CHAPTER 8

SUMMARY AND SCOPE FOR FUTURE WORK

8.1 Summary

Present work explains design and synthesis modalities of nanoparticle based drug or drug delivery systems, which can either be effectively used to target cancerous tumors or pathologically important microorganisms. First phase of thesis deals with magnetic drug delivery systems designed to irradiate cancerous tumors without significant damage to healthy cells. In the present work, three different approaches have been adopted to create novel drug delivery systems, which will have an edge over the conventional drug delivery systems produced / researched so far.

In the first approach, magnetically guided drug delivery system has been designed, which can guide a photosensitizer drug to a desired pathogenic sight in the body. The composite system is made up of three distinct parts namely, magnetic core, protective shell and a photosensitizer drug. The prime advantage of the system is each constituent is highly biodegradable and well suited with the body fluid for \textit{in-vivo} applications. Magnetite nanoparticles having average particle size of 10 nm and polydispersity index of 0.3 have been produced under mild reaction conditions at room temperature by following the well-established chemical coprecipitation method. Inorganic silica is chosen as protective shell because of the flexibility that is available in working with these porous structures. Synthesis protocols have been established to tailor the morphology of these core-shell structures in the form of spheres and capsules. A photosensitizer drug “methylene blue”, which has highest efficiency of singlet oxygen ($^{1}\text{O}_2$) generation, is loaded in a tunable fashion in the pores of these core-shell nanostructures.

Various physical and chemical characterization tools are used to confirm the formation of core-shell structure and their drug loading efficiency. High magnetic strength of the designed drug delivery system provide them an edge over other similar drug delivery systems as they all suffer from poor magnetic strength. Unique advantage with the present synthesis method is it can completely suppress the
formation of free silica nanoparticles during the hydrolysis and condensation of organosilicates.

The second approach is based on the special property of cancerous tumors called “enhanced retention and permeability effect (EPR)”. Majority of the commercially available drug delivery systems for cancer therapy are based on this concept. However, the concept has limited success partially because of the lack of the specificity and partly due to the absence of control mechanism over the release of the drug. To overcome these difficulties, in our approach, a multifunctional magnetic polymeric nanoparticle based drug delivery system has been designed and developed. The ultrafine size of multifunctional nanoparticles makes them highly susceptible to EPR effect. The ability of magnetic guidance and thermally triggered drug release mechanism provides them unique characteristics of controlled guidance to the pathological site and drug release in a predefined programmed manner.

Ultrafine magnetite nanoparticles (10 nm) are produced by chemical coprecipitation. These nanoparticles are prone to form aggregates because of their high surface activity. To overcome this problem, a short chain length molecule (oleic acid) is chemically adsorbed on the surface of nanoparticles, which prevents their agglomeration due to the steric repulsion. However, oleic acid coating makes them hydrophobic. For biomedical applications, drug carrier needs to be hydrophilic. Hence, to make them water dispersible, pluronic F-127 is physically anchored at the tail of oleic acid molecules by hydrophobic-hydrophobic interaction. In order to enhance the loading efficiency of the nanostructures and make them thermoresponsive, block-co-polymer (PEO-PLGA-PEO) is produced by emulsion-precipitation method. Antineoplastic drug doxorubicin is also grafted simultaneously into these polymeric nanostructures.

Designed nanostructures are thoroughly investigated by various physical and chemical characterization techniques after each stage of preparation to optimize their properties, which broadly affect their targeting efficacy. In-vitro experiments are also performed to determine drug loading efficacy and kinetics of drug release. Burst release for the initial few hours followed by slow and control release over a prolonged period are the most lustrous property of this drug delivery system. Thermoresponsive
nature of nanocarriers along with magnetic guidance and susceptibility towards EPR effect make them unique magnetically guided thermo-responsive drug delivery system.

Third approach is based on folate-mediated endocytosis. Most of the cancer tumors over express folate receptors, which are minimally present on healthy tissues. We have designed an active targeted drug delivery system, which can effectively target these folate receptors. Ultra-small size of drug delivery system and ability of magnetic guidance provides additional advantages over the conventional FR based drug-targeting systems.

Monodisperse ultra-small (7-8 nm) magnetite nanoparticles with high saturation magnetization (80 emu/g) constitutes the core of the functional drug delivery system. To overcome the opsonization i.e. to enhance the blood circulation time of drug delivery system, these ferrite cores are further coated with a fine layer of low molecular weight polyethylene glycol (PEG). Folic acid, a small molecule vitamin can effectively been taken up by folate receptors. Hence, it is chemically bound on the surface of PEG coated magnetite nanoparticles. Low activity of carboxylic acid group of folic acid made it difficult to conjugate them with PEG. To overcome this difficulty, a dark reaction condition, which produces activated folate that can easily bind with the PEG molecule has been established. Antineoplastic drug doxorubicin is grated into the pores of the composite nanoparticles. Physical and chemical characterization tools like XRD, TGA, TEM, VSM, FTIR, Uv-visible spectroscopy, etc. are used to characterize the drug delivery system. An in-vitro drug release study has also been exercised to understand the kinetics of release mechanism from the composite nanoparticles. Slow and controlled drug release, ability to be endocytosed by means of folate receptors, high loading efficiency and strong magnetic properties make this drug delivery system, a potential candidate for active targeting of various cancerous tumors.

Magnetic drug targeting is a complex technique. The success not only depends on the design of the drug delivery system but also on how it is delivered to the pathogenic site. Various physical parameters got involved in successful targeting of the tumor. Therefore, it is always advantageous to have an in-vitro physical simulation data of a particular parameter. One such parameter is the magnetophoretic mobility of the magnetic drug delivery system. In the last phase of the thesis, effect of
magnetic field and direction of flow on the magnetophoretic mobility has been evaluated by studying in-field rheological properties of model and biocompatible magnetic fluids.

