV and infrared region. UV-Visible spectroscopy includes the absorption spectroscopy in the ultraviolet-visible spectral domain. In the thesis of the electromagnetic field range, atoms experience electronic transition. The energy in a quantum (Planck's Law) is defined by the condition:

$$E = h\nu = hc/\lambda$$

Where h is Planck's consistent, ν is frequency and λ is wavelength of the incoming photon, and c is the speed of light, E is the energy. A light emitted from a visible as well as UV light source (hued red) is isolated into its component wavelength by a

prism or diffraction grating. Each monochromatic beam is divided into two equivalent force beams by a half-reflected device. First beam, the specimen beam passes through a small transparent beaker that contains a solution of the compound. The other beam which is the reference beam passes through an identical beaker containing only solvent. The intensity of the reference beam and specimen beams are denoted as I_o. The Beer-Lambert law defines that the absorbance of a solution is proportional to the convergence of the absorbing species in the arrangement and the path length. In this manner, for a fixed wavelength, UV/VIS spectroscopy can be utilized to decide the concentration of the absorber in solution. It is important to know how rapidly the absorbance changes with concentration. The measure of light I, transmitted through a solution of absorbing compound in a transparent solvent can be identified with its concentration by Beers Law:

$$log\ I/I_O = A = \varepsilon\lambda\ bc$$

where A is the absorbance, I_O is the incident light intensity, b is the cell path length in cm $\varepsilon\lambda$ is the molar absorptivity, and $\varepsilon\lambda$ is a function of wavelength, c is the solution concentration in moles/litre, which has units of litre/mole/cm.

3.5.3. Fourier Transform Infrared Spectroscopy

FTIR Spectroscopy is utilized essentially for subjective and quantitative examination of organic compounds, and furthermore to determine the chemical structure of numerous inorganics. Fourier transform infrared spectroscopy (FTIR) is a method which is utilized to get an infrared range of emission, photoconductivity absorption or Raman scattering of gas, solid or liquid. Infrared energy is absorbed by chemical bonds at particular frequencies (or wavelengths) ,the fundamental structure of mixes can be dictated by the spectral areas of their IR absorption. FTIR is configured as Michelson Interferometer. The interferometer comprises of a beam splitter, a fixed mirror, and a mirror that interprets forward and backward. The beam splitter is made of a unique material that transmits half of the radiation striking it and reflects the other half. Radiation from the source strikes the beam splitter and isolates it into two beams. One beam is transmitted through the beam splitter to the fixed mirror and the beam is reflected off the beam splitter to the moving mirror. The moving and fixed mirrors reflect the radiation back to the beam splitter. Once more, half of this reflected radiation is transmitted and half is reflected at the beam splitter, bringing about one

beam going to the detector and the other back to the source.

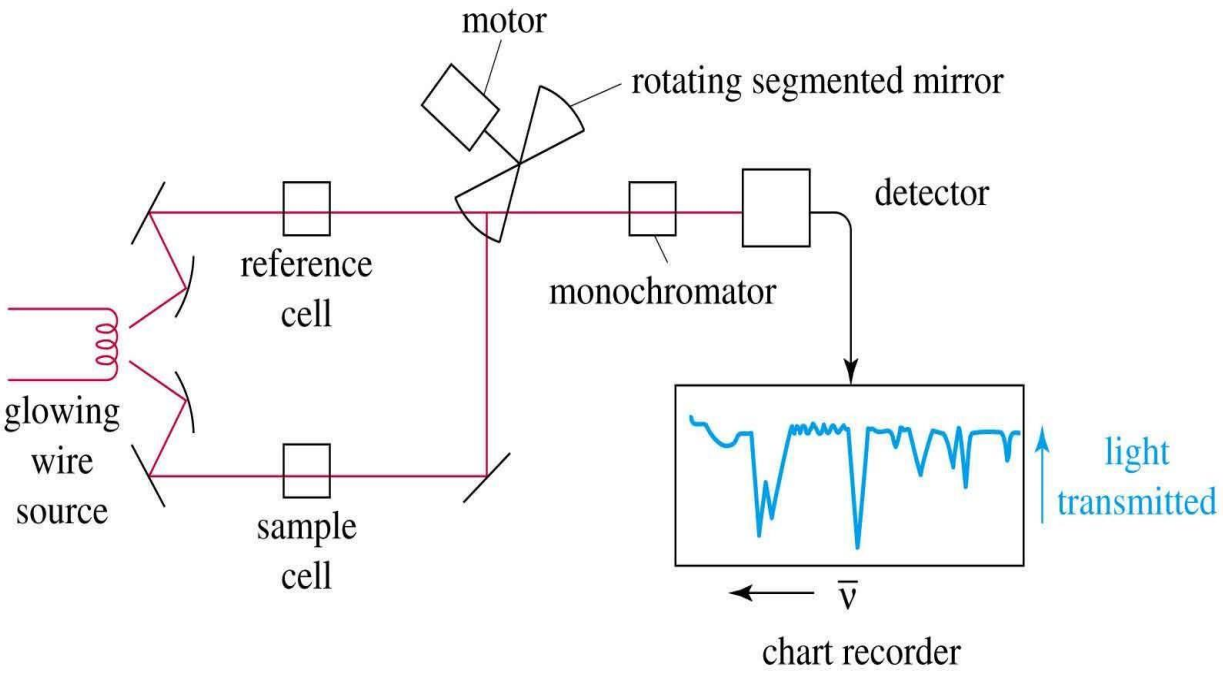

Fig.3.5.3: Schematic representation of Fourier Transform Infrared Spectroscopy.

