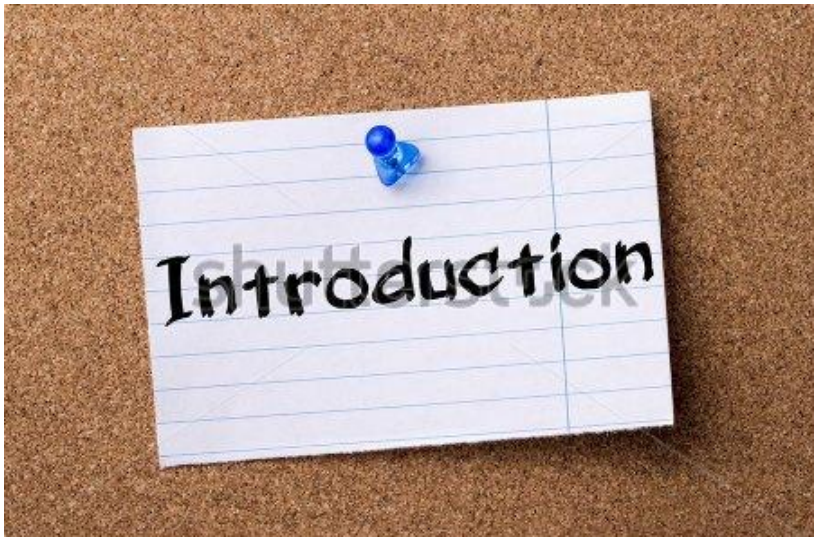
Chapter 1



1.1 Background

Various therapeutic agents were developed and used in medical science with the advances in science and technology. The United States Food and Drug Administration (FDA) approved new drug for their clinical use in every year, for example they approved 27-45 new drugs in 2011-2015. Each drug demands a delivery system for their administration to the patient. Various kinds of dosage forms are used like tablets, capsules, ointment, creams, aerosols, injections, and suppositories for drug delivery purpose [Shen et al., 2003]. Conventional drug carriers release the drug abruptly, resulting loss of therapeutic activity which is the major drawback of the conventional drug carrier requiring readministration of the drug. Several dosages are required to main the therapeutically effective drug level in plasma. Readministration of the drug sometimes might be causes fluctuations of drug level in plasma concentration which results side effects or lack of expected therapeutic assistance to the patient. Therefore, it is a matter of great challenge for the researchers involved in pharmaceutical studies to design a drug delivery system which can release the drug at regular time and intervals, with control rates, along with the targeting to specific sites [Alhaique et al., 2016].The first concept of exploiting polymers for designing the therapeutic agent carrier was examined in 1960s applying polymers as blood plasma expanders, injectable depots and wound dressing [Larson et al., 2012]. Recently, biopolymers are drawing great attention in biomedical application. Biopolymers are naturally formed during the growth cycles of all organisms [Chandra et al., 1998]. Biopolymers are becoming much more attractive day by day because of their inexpensiveness, biocompatibility and biodegradability. Researchers are trying to design advance drug delivery system through the fulfillment of pharmaceutical requirements such as improved bioavailability of drugs, excellent

compatibility between drug and matrix, adequate drug stability, sustained or controlled drug release.

1.1.1 Sustained or controlled drug delivery systems

Controlled drug delivery term is mainly used by the biomedical researcher in order to express the advance type of drug delivery system over classical drug delivery system. The controlled drug delivery can be defined as "Delivery of the drug at the right place, at the right concentration for the right time of period" [Kumar et al., 2011]. One of the most demanding features of drug therapy is drug bioavailability, which is oftenly diminished by the physiological barriers and blood clearance after drug administration [Luo et al., 2014; Kim et al., 2013]. This phenomenon limits the sustainability of the therapeutic effect resulting readministration of the drug [Kolhe et al., 2003]. Most of the conventional drug delivery systems are given in the form tablet or capsules which release the drug immediately after the administration leading to rapid and complete systemic drug absorption [Narasimharao et al., 2011]. Complementary pharmacodynamic starts after rapid absorption of the drug. Plasma drug concentration decreases according to the drug's pharmacokinetic profile when drug is completely absorbed [Steve et al., 2004]. Fluctuation of drug level in plasma may cause unnecessary side effects or loss of therapeutic advantage to the patient [Steve et al., 2004].

