1
Introduction

1.1 INTRODUCTION

Design and synthesis of potent Drug Delivery Systems (DDSs) are extremely important in pharmaceutical applications. Biodegradable and biocompatible drug carriers with targeted and stimulus-responsive properties have been developed in last few decades through innovations in material chemistry [Allen, 2004; Zhang et al., 2013]. Various drug carriers like nanostructures, including polymers [Jain, 2000], dendrimers, silica or carbon based nanoparticles [Skorupska et al., 2014] and liposomes [Almeida and Souto, 2007] have been investigated as drug delivery systems. Consequently it has become important to analyze drug carriers in terms of their (a) structure (b) interaction with drug molecules (c) drug loading capacity (d) functionality and (e) drug release kinetics. Over the last few decades the literature has majorly focused on the aforementioned issues while designing and characterizing DDS. On the contrary a very few attempts have been made to analyze physical and chemical behavior of a drug molecule in presence of such drug carriers. It is worth mentioning that dynamics and molecular interactions of the drugs that determine the relevant biological activities can be altered in presence of drug carriers. The present thesis therefore aims to highlight the molecular dynamics and interactions of a drug molecule within a drug carrier; together representing the Drug Delivery System (DDS).

According to the definition of World Health Organization (WHO), a drug is any substance or product that is used to modify or explore physiological systems or pathological states for the benefit of the recipient. In the context of medicine, a chemical used for the prevention, diagnosis or treatment of diseases is called drug. The conventional routes for drug administrations are oral, injection and transdermal based delivery. These types of approaches have several limitations like repeated administration resulting fluctuating concentration of the drug in the patient that leads to unfavorable condition and adverse side effects to the patient. To combat such situation development of drug formulation has gained recent attention in the pharmaceutical industries. Accordingly designing of DDS has emerged as an important field of research. DDS are composed of a drug associated with a suitable drug carrier which releases the drug at a specific rate and at a specific site. Over past few decades, newer approaches have been developed for the targeted delivery and controlled release of the drugs through matrix entrapment [Schwardt et al., 2010]. The major aim of drug delivery and formulation is to maintain the desired therapeutic concentration at the specific site with minimal adverse effects along with masking of bitter taste and unpleasant odor of a drug, enhancement of bioavailability, prevention of drug degradation and increment of uptake at the diseased site. Figure 1.1 describes the desirable properties of an efficient drug carrier while in the subsequent sections various drug carriers are briefly highlighted.

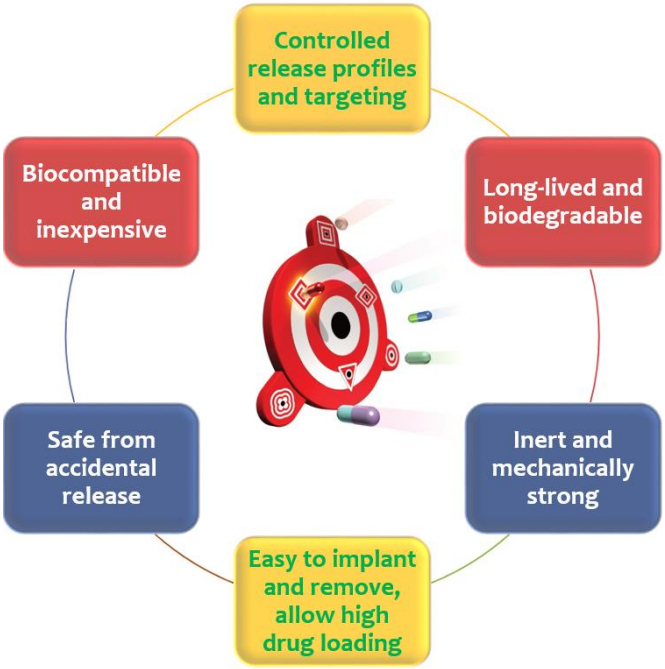

Figure 1.1 : Desirable properties of an efficient drug carrier.

1.2 CARRIER BASED DDS$_S$: CATEGORIES AND USAGES

A wide variety of carrier based DDSs have been developed and studied in last few decades having unique advantages and disadvantages. The most popular DDS that have been discussed in recent past are liposomes, monoclonal antibodies, microspheres and nanoparticles [Bhagwat, 2013; Dutta, 2007; Allen, 2014] as depicted in Figure 1.2.