Highly stable magnetite based model (in kerosene) and biocompatible (in water) magnetic fluids are produced. Structural properties of magnetite nanoparticles are obtained from powder XRD measurements and magnetic properties are evaluated on VSM. In-field viscosity measurements of both the fluids are carried out on capillary viscometer. The effect of direction of the magnetic field and diameter of the capillary on the rheological properties has been investigated. Magnetophoretic mobility of the system has been derived for these conditions. The obtained results are in good agreement with the Shliomis theory of ferrohydrodynamics. Hence, it is concluded that the theory can be used in predicting rheological behavior of magnetic fluids under external magnetic field and could provide useful insight in designing a magnetic field assisted drug delivery experiments.

Development of multidrug resistant strains of microbes to current antibiotics is a serious problem in public healthcare. An immediate attention is required to tackle the challenges laid by this new crop of notorious pathogens. Silver is known for its antimicrobial properties since ancient times. Silver in nano form can further enhance its activity against microbes. Hence, in the recent time market related to the products of personal hygiene are flooded with silver nanoparticle based products. However, recent study on toxicity of silver has red signaled their wide spread use in medical products. Unavailability of technologies for the safe disposal or recycling of these potentially hazardous nanostructures may further decline their importance.

In the second phase of the thesis, an alternative approach is devised to fight against microorganisms with highly reactive monodisperse magnetic-silver core-shell nanostructures. These nanoparticles can be safely recovered from the medium and could be recycled by means of magnetic field. Magnetite nanoparticles are produced by the thermal decomposition of organometallic complex of iron. A shell of silver is formed over it by thermal reduction of silver nitrate with oleylamine. A facile phase transfer mechanism has also been developed to convert these hydrophobic nanoparticles into hydrophilic ones. Structural characterization is carried out by powder XRD and transmission electron microscopy measurements. Single surface
plasmon resonance (SPR) peak in the UV-visible spectrum of core-shell nanostructures confirms the formation of silver shell. The quantitative analysis of core-shell nanostructures is done through inductively coupled plasma atomic emission spectroscopy. Magnetic properties of the composite nanoparticles are also investigated through VSM measurements. Antibacterial activity of silver nanoparticles and magnetic-silver core-shell nanostructures are investigated against four sets of bacterial and one set of fungal strains. The minimum inhibitory concentrations (MICs) are in the range of 100 – 350 µm/mL and 2000 µm/mL for bacterial and fungal strains, respectively. Recycling of core-shell nanostructures with the help of magnetic field is demonstrated successfully. A qualitative explanation of antibacterial action of silver nanoparticles on microbes is also described. The designed nanostructures have higher antibacterial efficiency than any conventionally used antibiotics. The recycling efficiency of the nanostructures makes them more economical and environmental friendly system, which can tackle the challenges laid down by the nanotechnology.

8.2 Scope for Future Work

Magnetic drug delivery systems will emerge as an important weapon to fight against cancer. The efficiency of singlet oxygen generation and its decay kinetics are two of the most important parameters for any PDT system. Hence, evaluation of these parameters for the designed drug delivery system in chapter 3 and their in-vitro and in-vivo toxicity would be an assignment of very high importance. An attempt should be made to load multiple chemotherapeutic drugs simultaneously in a drug delivery system to enhance the mortality of these systems. Especially syntheses conditions need to be developed to load recently developed chemotherapeutic drugs, which are hydrophobic in nature. Evaluation of In-vitro and in-vivo clinical toxicity of the passive and active drug delivery systems explained in chapter 4 and 5 should be evaluated against clinically important cancers. Results of such studies will provide important clues for further enhancement in the efficiency of these drug delivery systems.

Physical simulation is a very important tool in designing practical drug delivery systems. Magnetic drag force required for the controlled movement of the nanoparticle based drug delivery system is an important aspect of the drug targeting. The drag force required for the transportation not only depends on physical and
magnetic properties of nanostructures but it also depends on direction of viscous drag force. Therefore, it is advantageous to have a dedicated study to verify the existing theories on magnetohydrodynamics so that appropriate mathematical model can be developed, which can provide critical information in designing drug delivery experiments. Another important aspect, which can be studied through physical simulation, is the mechanism of drug release. There are various mechanisms, which can be used to control the release behavior of drugs from the magnetic nanoparticle based drug delivery systems. Few important parameters whose effect on drug release would be studied by physical simulation are pH, temperature and ac magnetic field. This assignment would provide an important piece of information, which might play a crucial role in designing a successful in-vivo drug delivery experiment.

Enhanced antibacterial activity of metal nanoparticles in general and silver nanoparticle in special made them attractive candidate, which can replace narrow target antibiotics. However, clinical toxicity of these nanostructures and their impact on environment is already a deep matter of concern amongst clinicians and environmentalist. Therefore, a complete study devoted towards the environmental effect of metal nanoparticles especially on environmental friendly microbes will provide critical information about the fate of this future medicine. The mode of interaction of metal nanoparticles with microbes is still an open subject. Different research groups have proposed various mechanisms of antimicrobial action of nanoparticles; however, none of them can give the satisfactory explanation of the observed facts. Therefore, a dedicated investigation on interaction of nanoparticles with microbes will be a worthy assignment. Development of strains of microbes that are resistant to metal nanoparticles has so far not been reported and most of theresearches believe that it is highly unlikely to have microorganism will develop resistance against these broad targeting metal nanoparticles. However, this belief needs to be tested at industrial scale for the practical implementation of these nonconventional medicines.