PREFACE

Research related to the development of novel biomedical technologies involving in-vivo use of magnetic nanoparticles present relatively preliminary and multidisciplinary attempts to overcome the major chemotherapeutic drawbacks related to its spatial non-specificity. In the last decade, nanotechnology has developed to such an extent that it has become possible to fabricate, characterize and specially tailor the functional properties of nanoparticles for biomedical applications and diagnostics. Nanosized particles have physical and chemical properties that are characteristic of neither the atom nor their bulk counterparts. Quantum size effects and the large surface area of magnetic nanoparticles dramatically change some of the magnetic properties and exhibit superparamagnetic phenomena and quantum tunneling of magnetization, because each particle can be considered as a single magnetic domain. Based on their unique mesoscopic physical, chemical, thermal and mechanical properties, superparamagnetic nanoparticles offer a high potential for several biomedical applications, such as

- cellular therapy, cell labeling, targeting and as a tool for cell-biology research to separate and purify cell populations
- tissue repair
- therapeutic gene and radionuclide delivery
- radio frequency method for the catabolism of tumors via hyperthermia and
- magnetic drug targeting

In the past few years, considerable interest has been devoted towards the design of new drug delivery systems with the aim of specifically target the drug to a tumor site. Magnetic drug targeting is a viable route to increase the effective use of a drug and minimize undesirable side effects and toxicity. For this application, the drug delivery systems must be superparamagnetic, biocompatible and hydrophilic in nature. Such drug delivery systems should have high drug loading efficiency and controlled drug release profile. Iron oxide nanoparticles offer exciting new opportunities toward developing effective drug delivery systems, as it possesses higher saturation magnetization and has no coersivity & remanant magnetization
below a critical size of 15 nm. Their biocompatibility is already proven. The surfaces of these particles could be modified through the creation of few atomic layers of organic, inorganic or oxide surfaces, suitable for further functionalization by the attachment of various bioactive molecules. Magnetic nanoparticles having suitable surface characteristics have high potential for the use in in-vitro and in-vivo applications.

The aim of the present research is to design magnetic drug delivery systems for non-invasive treatment of cancerous tumors and to study certain aspects of magnetic drug targeting. In this thesis three biocompatible magnetic drug delivery systems are designed by surface functionalizing magnetite core with i) inorganic silica matrix ii) biodegradable thermo-responsive block – copolymer (PLU-PLGA-PLU) and iii) activated folate. The design of each drug delivery system is described in individual chapters along with their physical and chemical properties. Physical simulation study of magnetic drug targeting is also included in this thesis. Second phase of the work include a detailed study on the design, fabrication and antimicrobial activities of metal-magnetic oxide core-shell nanostructures that can effectively neutralize clinically important organisms, which are posing great threat against conventional and narrow target antibiotics.

Fundamental aspects of drug targeting in general, and magnetic drug targeting as a special case as well as problems and recent advances in the development of drug delivery systems (including photodynamic, passive and active drug delivery systems) are appraised in chapter I. In this chapter, some of the recent reviews on application of magnetic fluid in drug delivery system along with their merits and demerits are also included. This chapter concludes with the description of state of art in the development of metal nanoparticle based nonconventional antibiotic systems.

Characterization of the materials synthesized for biomedical applications is a key issue in research. Chapter II provides information about various experimental techniques used thoroughly to characterize magnetic drug delivery systems and their constituents. Each technique is explained along with their principles, instrumentation and method of sample preparation. It includes structural characterization by powder X-ray diffraction (XRD), magnetic properties by DC magnetization measurements, chemical properties by Fourier transform infrared spectroscopy (FTIR), UV-visible
spectroscopy (UV-vis) and inductively coupled plasma atomic emission spectroscopy (ICP-AES), thermal stability by thermogravimetric analysis (TGA), microstructural analysis by optical microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Hydrodynamic properties are determined by Dynamic light scattering (DLS) and viscosity measurements.

The non-invasive methods used for the treatment of cancerous tumors have received considerable attention includes photodynamic therapy (PDT) and controlled delivery of therapeutic agents at the tumor site by means of active and passive drug targeting. Photodynamic therapy is based on the concept that photosensitizer drug molecules are chemically conjugated with superparamagnetic nanoparticles and targeted to the tumor site by means of external magnetic field. At the tumor site, the photosensitizer molecule generates reactive oxygen species upon irradiation, which can irreversibly damage the tumor tissues. The scope of chapter III is to explain design, construction and characterization of a magnetic drug delivery system, which can be utilized for photodynamic therapy.