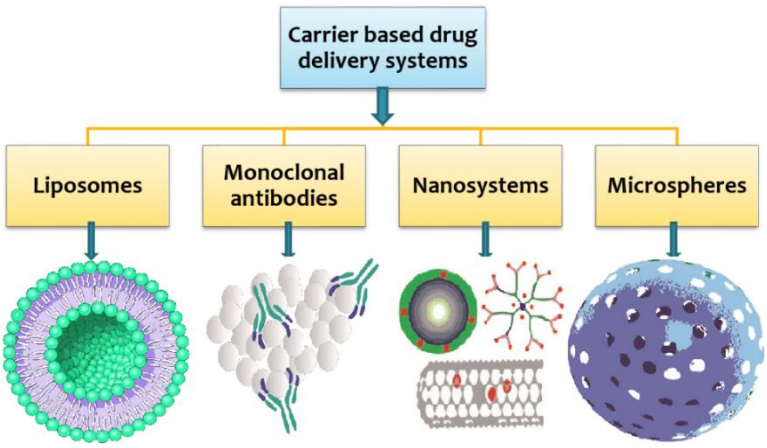

Figure 1.2 : Types of carrier based drug delivery systems

Among the various newly discovered drug carriers, nanosystems (supramolecular and nanoparticle based DDS) and polymeric microsphere based DDS have gained considerable attention due to their higher biocompatibility, endurance and biodegradation properties with minimal or no side effects. In the forthcoming sections very brief introduction of

supramolecular, polymeric and nanoparticle based DDSs have been provided. Liposomes and monoclonal antibody based DDS are beyond the scope of the present thesis and will not be discussed further.

1.2.1 Supramolecular DDS

Supramolecular chemistry is one of the most flourishing field of research. The formation of "host-guest" complexes was a breakthrough development in this field where a host selectively binds to a guest. Supramolecular host *viz.* cyclodextrin (CD), cucurbituril (CB), calixarenes, and pillararenes are the most popular excipients to carry various hydrophobic drugs and proteins [Miyauchi et al., 2005]. Formation of the inclusion complex with these host candidates enhance the solubility and stability as well as the permeability through biological membranes of hydrophobic drugs [Uekama et al., 1998]. Further due to their high biocompatibility these supramolecules are considered as GRAS (Generally Recognized As Safe) drug carriers by Food and Drug Administration (FDA) [Kurkov and Loftsson, 2013].

Among the different studied hosts, cyclodextrins (CDs) are most promising and most explored macrocycles [Uekama et al., 1998; Kurkov and Loftsson, 2013; Zhang and Ma, 2013]. Cyclodextrin based drugs can be administered by oral, transdermal, nasal and rectal delivery [Hirayama and Uekama, 1999; Loftsson and Brewster 2011; Matsuda and Arima, 1999; Loftsson, and Jarvinen, 1999]. The main advantages of cyclodextrins based drug carriers are (i) high bioavailability and biocompatibility (ii) low toxicity (iii) available in variable cavity sizes (iv) prevention of drugs from biodegradation [Szejtli, 2004; Anjana et al., 2013]. Besides these advantages the inexpensive synthesis process at large scale makes them an appealing candidate among other supramolecules namely cucurbutril, calixarene and pillararenes [Webber and Langer, 2017].

1.2.2 Polymeric DDS

Polymers have been used in pharmaceutical industries for the site specific delivery of drugs over an extended period of time with improved patient compliance [Pillai and Panchagnula 2001; Liechty, 2010; Srivastava et al., 2016]. The major polymeric candidates used as drug carrier are broadly categorized as hydrogels, micelles, polyplexes, dendrimers, cellulose based and polyester-based polymers. [Brocchini and Duncan, 1999; Liechty et al., 2010; Srivastava et al., 2016].

Hydrogels are composed of the hydrophilic polymers and used as the carrier of biological fluids and proteins [Peppas et al., 2000; Kamei et al., 2009]. Polyethyleneimine (PEI) and DNA based polyplexes are utilized in the gene delivery [Gosselin et al., 2001] while polymeric micelles are also explored as an efficient drug carrier [Ruel-Gariépy and Leroux, 2004]. Dendrimers have been utilized as the carriers for anticancer drugs and genes [Liechty et al., 2010; Madaan et al., 2014]. Cellulose based polymers particularly polyethylene glycol (PEG) has been reported as the carrier of various therapeutics *viz.* asparaginase, interferon and adenosine deaminase [Duncan, 2006; Pasut and Veronese, 2007; Heredia and Maynard, 2007].

Among these several possibilities polyester based polymers *viz.* poly-lactic acid (PLA), poly-glycolic acid (PGA) and their copolymer poly-lactic-co-glycolic acid (PLGA) are the most widely used polymeric candidates in DDSs [Griffith, 2000; Nagarwal et al., 2011]. More specifically PLGA that was discovered as surgical sutures in the 1960's is the most investigated drug carrier among all the polymeric candidates [Makadia and Siegel, 2011]. It has been approved by the FDA and European Medicine Agency (EMA) to be used as potent drug carrier due to the incredible biocompatibility, tunable structure, availability of different molecular weights, ease of functionalization and sustained release profile [Taluja et al., 2007; Lu et al., 2009; Roointan et al., 2018].