3.5.4. Vibrating Sample Magnetometer:-

A vibrating sample magnetometer (VSM) is an instrument that is utilized for the characterization of magnetic samples. If a sample of any substance is placed between the poles of an electromagnet in a uniform magnetic field, an induced dipole moment will be produced. If specimen vibrates with sinusoidal motion an electrical signal in the form of sinusoidal wave will be produced in an appropriate placed pick-up coil. The signal has same vibrational frequency and its amplitude will be directly proportional to the relative position about the pick-up coils system, magnetic moment and its amplitude. The sample is placed in a small sample holder that is situated at the end of a sample rod placed on an electromechanical transducer. The transducer is directed by a power amplifier which is further directed by an oscillator. The sample vibrates perpendicular to the magnetic field along the Z axis. The previously generated signal in the pick-up coil system is fed to a differential amplifier and the output of the differential amplifier is finally fed into a tuned amplifier and an internal lock-in amplifier that receives a reference signal supplied by the oscillator. The output of the magnetometer or output of this lock-in amplifier is proportional to the magnetic

moment of sample being studied.

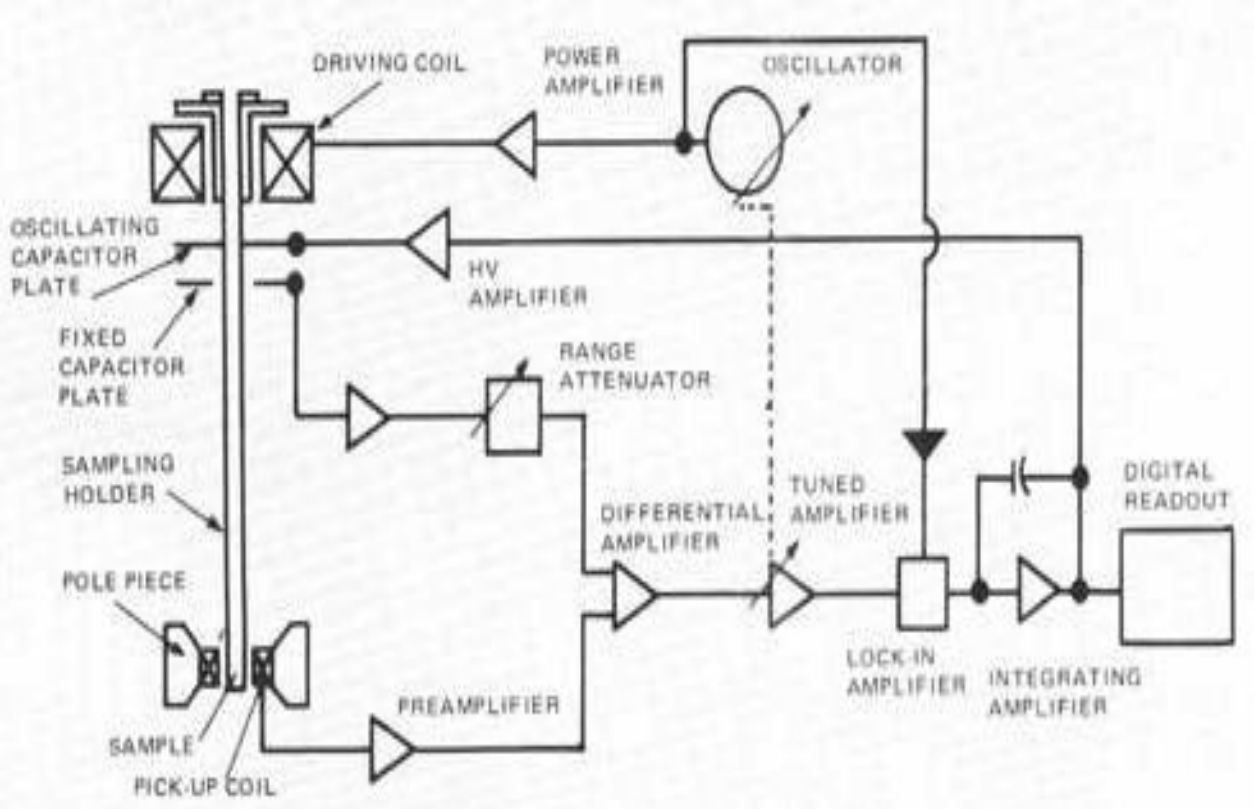

Fig.3.5.4: Schematic representation of Vibrating Sample Magnetometer

3.5.5. Dynamic light scattering (DLS)

In this technique by measuring the irregular changes in the intensity of light scattered from a solution or suspension the particle size about 1nm can be measured. This technique is called dynamic light scattering (DLS) and it is also known as quasi-elastic light scattering (QELS) and photon correlation spectroscopy (PCS). Light from the laser (monochromatic source) is shot into the sample in the cell. The light as scattering signal is collected at any of two detectors, either at a 173 degree (back angle) or 90 degree (right angle) scattering angle. The provision of two detectors gives us the freedom to choose the measurement conditions. The liquid viscosity and refractive index are necessary in order to interpret the measured results.