Out of large family of superparamagnetic materials, magnetite is selected as core because

- It is the only biocompatible magnetic nanoparticles, which is approved for *in-vivo* use by US federal agency of food & drugs (FDA)
- It has high magnetization and magnetic susceptibility for an effective magnetic drug targeting
- It can be synthesized in variety of shapes, size & in bulk quantities, and
- can be produced with particle size ranging from few nanometers to tens of nanometer.

However, bare magnetite cannot be used in drug delivery systems, as they have large surface to volume ratio, which leads them to form aggregates. The agglomeration will increase the probability of plasma adsorption and hence they are quickly cleared from the blood stream by the macrophages of mononuclear phagocyte system before they can reach to the target site.
To overcome these problems, magnetite nanoparticles produced via chemical coprecipitation technique are surface functionalized with inorganic silica. There are several advantages of silica as the stabilizer. Unlike polymers, it is not subject to microbial attack and it neither swells nor changes porosity in response to the environmental pH. Silica is chemically inert and therefore, dose not affects the redox reaction at the core surface. The nonmagnetic shell can suppress magnetic dipolar interaction and prevent the agglomeration of magnetic nanoparticles. Silica shell acts as a stabilizer and limiting the effect of outside environment on the core particles. These properties of silica-coating also increase the circulation time of magnetic nanoparticles in the blood stream, which is a crucial factor for drug targeting.

In this work, surface functionalization of magnetite nanoparticles with silica shell is achieved by hydrolysis and condensation of tetraethyl orthosilicate (TEOS). Concentration of TEOS is optimized to avoid the formation of free silica during the encapsulation of magnetite nanoparticles inside the silica cage. A photosensitizing drug ‘methylene blue’ is entrapped in the growing silica cage by demethylation of methylene blue in high pH medium of ethanol-water. Kinematical conditions have been established for tunable loading of photosensitizer in nanospheres and nanocapsules of Fe$_3$O$_4$-SiO$_2$ core-shell nanostructures.

Phase of magnetite and its surface functionalization with silica is confirmed by XRD measurements. Superparamagnetic nature of silica encapsulated magnetite nanoparticles is also confirmed by magnetization measurement at 300 K on indigenously built vibrating sample magnetometer. To study the nature of binding of silica with magnetite an FTIR spectrum is recorded in the mid-IR region of the electromagnetic spectrum. The absorption peaks corresponding to the stretching vibrations of Fe-O-Si and Si-O-Si approves the chemical binding of Si with magnetite and formation of the silica shell, respectively. Entrapment of methylene blue in silica matrix is confirmed from the analysis of UV-visible spectrum of drug delivery system. Optical microscopy, SEM and TEM techniques are used to study the surface morphology of silica encapsulated magnetite nanoparticles.

Controlled drug delivery by means of magnetic field holds immense potential to improve the treatment of cancer. One approach is to package the drug in polymer-coated magnetic nanoparticles and target them to tumor site by means of external
magnetic field. Chapter IV deals with the design and relevant characterization of biocompatible, polymeric magnetic drug delivery system loaded with an anticancer therapeutic drug ‘doxorubicin’. It is very important that polymer used in this system should be biodegradable and can be eliminated by the physiological system without leaving any residues. In addition, the use of polymeric nanoparticles in-vivo requires them to be hydrophilic with a pH close to physiological pH (~7). Magnetic drug targeting is accomplished by conjugating the drug to a polymer matrix and encapsulating superparamagnetic nanoparticles inside it, thereby allowing the drug to be released in a pre-designed manner.

Magnetite nanoparticles are produced by chemical coprecipitation method and coated with a monolayer of oleic acid to avoid their agglomeration due to magnetic dipolar interaction. Surfactant coating renders the surface of nanoparticles hydrophobic. As mentioned previously, for biological applications nanoparticles must be hydrophilic in nature. To make them hydrophilic, a facile phase transfer mechanism is developed by using biocompatible block copolymer Pluronic F-127. Pluronic functionalized nanoparticles are encapsulated in a rapidly biodegradable matrix of PLGA (poly lactide-co-glycolide) along with the anticancer drug doxorubicin. The thermoresponsive nature of the drug delivery system is attributed to the formation of block-copolymer PLU-PLGA-PLU.

Structural and magnetic properties of designed drug delivery system are obtained by XRD and VSM measurements. FTIR spectroscopy is an appropriate technique to understand chemical adsorption or functionalization of nanoparticles with polymers. To understand this, FTIR spectrum of composite nanostructures is recorded. To determine the amount of various coatings mass loss as a function of temperature is recorded on TGA. The observed losses are in agreement with the calculated weight proportions. Encapsulation of doxorubicin in the PLGA matrix is confirmed by UV-visible spectroscopy. Dynamic light scattering is employed to determine the physical size of the drug delivery system and their thermoresponsive nature. Drug loading efficiency and drug release profile is also determined using UV-visible spectroscopy.