1.2.3 Nanoparticle based DDS

Nanoparticles (NPs) based DDS are considered as colloidal drug carriers that impart improved pharmacokinetic and pharmacodynamic properties to the drug. Nanomedicines have significantly benefited by the unique properties of these NPs *viz.,* large surface to volume ratio, modifiable shape and size, enhanced bioavailability by increasing the aqueous solubility of drug, increased resistance time, site specific delivery, ability to cross cell and tissue barriers, reducing the overall amount of drug used, fewer side effects and reduced number of repeated administration. As a consequence several research groups besides developing synthetic methods and characterization techniques have also shown interest in analyzing these nano-delivery systems with respect to drug loading efficiency, release mechanism, interaction with biomolecules and cytotoxicity [Orfi, 2016; Bouttefeux et al., 2016; Muhammad et al., 2011; Tan et al., 2014; Ramezanpour et al., 2016]. Various nanoparticles *viz.* carbon nanotubes, gold nanoparticles, metal oxides nanoparticles and quantum dots have been investigated in past few years. Carbon nanotubes (CNTs) were used in the targeted delivery of various anticancer drugs including paclitaxel, cisplatin and doxorubicin [Lay *et al.,* 2011; Chen et al., 2017]. Gold nanoparticles (Au NPs) have investigated as a potent drug delivery and medical imaging agent due to their special optical, electronic and sensing properties [Ghosh et al., 2008; Kong et al., 2017]. Quantum dots were used in cellular imaging and drug delivery due to their fluorescent semiconducting nature [Probst et al., 2013]. Metal oxides nanoparticles *viz.* SiO_2, Fe_2O_3, TiO_2 and ZnO have been studied as efficient carrier for several drugs in recent years [Xu et al., 2006; Slowing et al., 2008; Wahajuddin and Arora, 2012; Watermann and Brieger, 2017].

In the present thesis we have chosen cyclodextrin based supramolecular nanospheres, PLGA based microspheres and ZnO nanomaterial based drug carriers to analyze the molecular interaction and related drug behavior within these cavities.

1.3 STRUCTURE, DYNAMICS AND MOLECULAR INTERACTION OF DDS: QUALITATIVE AND QUANTITATIVE ANALYTICAL TOOLS

The unique properties of modern drug delivery systems have compelled researchers to characterize these systems at molecular level both by experimental and theoretical methods. DDSs have been analyzed in terms of their size, stability, loading capacity and drug release mechanism by employing various analytical techniques. The major analytical techniques used for this purpose are optical spectroscopy, thermal analysis, microscopic methods, X-Ray diffraction and NMR spectroscopy both in solution and solid state. In the following sections a very brief overview of these methods have been provided with emphasis on NMR as a technique that not only determines the structure of the drugs but also enables characterization of molecular interaction and dynamics of the drug within the drug carrier. Quantum chemical calculations and molecular dynamics simulations have also been employed for extensive analysis of drug-drug carrier interaction [Nagaraju and Sastry, 2009; Lipkowitz, 1998; Jimenez and Alderete 2008]. In all these methods drug-drug carrier interaction has been visualized as a complexation process whose association constant depends on the physical properties of the solution.

1.3.1 Optical spectroscopic, thermal and Microscopic techniques

Different optical spectroscopic methods such as UV Visible, fluorescence, infrared (IR) and Raman spectroscopy have been extensively used in characterizing DDS in terms of qualitative understanding of drug encapsulation and release as well as quantitative determination of complexation stoichiometry and association constant. Ample examples are available in literature where stoichiometry and association constant of supramolecular inclusion complexes are reported using the continuous variation method and spectral shift via UV-Vis. and

fluorescenece spectroscopic techniques [Bettinetti *et al.,* 1989; Al-Rawashdeh, 2005; Radi and Eissa, 2010; Mura, 2014]. UV-DRS, FTIR and Raman spectroscopic analysis have been also used to study DDS; the former to decipher optical properties of metal oxide based nanoparticles such as ZnO [Indubala et al., 2018] while the latter two methods are used for polymeric DDSs [Guimarães et al., 2015].

Stability related information of the drug carrier in presence and absence of a loaded drug can be obtained by thermal analysis. In particular Differential Scanning Calorimetry (DSC) is widely used techniques for this purpose. DSC investigation of supramolecular, polymeric and nanoparticle based DDSs have been reported in many literatures. [Yang et al., 2011; Nair et al., 2011; Gasmi et al., 2015; Tsonos et al., 2015].

Besides stability, surface morphology and structure are important aspects of these DDS. Molecular and crystal structure of solid DDS formulation in one hand has been unraveled by single crystal and powder XRD methods [Srinivasan and Stalin, 2014] while on the other hand surface morphology and adsorption behavior have been evidenced in literature by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) [Kang et al., 2004; Li et al., 2008; McCall and Sirianni, 2013; Lu et al., 2015; Steffy et al., 2018].