Figure 1.1 shows a graph of drug concentration in plasma verses desired time of action. There are couples of steps for absorption of drug. First is "ineffective level" where concentration of the drug is not sufficient to show its desired activity. Second is "therapeutic level" in which the concentration of the drug is enough for proper action of drub. Third is "toxic level" where concentration of the drug may be toxic for the patient. In case of conventional drug delivery system like injection, the drug concentration starts from ineffective level, reaches to the therapeutic level and reaches to

the toxic level crossing the therapeutic level, again return back to the ineffective level passing from the desired level. Therefore, to maintain the concentration of the drug inthe

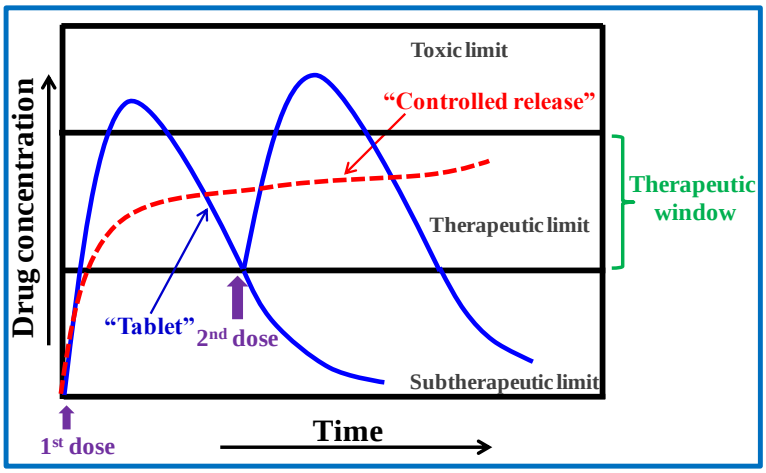


Figure 1.1: A Schematic representations of controlled drug delivery system over conventional one [Bajpai et al., 2008]

therapeutic level repeated doses are required. In case of controlled drug delivery system, constant predictable release can be achieved within therapeutic level for longer period of time in a single dose, which is great advantage of the controlled drug delivery system as opposed to conventional drug delivery system. The important advantages of the controlled drug delivery system are listed below [Bajpai et al., 2008]:

(i) Concentration of the drug in the blood stream remain constant resulting least fluctuation.

(ii) Predictable release rate for longer period of time.

(iii) Reduce the possibility of readministration and eliminate the side effects.

(iv) Protect the therapeutic agent having very short half-life.

(v) Optimize the drug therapy and superior patient compliance.

(vi) Solve the drug solubility problem and consequently, drug wastage.

1.2 Polysaccharide and its contribution in human life:

Polysaccharides are the class of organic compound exclusively found in plant kingdom. They are known as the complex carbohydrate polymer containing monosaccharide units linked together by glycosidic bonds into linear or branched chains of different length, and are classified on the basis of their main building units, i.e. monosaccharide components, type of linkages and the anomeric configuration of glycosidic linkages. Polysaccharides are highly eco-friendly, renewable and inexpensive organic compound in this earth. Polysaccharides are the major constituents of food, energy source and fiber [Bhattia et al., 2013]. The fibers help in digestion, while polysaccharides are first time use as fuel by man. In principally, the contribution of polysaccharides in human life is endless and they bound us by all the way in every aspect. Even in this era of synthetic materials, polysaccharides are heavily used in textiles, paper, glue, food and food additives without causing any environmental problem [Yapo et al., 2011; Markov et al., 2011, Gregorova, 2015].