The obtained optical signal indicates random changes because of the random change in relative position of particles.

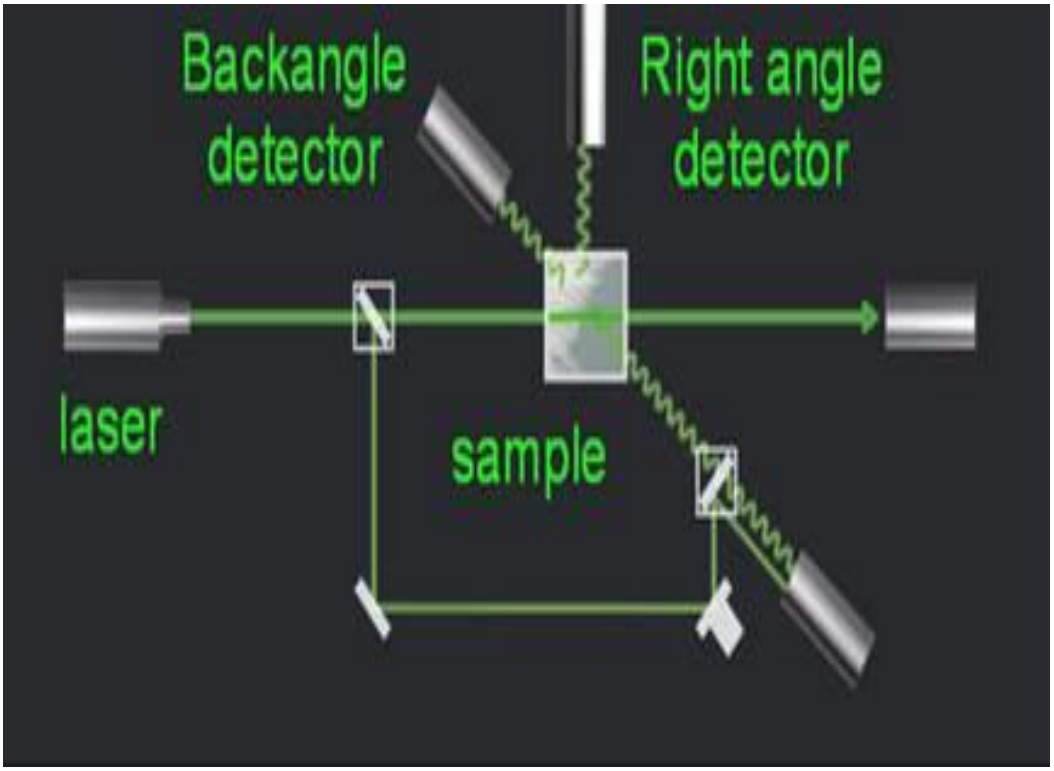

Fig.3.5.5: Schematic representation of Dynamic light scattering

Chapter- 4

Result and discussion

X-ray diffraction:-

Fe_3O_4 nanoparticles were synthesized by co –precipitation method by constant stirring at room temperature The XRD patterns of the Fe_3O_4 nanoparticles and silica-coated Fe_3O_4 nanoparticles are shown in figure 4.1.(a).For the first sample all the six peaks observed at 2θ ~35.65° (311), 62.96° (440), 57.34° (511), 30.35° (220), 43.37° (400), and 53.82° (422) indexed to a pure cubic spinel structure. The pattern is in good agreement with the Joint Committee on Powder Diffraction Standards (JCPDS) card number 01-075-0449.