Although these above mentioned techniques are suitable in providing insights related to complexation, encapsulation energetics, thermal stability, morphology, size, shape, amount of drug loading, and the changes induced in the properties of a drug carrier due to the presence of the drug [Chaisri et al., 2011; PS and VG, 2013; Souza et al., 2014; Masloub et al., 2016] but such methods rarely highlighted information related to the structural rearrangement, motional dynamics, local organization of the loaded drug molecules and the possible intermolecular interactions within the drug carrier. It would be extremely valuable if one is able to predict the above mentioned properties of a drug within the drug carrier since such information will facilitate designing of better manufacturing strategy and determination of self-stability as well as drug release kinetics. Towards this end, solution and solid state NMR methods offer complete quantification of molecular mobility, internuclear distances, diffusion and exchange kinetics of DDS with high accuracy and reproducibility making it the technique of choice to study pharmaceutical composites [Saindon et al., 1993; Brown and Spiess, 2001; Lee and Ooya, 2012]. In the forthcoming sections NMR investigation of DDSs, the major focus of the present thesis will be thoroughly discussed.

1.3.2 NMR investigation of DDS

Nuclear Magnetic Resonance spectroscopy provides comprehensive analysis of structure, dynamics and molecular interaction of simple to complex molecular system in both solution and solid state. Moreover continuous developments in methodology and instrumentation of NMR spectroscopy have made it one of the most suitable method of choice for structural analysis of drug delivery systems at atomic resolution [Clore and Gronenborn 1994; Diercks et al., 2001; Coles et al., 2003; Pellecchia et al., 2008; Pandya et al, 2018]. Further, NMR can provide valuable information related to the motional dynamics and molecular interaction of the drug molecules especially the week intermolecular interactions in case of host-guest inclusion complexes that are critical in different stages of drug development [Fejzo et al., 1999; Coles et al., 2003; Kessler et al., 2003; Schwardt et al., 2003; Beusekom, 2007; Jahnke et al., 2005; Bober et al., 2017; Akash et al., 2018]. The major NMR parameters in the study of DDSs include chemical shift changes, relaxation times (T_1 and T_2), magnetization transfer (NOE, STD and chemical exchange) and translational diffusion [Stockman and Dalvit, 2002; Konstantin, 2003]. The following flow diagram summarizes the various aspects of new drug development that can be investigated by NMR.

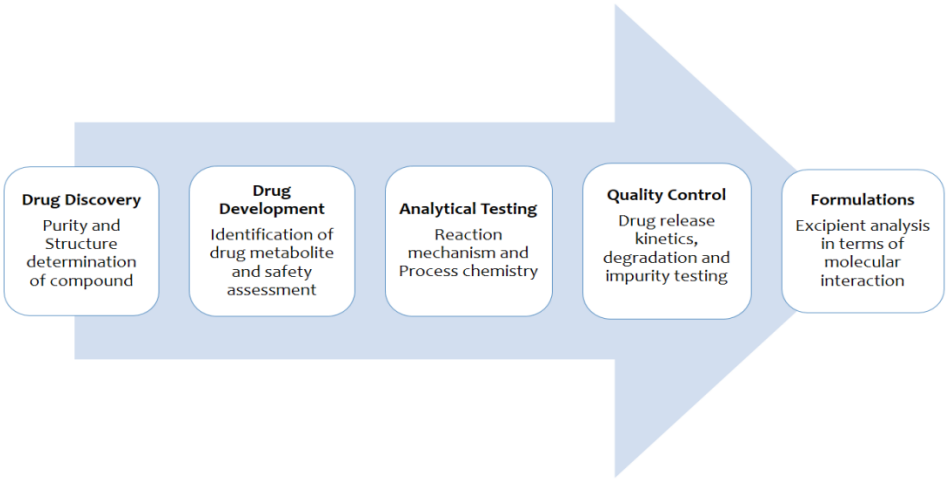

Figure 1.3 : Drug discovery and development processes that can be investigated by NMR.

In the following sections we have discussed the various NMR methods that have been used in literatures to investigate the DDSs both in solution and solid states.

1.4 SOLUTION STATE NMR METHODS TO INVESTIGATE DDSs

Solution state NMR spectroscopy is majorly used to indentify changes in chemical shift position of a drug or drug carrier upon encapsulation or binding interaction. Monitoring chemical shift changes as a function of host:guest concentration ratio helps determination of stoichiometry and binding constant. [Powers, 2009; Y. Li and Kang, 2017]. On the other hand, structure determination of DDSs is an indispensable prerequisite for pharmaceutical industries. However, structural analysis is an intriguing and challenging task since drug and drug carrier complexes are intrinsically dynamic due to the labile nature of non-covalent interactions resulting instantaneous information on structural changes to be difficult to collect. Spin-spin couplings information and Nuclear Overhauser Enhancement (NOE) measured for the drug delivery system enrich structural elucidation. In addition relaxation based experiments indicate the motional behavior of the drug in the free state as well as within the carrier. Figure 1.4 represents the various timescale probed by different NMR experiments that are relevant for DDS.