Active targeted delivery of anticancer drugs is a promising approach towards the development of new drug carriers. Intracellular uptake of nanoparticles into cells
can be accomplished by liquid phase endocytosis, receptor-mediated endocytosis or phagocytosis. A logical method to promote cellular internalization of nanoparticles is to modify their surface with a ligand such as folic acid, which can be efficiently taken up by the cells through receptor-mediated endocytosis. Folate receptors are over expressed on the surface of cancerous tumors and rarely expressed on healthy normal cells. Chapter V describes design of a magnetic drug delivery system whose function is based on receptor-mediated endocytosis. In the previous two drug delivery systems, magnetite nanoparticles are produced by chemical coprecipitation route, which generally yields polydispersed nanoparticles with a polydispersity index of 0.2-0.5. Saturation magnetization, magnetic susceptibility and magnetic anisotropy are strongly depends on size and size distribution of nanoparticles. Therefore, to improve drug-targeting efficiency it is desirable to have monodispersed particles.

Monodispersed magnetite nanoparticles are produced by thermal decomposition of organometallic complex of iron. In order to increase their blood circulation time and to make them hydrophilic, a low molecular weight, rapidly biodegradable protein polyethylene glycol (PEG 600) is coated on the surface of magnetite. Use of short chain length PEG, does not increase much overall size of the magnetite nanoparticle conjugates and helps in delaying their recognition by the mononuclear phagocyte system. Activated folate is chemically tagged on PEG functionalized magnetite nanoparticles. The anticancer drug doxorubicin is loaded into the folic acid modified PEG coated magnetite nanostructures.

Spinel phase of magnetite nanoparticles is verified through XRD measurements. Magnetization measurement confirms that magnetite nanoparticles are superparamagnetic and have a moderately high saturation magnetization (80 emu/g). Surface functionalization of nanoparticles is confirmed by FTIR and conjugation of doxorubicin is confirmed through UV-VIS spectroscopy. Hydrodynamic size is obtained by DLS measurements. Drug loading efficiency and drug release profile is also determined by using UV-VIS spectroscopy.

Ferrofluid offers certain attractive application potentiality in biomedicine and biotechnology. Cell separation and targeted drug delivery are two of the most prominent applications of ferrofluid. The former is already commercially exploited while the later one is still require extensive investigations. In these applications,
transport property of the ferrofluid under the influence of an external uniform or gradient magnetic field plays a vital role. For example, nanomagnetic particles in the biocompatible ferrofluid tagged with anticancer drug are injected intravenously or directly to a specific site in a targeted drug delivery system. In the former case, the ferrofluid has to flow along with the blood flow.

Rheological properties of blood have been well reported by several authors. In all these studies, effect of external magnetic field on the viscosity is not taken into the account. Shliomis has shown that Rosensweig’s quasi-stationary model for ferrohydrodynamics is not valid when external magnetic field effect on viscosity is taken into the account. When nanomagnetic particles attached with drug molecule are injected in human artery system, the flow behavior of these particles will be quite complex. Thus, geometry of the magnetic field is important when designing a magnetic drug targeting experiment. In chapter VI systematic investigation of the field induced hydrodynamics of water and kerosene based ferrofluid in capillary vessels by studying it’s in-field viscosity as a function of capillary diameter (0.5-2.0 mm) and angle (0-90°) between the vorticity of flow and the direction of external magnetic field is carried out. Magnetophoretic mobility is also determined, which is an important parameter for designing magnetic drug targeting system.

Development of new strains of microorganisms that are resistant to current antibiotics poses a biggest challenge in public health care. There is an urgent and inevitable need for the development of an alternative technology, which can overcome the drawbacks associated with narrow target antibiotics. Recent research on antimicrobial properties of metal nanoparticles (especially silver nanoparticles) shows promising results. However, environmental and clinical toxicity of these potentially hazardous nanostructures is a big concern and needs an immediate attention.

Chapter VII of this thesis explains fabrication of silver and magnetite-silver nanostructures. It also includes relevant characterization of these multifunctional nanoparticles. The antibacterial and antifungal action of silver and magnetic silver nanoparticles have been evaluated against clinically important strains. A mechanism has been devised for successful recycling of core-shell nanostructures by means of external magnetic field. The process can be used to prevent the accumulation of these
toxic nanostructures in environment and thus provides a clean alternative for their use at industrial scale.

The thesis concludes with chapter VIII, summarizing the present investigation along with some of the future perspectives in this lucrative and challenging field of science and technology.