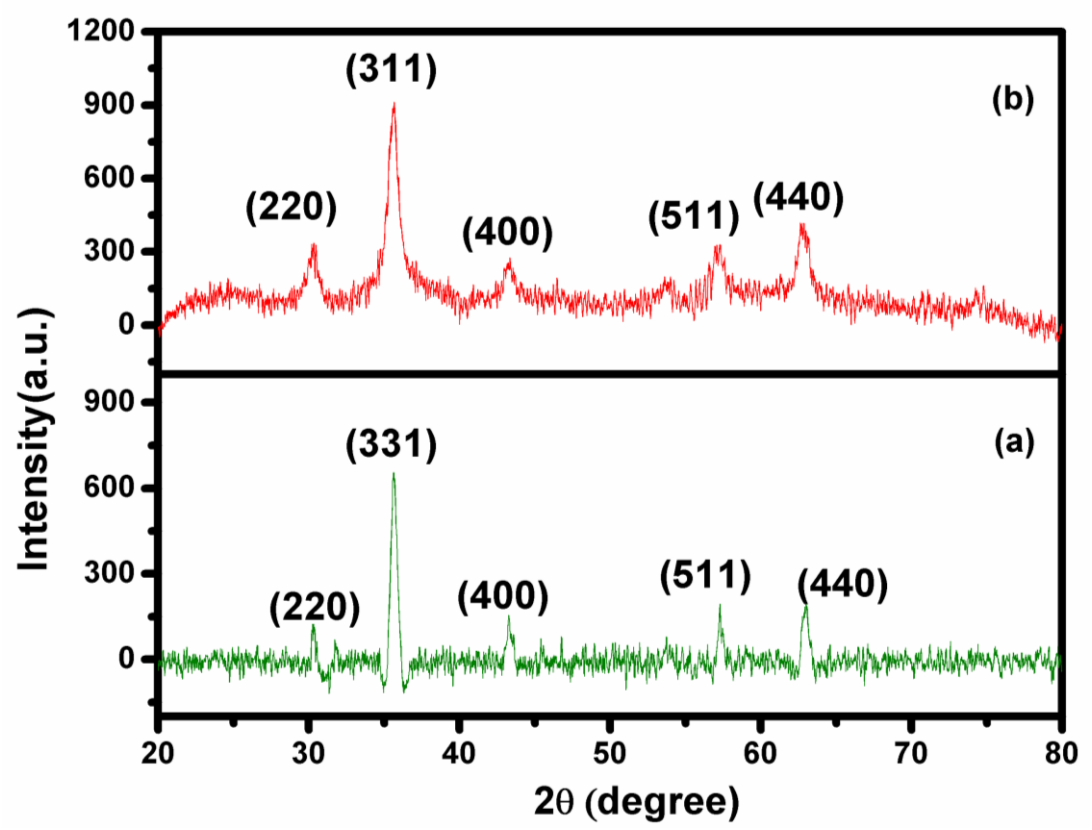

Fig.4.1: XRD patterns of uncapped and silica capped Fe_3O_4 nanoparticles.

For second sample all the peaks in the XRD patterns agree well with Joint Committee on Powder Diffraction Standards (JCPDS) card number 01-088-0315. For this sample all the five peaks observed at 2θ ~35.69° (311), 62.74° (440), 57.07°

(511), 30.30° (220) and 43.20° (400) are due to Fe_3O_4 nanoparticles. The broad hump centered at 23° is due to the presence silica which is amorphous in nature. The average crystallites size (D) of base and silica-coated Fe_3O_4 nanoparticles has been calculated using Debye-Scherrer formula:-

$$D=0.9\,\lambda / \beta \cos\theta$$

Where, β is the full width at half maxima (FWHM) of highest intense peak, λ is characteristics wavelength, and θ is Bragg's angle and the calculated values are represented in table 1.

Table 1: Crystallite size calculated from x-ray diffraction

S.No	Sample specification	Crystallite Size (nm)
1.	Fe_3O_4	8.2
2.	Fe_3O_4@SiO_2	9.4

TGA: - (Thermo gravimetric analysis)

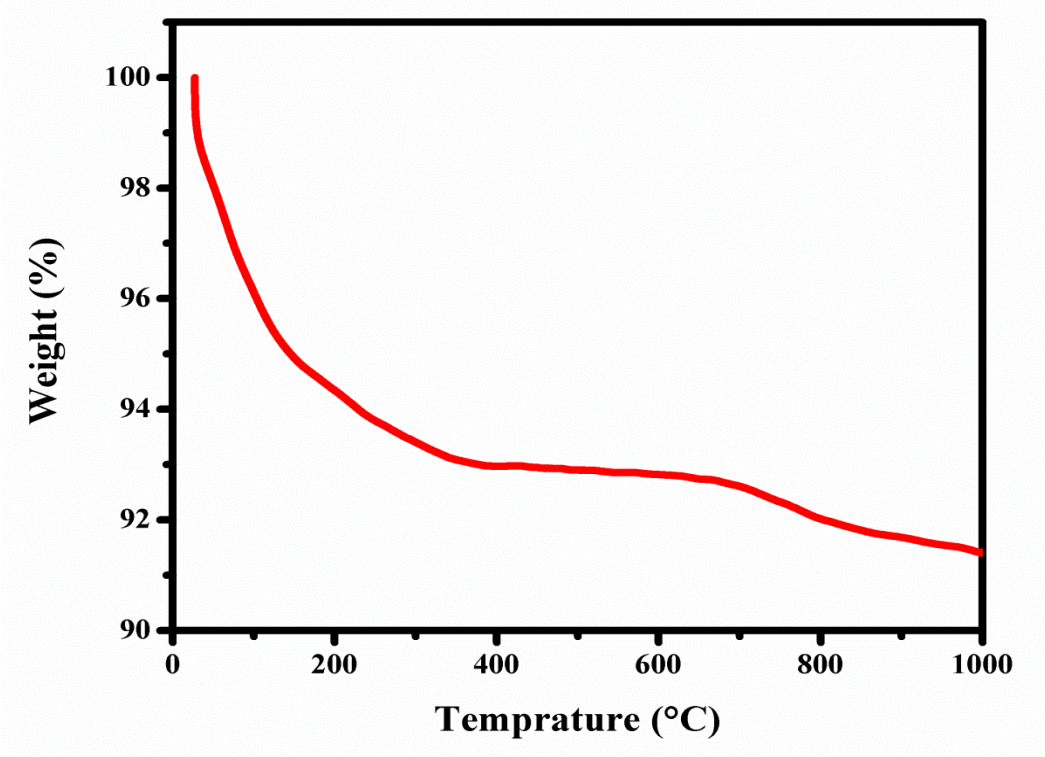

Fig.4.2: TGA analysis of Fe_3O_4 @SiO_2 nanoparticles

It is a suitable characterization technique used to measure the proportion of weight of an individual component in the nanoparticles. TGA of synthesized silica –coated Fe_3O_4 shows weight loss of 8.6% in the 30-400 °c temperature range because of silica coating at the surface of nano- particles and above 400 °c it is almost stable without any significant loss [3].