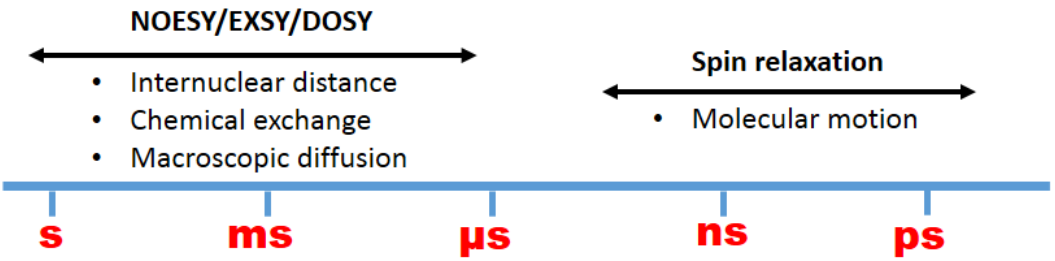

Figure 1.4 : The NMR methods used to probe the various molecular motion and respective timescale.

In the following sections NMR methods *viz.* chemical shift, relaxation time, magnetization transfer and diffusion based methods have been discussed to summarize the current status of NMR literature in the area of DDS.

1.4.1 Chemical Shift based studies

Chemical shifts were used in the quantitative analysis and purity test of DDSs in several studies [Rudin and Widmer, 2000; Holzgrabe et al., 2005; Malet-Martino and Holzgrabe, 2011; Yang et al., 2015; Toman et al., 2016]. Chemical shifts depend on the surroundings of the nuclei and therefore it can be used to monitor the changes induced by intermolecular interactions in DDSs Chemical shifts have already been examined to find out the molecular aggregation [Sacco and Holz 1997; Schreier et al., 2000; LaPlante et al., 2013; Pandya et al., 2018] and molecular recognition [Liu et al., 2007]. Further, the chemical shifts can be used to determine the stoichiometry and binding constants of the host-guest complex [Fielding, 2000; Ngowe et al., 2001; Dodziuk, 2006].

Cyclodextrin inclusion complexes have been investigated by observing the chemical shift changes, since the complex formation between drug and cyclodextrin results in chemical shift changes of both the drug and CD protons [Dodziuk, 2006]. It has been reported that during the inclusion of drug into CD cavity the inner cavity protons of CD (H_3 and H_5) experienced the highest chemical shift changes compared to the outer protons. Demarco and Thakkar first noticed the chemical shift changes in the CD inner cavity protons (H_3 and H_5) in the presence of a variety of aromatic compound in aqueous solution and envisaged from these observation that the aromatic ring of the test molecule is positioned inside the CD cavity [Demarco and Thakkar 1970]. This observation later become the basis for NMR spectroscopic study of the CD inclusion complexes [Fronza et al., 1992; Schneider et al., 2002; Bertacche et al., 2006; Nicolescu et al., 2010; Floare et al., 2013; Louiz et al., 2015; Viswalingam et al., 2016; Majhi et al., 2018]. The change in chemical shift data obtained from ^{1}H NMR titration experiments using continuous variation method can be used to determine stoichiometry and binding constant of the complex [S. Li and Purdy, 1992; Louiz et al., 2015; Viswalingam et al., 2016]. NMR spectra of CD complexes commonly exhibit fast exchange of guest molecule with the host resulting an averaged chemical shift on NMR time scale [Berg et al., 1996; Pîrnău et al., 2009; Zhao et al., 2016].

1.4.2 NMR Relaxation based studies

NMR relaxation rates are important to probe the changes in molecular motion of guest molecule due to the intermolecular interaction with the host [Schneider et al., 2002; Pons and Millet, 2001; Francisco et al., 2012]. The relaxation studies of CD inclusion complexes have been reported by several groups. The relaxation time of the guest molecule tends to decrease in presence of CD confirming the interaction between the guest and CD [Grillo et al., 2007]. Similar decrease in protons T_1 values have been observed for 1:1 dapsone:β-CD complex as reported by Francisco and co-workers [Francisco et al., 2012]. They have observed that once the dapsone molecule get encapsulated in the β-CD cavity, their T_1 values become of the same order as of β-CD confirming the change in mobility of dapsone after encapsulation. Crescenzi and co-workers have studied the chitosan-cyclodextrin network using ^{1}H NMR relaxation measurements. They have measured the longitudinal and transverse proton relaxation times of chitosan-cyclodextrin network and presented two kinds of water with different modes of relaxation at different temperatures [Paradossi et al., 1997]. Gafni et al. have highlighted the significance of hydrogen-bonding in enantiomeric differentiation of guest molecules in CD cavity using the relaxation rate measurements [Gafni et al., 1998].

Anczewski et al. have studied the 1:2 complex of two enantiomer of camphor with α-CD using longitudinal and transverse relaxation rates measurement to understand the chiral recognition. Based on the difference in relaxation rates and respective internal motion of methyl groups they have proposed two different orientation of the camphor enantiomers in α-CD cavity [Anczewski et al., 2003]. Rotational dynamics of 1-adamantanecarboxylic acid inside the β-CD cavity have been investigated by Tošner and co-workers by using multiple-field ^{13}C NMR relaxation [Tošner et al., 2006]. Ceborska et al. have measured the T_1 values of folic-acid in free and it's complex forms with α-, β- and γ-CD. They have confirmed the formation of complex by monitoring the changes in T_1 values [Ceborska et al., 2012]. Dushkin et al. have investigated the

complexes of polysaccharides with various drugs using the spin-lattice (T_1) and spin-spin relaxation (T_2) times to confirm the complex formation and observed a notable decrease in relaxation times of drugs after the complex formation [Dushkin et al., 2013; Chistyachenko et al., 2015].