The water soluble polysaccharides are rapidly used in different industry and agricultural fields as flocculants [Bratby et al., 2006], adsorbents [Parkar et al.,], controlled drug delivery system [Pal et al., 2008, viscofiers [Wunderlich et al., 2011]. Now a day, great attentions have been paid to the polysaccharides in biomedical application, especially in drug delivery [Das et al., 2015] because of their inherent biocompatible and biocompatible and biodegradable nature.

1.3 Biopolymers and its application in biomedical field

Biopolymers are defined as naturally occurring polymer during the growth cycles of all organisms. The well-known naturally occurring biopolymers are chitin, chitosan, dextran, hyaluronic acid, cellulose, chondroitin sulfate, pullulan, and alginate [Oh et al., 2009].

1.3.1 Chitin

Chitin is a long-chain biopolymer of N-acetylglucosamine, mostly found in many sources throughout the natural world [Kumar et al., 2000]. The structure of chitin is shown in **Figure 1.2**.Chitin is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans (e.g., crabs, lobsters and shrimps) and insects, the radula's of mollusks, and the beaks of cephalopods, including squid and octopuses. Chitin is second most abundant natural polysaccharide in earth after cellulose. Chitin is enormously used in several medical and industrial purposes due to its intrinsic biofriendly properties and abundant availabilities [Rinaudo et al., 2006].

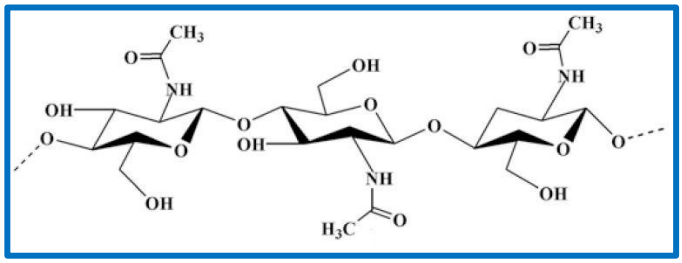


Figure 1.2: Structure of chitin [Kumar et al., 2000].

1.3.2 Chitosan

Chitosan is a linear biopolymer obtained from deacetylation of chitin. The chemical structure of chitosan is shown in **Figure 1.3.**Chitosan is treated as appropriate biomaterials from biomedical applications because of its high biocompatibility, biodegradability, antibacterial, non-antigenicity and blood coagulation properties [Muzzarelli et al., 1988; Muzzarelli et al., 1987; Muzzarelli et al., 2009; Jayakumar et al., 2007; Jayakumar et al., 2005]. Chitosan also serves as a drug delivery vehicle for nasal, oral, transdermal, and ocular drug delivery [Casettari et al., 2014; Tang et al., 2014; He et al., 2009; Jana et al., 2014; Fuente et al., 2006]. It can also be used for the delivery of insulin, genetic material, DNA, vaccines and human hormone [Mukhopadhyay et al., 2013; Mukhopadhyay et al., 2014; Mao et al., 2010].The chemical properties of chitosan

help to support cell attachment and proliferation. Chitosan having polysaccharide backbone is structurally similar to glycosaminoglycans, the major component of the extracellular matrix of bone cartilage. The antibacterial properties of chitosan make them suitable for designing scaffolds for regeneration of alveolar bone as they are highly susceptible to bacterial infection [Dash et al., 2015].

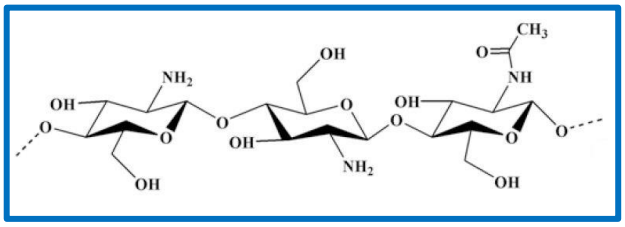


Figure 1.3: Structure of Chitosan [Muzzarelli et al., 1987].