VSM (Vibrating Sample Magnetometer)**:-**

The magnetic properties of magnetic nanoparticles are measured by vibrating sample magnetometer. Magnetization of Fe_3O_4 nanoparticles is a function of applied filed. The saturation magnetization of Fe_3O_4 nanoparticles is 34.5 emu/g as shown in figure 4.2. It is superparamagnetic in nature.

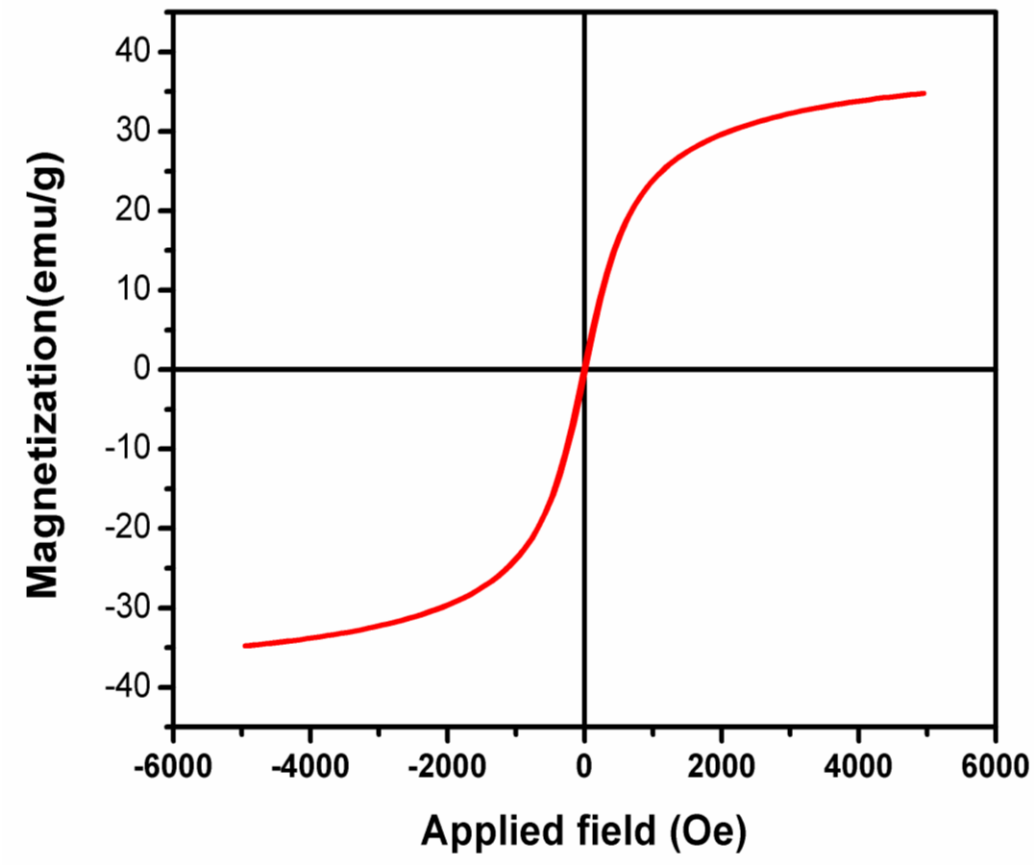

Fig.4.3: VSM of Fe_3O_4 nanoparticles

FTIR study:

FTIR spectroscopy is used to determine the adsorption of inorganic or organic/polymer components on the surface of nanoparticles.