In a recent study Du and co-workers have investigated the inclusion complex of valsartan drug with HP-β-CD using spin-spin relaxation time (T_2). A shortened T_2 has been observed for valsartan after encapsulation in HP-β-CD cavity suggesting a direct evidence of the complex formation [Du et al., 2016]. Ruggiero et al. have characterized iron oxide and paclitaxel loaded PLGA by ^{1}H Nuclear Magnetic Relaxation Dispersion (NMRD) profiles and correlate the data with heating potential in alternating magnetic field [Ruggiero et al., 2016].

1.4.3 Magnetization transfer based methods

(a) Nuclear Overhauser Enhancement (NOE)

Detection of the through-space dipole-dipole interactions via magnetization transfer between guest and the host protons that are spatially proximal to each other is another important probe for the structural and conformational analysis of complexes [Neuhaus and Williamson, 2000]. In 1D and 2D Nuclear Overhauser Enhancement Spectroscopy (NOESY) and Rotating frame Overhauser Enhancement Spectroscopy (ROESY) experiments, such dipole-dipole interaction is manifested via NOE cross peaks. Cross peaks are in general observed between nuclei that are upto 5 Å closer in space.

Since the pioneering work of Bergeron and Rowan, nuclear Overhauser enhancement (NOE) based experiments have been extensively used for the structure elucidation of supramolecular DDSs [Bergeron and Rowan 1976; Schneider et al., 2002]. The cyclodextrin DDSs lies in the intermediate motional regime making ROESY as the most preferable experiment to investigate interaction between drug and CD [Inoue, 1993; Schneider et al., 2002]. The 2D ROESY analysis gives information about the part of the drug molecule entering the CD cavity, mode of entry and orientation of the drug in drug:CD complex. Based on the intensity of the cross-peaks between drug and CD inner protons (H_3 and H_5) in 2D ROESY spectrum, conformation of the complexes were proposed by number of researchers [Bednarek et al., 2002; Fernandes et al., 2003; Bergonzi et al., 2007; Voulgari et al., 2007; Cruz et al., 2008; Sohajda et al., 2010; Zhao et al., 2016]. 1D NOE experiments were also used for obtaining information on the topology of the inclusion complexes of drugs with CD [Mulinacci et al., 1993; Matsui et al., 1994; S. A. Fernandes et al., 2007]. Computational studies are complimentary to the observed NOE. The results of computational studies show the feasibility of formation of complex and the favored mode of insertion of guest into CD. In case, all the intermolecular contacts extracted from the NOE spectrum are coherently justified by the minimum energy geometry obtained from computational calculations, it is considered as the potential structure present in the solution [Amato et al.,1992; Salvatierra et al., 1996; Carofiglio et al., 2002; Upadhyay and Kumar, 2009; Jahed et al., 2014; Adhikari et al., 2018].

Further, the NOE cross-peaks can be used to determine internuclear distances between interacting molecules [Pinto et al., 2005]. The precise information of the orientation of guest molecule inside the host can be illustrated by using these distances beyond just qualitative structural analysis. However, it is to be pointed out that accurate measurement of such distance parameters is a challenging task. Analysis of 1D and 2D NOE cross peaks have considered initial rate approximation. In case of host-guest complexes one needs to assume the two molecules as a single entity to extract the distance information.

(b) Chemical exchange

Chemical exchange is the process in which an atom or a group of atoms shuttle between two different chemical environments. It is a dynamic process and can occur in the timescale of microseconds to seconds range. Chemical exchange cross peaks are observed in the NOE experiment with opposite phases. In case of drug delivery system, the encapsulated drug exhibits moderate to fast chemical exchange between its free and bound forms. However, in many cases slow exchange gives rise to separate chemical shift position for the free and bound forms. The interaction of monoclonal antibody with an antigen (cytochrome c) has been investigated by hydrogen-deuterium (H/D) exchange analysis [Paterson et al., 1990]. Hwang and co-workers have proposed the use of phase-modulated CLEAN chemical exchange spectroscopy (CLEANEX-PM) to investigate water and amide proton exchange [Hwang et al., 1997]. Spencer and Fishbein have design and proposed the one-pulse experiments to measure chemical exchange in the intermediate exchange regime [Spencer and Fishbein, 2000]. Pons and Millet have thoroughly reviewed the NMR studies of the exchange dynamics in supramolecular complexes [Pons and Millet, 2001]. Momot and Kuchel have discussed the effects of chemical exchange between two pools in Stejskal Tanner plots and its application in micelles and cubosomes based drug delivery systems [Momot and Kuchel, 2003]. Wildes and Marqusee have examined the hydrogen exchange rates in a model ligand-protein system [Wildes and Marqusee, 2009]. Chemical exchange saturation transfer (CEST) has been used in recent years to characterize DDSs in terms of chemical exchange between bulk water protons and other exchangeable protons having different chemical shifts [Aime et al., 2005; Woods et al., 2006; Khemtong et al., 2009; Castelli et al., 2013]. Coelho et al. have studied the protons chemical exchange processes in bortezomib drug loaded polyethylene glycol DDS designed for cancer therapy [Coelho et al., 2015].