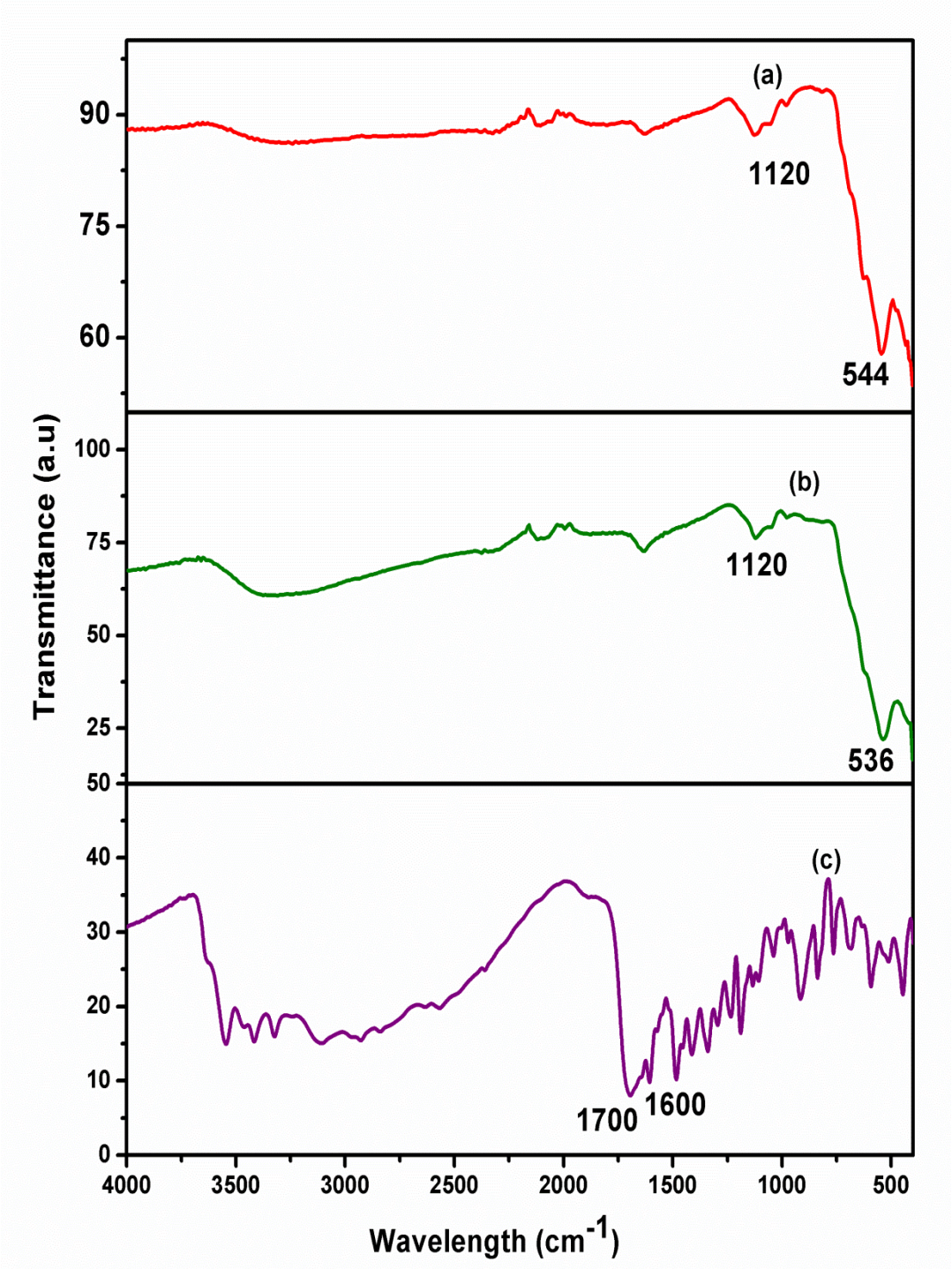

Fig.4.4: FTIR spectra of (a) bare nano particles (b) Fe_3O_4 nanoparticles coated with SiO_2 (c) Folic acid grafted on silica coated Fe_3O_4 nanoparticles

Peak of CMP at 536 cm^{-1} shifts to 544 cm^{-1} in the spectrum of silica-coated magnetic nanoparticles. The FTIR spectra of Fe_3O_4 nanoparticles and the silica –coated Fe_3O_4 particles represented in figure 4.3. Below spectra 4.3(b) identify that the Fe_3O_4 magnetic particles are coated by silica. Compared with these two spectra, peak at 1120 cm^{-1} is a direct evidence of the existence of characteristic Si–O–Si peak from figure 4. 2(a) and (b), we can also see that the characteristic Fe–O–Fe.

DLS (Dynamic scattering light):-

The size distributions of the histogram shows that silica coated Fe_3O_4 nanoparticles have larger particle size (109 nm) than Fe_3O_4 nano particles (12.15 nm) The reason behind the greater average particle size of silica –coated nano particles is the agglomeration of Fe_3O_4 nano particles inside the silica coating [19]. The dipolar attraction of the nanoparticles specific surface area and the high surface energy is reduced due the increase in particle size from 12.15 nm nanoparticles to 109 nm silica coated nanoparticles. The average size of folic acid coated nanoparticles size increased to 195nm and polydispersity remain same with silica coated nanoparticles~0.269 [30].

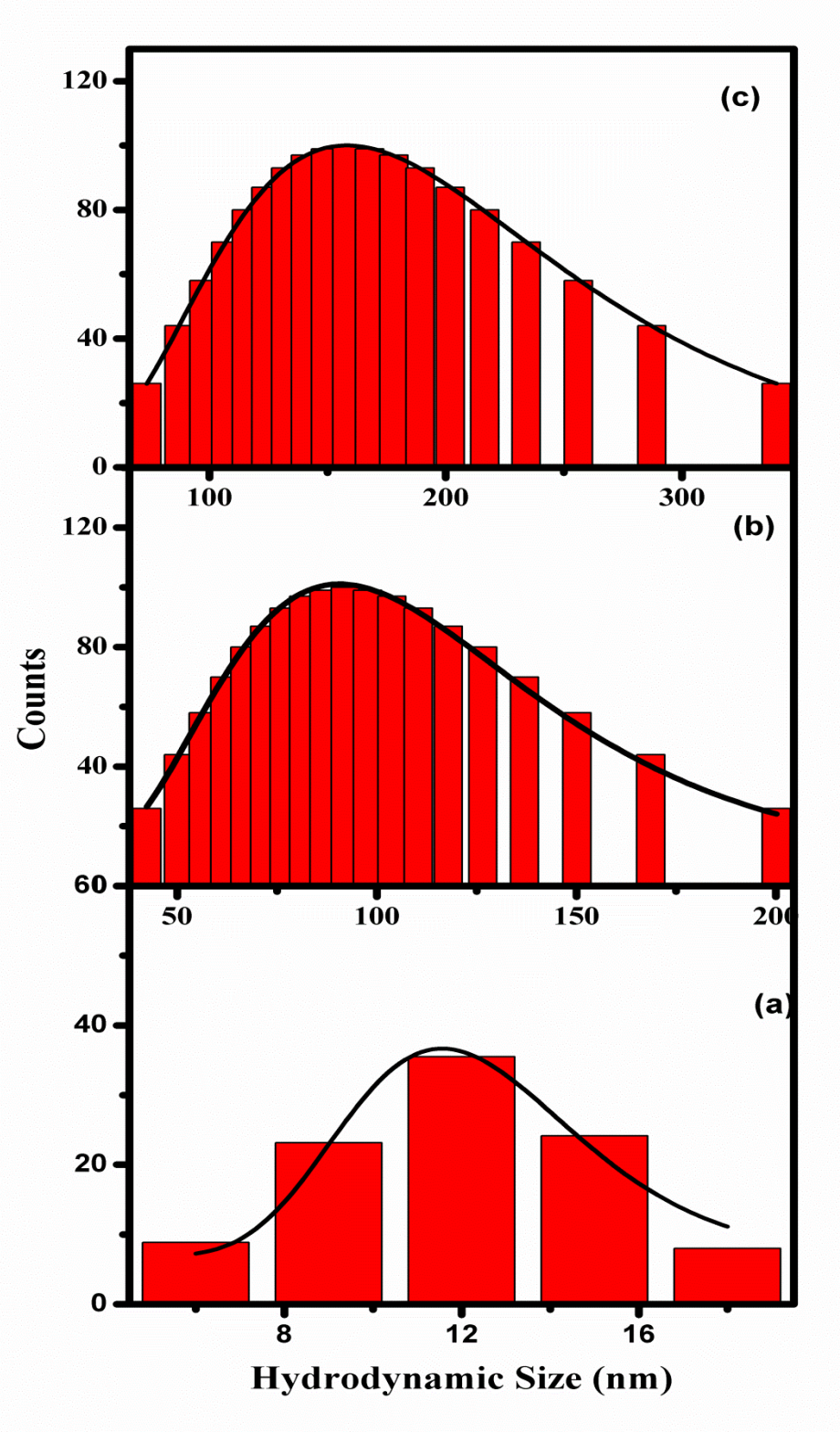

Fig.4.5: DLS of (a) bare nano particles (b)Fe_3O_4 nanoparticles coated with TEOS (c)Folic acid grafted on silica coated nano particles .

Drug content and Drug loading and Release Kinetics:-

The loading capacity of drug linked to core–shell nanoparticles is determined by UV-spectroscopy .Because the presence of magnetic nanoparticles interferes with the spectrum so the loading capacity of mythelenne blue drug is calculated as follows:-

$$Drug - loading\ efficiency\ (\%) = 100 \times (W1 - W2)/W1.$$

$W1$=weight of total drug, $W2$=weight of free drug (which is not attached to nano particles)

The free mythelenne weight (W free Drug) was calculated by Lambert–Beer law:-

$$A = \varepsilon cl,$$

Where A is absorptance, ε is molar absorptivity, λ is wavelength, l is the path length of the quartz cell and c is doxorubicin concentration cell. The value of drug content is given by:-

$$Drug\ content = (weight\ of\ drug\ in\ Nps/total\ weight\ of\ Nps) \times 100$$

To determine the response of drug release and its kinetics the same method for the drug loading capacity is applied. Two diffusion chambers are separated by dialysis membrane with a100 nm porosity and put into the glass beaker then fill it with a drug and nanoparticles. The entire volume of chamber was removed at different intervals of time and then replaced by fresh solution .This process is repeated with different concentrations of drug .To calculate the different amount of drug release optical density of sample is determined.

The experimentally observed and calculated values of drug contents are 6%, 3.9%, 1.1% for different value of drug concentrations. The drug loading efficiency is 100. The drug is continuously released for period of 100 min. Hence the drug release is mainly governed by the diffusion mechanism, while static magnetic field will be useful for targeting the nanostructure to the therapeutic site.