1.4.4 Diffusion coefficient based methods

Measurement of diffusion coefficients is an important parameter to study the interaction of a drug with drug carrier as NMR diffusion coefficient is altered due to these interactions [Price, 2003]. Further, translational diffusion can be used to determine shape and size of individual molecules as well as molecular aggregates [Johnson, 1999; Viel et al., 2003].

NMR diffusion has been extensively used for determining the association constant of inclusion complex between various drug and CD [Cameron et al., 2001; Cameron et al., 2002; Wimmer et al., 2002; Simova and Berger, 2005; Zhao et al., 2016]. Bakkour et al. have determined the association constants of the inclusion complex of CD with doxycyclin-hyclate drug by measuring diffusion coefficient that was comparable with the classical chemical shift based method (Scott's plot) [Bakkour et al., 2006]. Fernandes et al. investigated the complexation of tetracaine drug with β-CD and calix[6]arenes by Pulsed Field Gradient Stimulated Echo (PGSE) NMR. They have determined the fraction of complexed population and association constants with the help of experimentally measured diffusion coefficient [Fernandes et al., 2007].

In recent years, diffusion coefficient measurement has been used to understand the drug release behavior of a DDS. Soo-Jin and Ki-Seok reported that the drug release mechanism is hydration and diffusion mediated in polymer matrixes [Soo-Jin and Ki-Seok, 2011]. The PGSE-NMR has been used for the study of drug release mechanism in supramolecular, polymeric and hydrogels based DDSs by various groups [Amsden, 1998; Griffiths and Stilbs, 2002; Kwak and Lafleur, 2003; Momot and Kuchel, 2003; Occhipinti and Griffiths, 2008; Chamarthy and Pinal, 2008; Kim and Park, 2010; Soo-Jin and Ki-Seok, 2011].

1.5 SOLID STATE NMR METHODS TO INVESTIGATE DDS

Application of solid state NMR experiments to analyze solid pharmaceutics is relatively new however, the solid state NMR methods revealing both qualitative and quantitative information

have become very popular in the pharmaceutical analysis. Magic Angle Spinning (MAS) and high power proton decoupling are absolute requirement to monitor ^{13}C NMR spectra of pharmaceutical samples. MAS combined with cross polarization (CP) from ^{1}H to ^{13}C not only enhances the signal intensity but also removes different anisotropic interactions such as chemical shift anisotropy (CSA) which causes line broadening [Ziarelli and Caldarelli, 2006]. SS-NMR has been utilized in various applications related to DDSs *viz.*, differentiation of crystalline and amorphous phase, characterization of excipients and identification of impurities, characterization of drug-drug and drug-carrier interaction *etc.* [Byrn et al., 1999]. ^{13}C chemical shifts measurement by CP-MAS NMR has already been used for the quantification of solid state formulations [Harris et al., 2005; Ziarelli and Caldarelli, 2006]. The use of two dimensional heteronuclear correlation (2D HETCOR) NMR and spin lattice relaxation time measurements are also becoming popular to characterize solid state DDSs [Babonneau et al., 2010; Brown and Spiess, 2001; Spataro et al., 2018].

Relaxation measurements indicate molecular mobility of polymeric DDSs. Aso and Yoshioka have characterized Nifedipine-PVP (poly vinylpyrrolidone) and Phenobarbital-PVP solid formulations using ^{13}C-CPMAS and spin lattice relaxation times to understand the drug-PVP interactions and molecular mobility [Aso and Yoshioka, 2006]. Yuris et al. have studied the interaction of starch with mesona chinensis polysaccharide (MCP) using ^{1}H and ^{13}C NMR relaxation measurement. They have observed that the interaction between starch and MCP have affected the molecular mobility of water in starch-MCP gels [Yuris et al., 2019]. SS-NMR measurements are becoming promising tools to analyze nanomaterials as well. Both 1D and 2D Magic Angle Spinning (MAS) advanced solid state NMR methods are employed to investigate metal oxide nanoparticles to interpret their interaction with adsorbed or encapsulated molecules *e.g.*, the capping agents, the loaded drugs *etc.* [Holland et al., 2007; Juárez-Pérez et al., 2010; Ramos-González et al., 2012]. Multinuclear MAS SS-NMR techniques have been successfully used to characterize metal oxide based nanoparticles applied as solid DDS. ^{1}H, ^{13}C, ^{15}N, ^{29}Si, ^{31}P are some of the preferable nuclei in solid state that can be handled with more ease compared to exotic nuclei in terms of hardware, sensitivity and natural abundance *e.g.* ^{17}O, ^{19}F, ^{67}Zn, ^{49}Ti, ^{77}Se, ^{113}Cd, ^{139}La *etc.* [Brodard-Severac et al., 2008; Spataro et al., 2018]. 1D and 2D CP-MAS experiments have been employed to understand surface interaction of the metal oxide NPs with amino acids to unveil the nano-bio interface [Babonneau et al., 2010]. 1D MAS variable temperature measurements along with NMR relaxation analysis, 2D heteronuclear correlation and dipolar recoupling experiments have been proven to be extremely informative in case of mesoporous silica NPs in the solid state. Mesoporous silica based nanoplatforms have been characterized in terms of drug encapsulation and interaction with amino acids [Babonneau et al., 2004; Guo et al., 2016]. Marbella and Millstone have studied the noble metal nanoparticles using advanced SS-NMR techniques like rotational echo double resonance (REDOR) [Gullion and Schaefer, 1989; Marbella and Millstone, 2015].

1.6 SCOPE OF THE THESIS

The present thesis aims to investigate the structural and dynamic properties of supramolecular (cyclodextrins), polymeric (PLGA), and nanoparticle (ZnO) based drug delivery systems with a special emphasis on one dimensional NMR experiments both in solution and solid state. Various NMR parameters *viz.,* chemical shifts, relaxation rates, magnetization transfer due to Nuclear Overhauser Effect (NOE) and chemical exchange, diffusion and cross polarization have been explored and utilized to understand the structural and dynamic properties of these DDSs. ***Chapter 2*** presents a brief overview of the theoretical background of the above mentioned NMR parameters that have been used to investigate the dynamic processes in solution state DDSs. Also we have discussed solid state CP-MAS experiments that have been used for naomaterial based drug delivery systems.

In supramolecular DDSs the geometry of host:guest inclusion complexes were generally addressed by time consuming 2D NOE method. Extraction of internuclear distance from this NOE cross peaks albeit, did not receive much attention. We have determined the internuclear distance of these complexes with time efficient 1D NMR relaxation based methods. ***Chapters 3 and 4*** reports on encapsulation of paracetamol, aspirin and diflunisal within β-cyclodextrin (β-CD) and modified β-CD cavity and further measurement of internuclear distances using NMR relaxation approach. In chapter 3 we have demonstrated the applicability of nonselective (R_1^{ns}), selective (R_1^{se}) and bi-selective (R_1^{bs}) spin-lattice relaxation rates to infer dynamical parameters *e.g.*, molecular rotational correlation times of the encapsulated drugs. Moreover, analysis of cross-relaxation rates (σ_{ij}) extracted from the bi-selective inversion recovery experiment enables us to determine the host-guest internuclear distances. In ***Chapter 4*** a combination of R_1^{ns}, R_1^{se} and R_1^{bs} measurements has been proven to be sufficient to quantify molecular rotational correlation time (τ_c) and internuclear distances for diflunisal_β-CD complex while in case of diflunisal_HP-β-CD complex selective NOE experiment becomes a necessary choice to extract the distance data.

In case of polymeric DDSs information related to interior of the microsphere, local organization of entrapped molecules and possible intermolecular interactions within the cavity are rarely highlighted. Therefore, it would be extremely valuable to predict the inner environment and molecular dynamic properties of drug within the polymer cavity since such information will facilitate designing of better manufacturing strategy and determination of self-stability as well as kinetics of the drug release. Hence, we attempted to understand molecular dynamics of drug within the PLGA microsphere by employing 1D selective NMR methods in ***Chapter 5.*** We have highlighted the use of selective relaxation and selective NOE methods for encapsulated and free drug respectively to quantify the exchange dynamics observed among the mobile protons of the drug and the solvent inside the cavity. Analysis of the exchange rates confirmed existence of more than one kind of water population within the PLGA cavity.

In case of the nanoparticle based DDSs use of solid state NMR approaches to analyze ZnO based nanoparticles are scarce. Therefore, it would be of immense interest to apply these powerful solid state NMR experiments to envisage ZnO based nano-drug delivery systems in terms of its molecular interactions with drugs and biomolecules. We have investigated the anticancer drug loaded ZnO NPs using 1D SS-NMR methods to understand the intermolecular interactions in ***Chapter 6.*** This chapter focusses to understand the adsorption behavior of a set of anticancer drugs on ZnO nanoparticles proposed as probable drug delivery systems by employing solid state NMR, UV-DRS and XRD measurements. UV-DRS and XRD measurements of free and drug loaded ZnO nanoparticles confirmed adsorption of drugs. Further to analyze the nature of drug adsorption on the nanoparticle surface, ^{13}C CP-MAS and relaxation time were measured. ***Chapter 7*** summarizes the research work and is followed by bibliographic information.

...