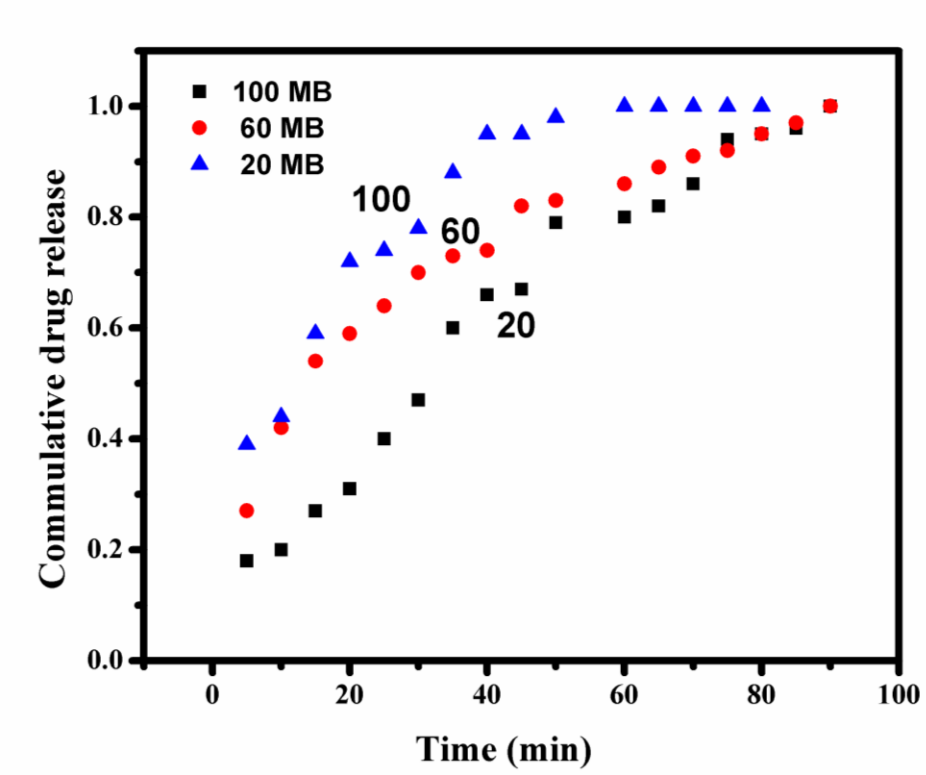

Fig.4.6: Drug release profile of drug @FA@si@Fe_3O_4 for different concentrations of drug

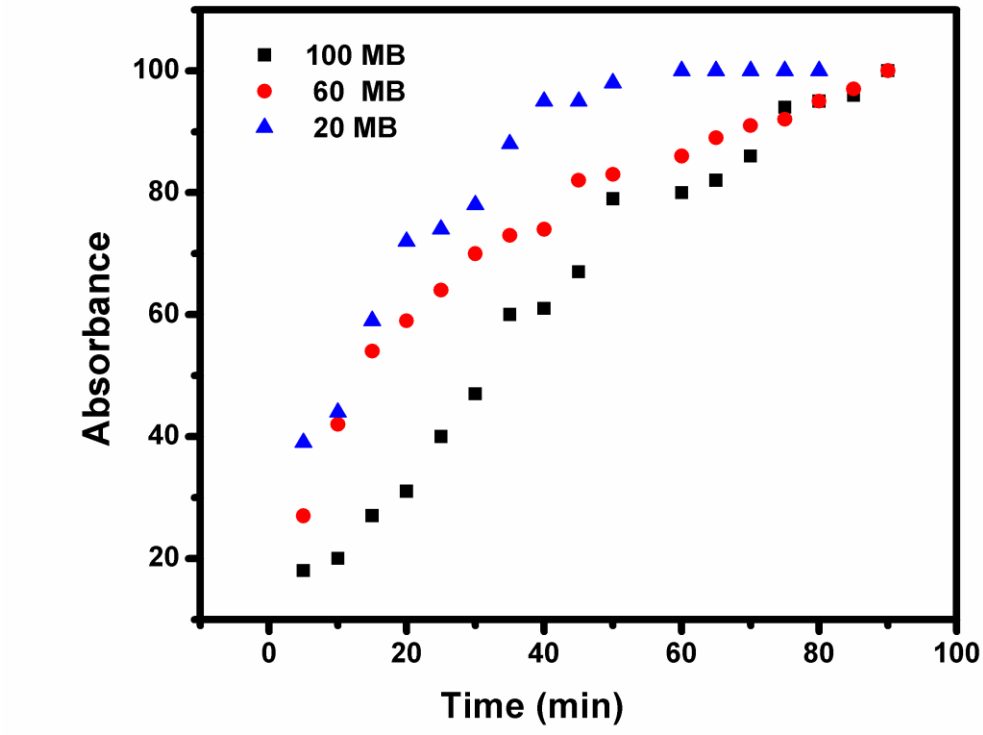

Fig.4.7: Drug loading profile of drug @FA@si@Fe_3O_4 for different concentrations

Conclusion:-

By co-precipitation method Fe_3O_4 Magnetite nanoparticles have been prepared and the silica particles are grafted on magnetic nanoparticles by process of hydrolysis and condensation of (TEOS) tetraethyl orthosilicate. Average size of nanoparticles is 8.4 nm, silica-coated nano particle is 9.2 nm and XRD confirms the spinel structure of Fe_3O_4 nanoparticles. The coating of silica and folic on magnetic nano particles is confirmed by FTIR technique. The magnetization value of Fe_3O_4 magnetic nano particles is 34.5 emu/g. TGA confirms the weight loss of the silica coated nano particles and DLS confirms the size of bare nano particles, silica coated nano particles and FA@si@Fe_3O_4 nanoparticles and also confirms the Coating of folic acid and silica on nanoparticles. UV –Visible spectrum proves the loading of methylene blue on folic and silica coated nanoparticles and the kinetics of drug release.

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