1.3.3 Dextran

Dextran is a one type of glucose homopolysaccharide having consecutive α-(1→6) linkages in their major chains, more than 50% of the total linkages. In addition to that Dextran also posses α-(1→2), α-(1→3) or α-(1→4) branch linkage in their side chain. The structure of Dextran is shown in **Figure 1.4**. Dextran is water soluble and stable in both mild acidic and basic conditions. It is easy to functionalize dextran because of the presence of large number of hydroxyl groups in their moiety. Dextran is extensively used in pharmaceutical field because of its non-toxic, biodegradable, and biocompatible in nature [Hovgaard et al., 1995; Jalaja et al., 2014; Mocanu et al., 2014; Zafar et al., 2014].

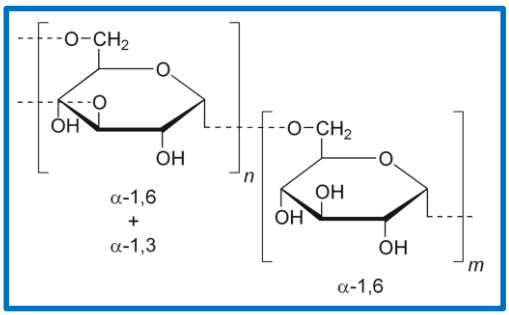


Figure 1.4: Chemical structure of Dextran [Hovgaard et al., 1995].

1.3.4 Cellulose

Cellulose is the most abundant naturally occurring organic polymer in the earth. Cellulose is a polysaccharide containing a linear chain of several hundred to many thousands of β-(1→4) linked D-glucose units having general formula $(C_6H_{10}O_5)_n$ [Nishiyama et al., 2002]. It is mostly found as structural component of the primary cell wall of green plants, many forms of algae and omycetes. Cellulose is odourless compound without any test. It is insoluble in water and most organic solvents.It is well

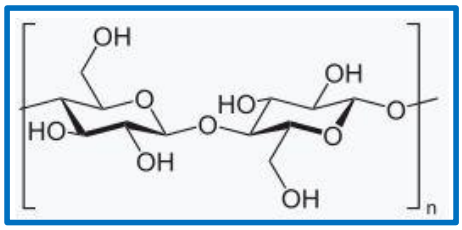


Figure 1.5: Structure of Cellulose [Nishiyama et al., 2002].

biocompatible and biodegradable in nature [Nishiyama et al., 2002]. Cellulose is broken down into its glucose unit when it is treated with concentrated acid at high temperature. Application of Cellulose has covered various areas such as tissue engineering, artificial bones, civil engineering, dentistry, food packaging, as stationary phase for thin layer chromatography and, as inactive fillers in drug tablet [Moon et al., 2011; Kaya et al., 2016**]**. The structure of cellulose is shown in **Figure 1.5**.

1.3.5 Hyaluronic acid

Hyaluronic acid or hyaluronan is linear polysaccharide containing repeating unit of β-1→4-D-glucuronic acid and of β-1→3-N-acetyl-D-glucosamine. The structure of hyaluronic acid is shown in **Figure 1.6**. It is found in tissue like skin, umbilical cord, cartilage, bird crests, vitreous humor and synovial fluid [Amado et al., 2016; Fakhari et

al., 2013]. Hyaluronic acid is collected through the extraction of animal tissue (bovine vitreous humour, rooster combs, and human umbilical cord). Now a day it is derived from bacteria (*Streptococcus zooepidemicus*) through the fermentation [Amado et al. 2016; Fakhari et al., 2013]. The Hyaluronic acid with high molecular weight has significant impact in the clinical and pharmaceutical sectors, including plastic surgery, major burns, treatment of arthritis and intra-ocular drug surgery [Amado et al. 2016]. It is highly biocompatible and biodegradable in nature. It can target specific cell surface receptors because of the presence of several functional groups on their backbone, especially it can bind to CD44 (a membrane glycoprotein receptor), which is over expressed on several cancerous cell surface. In this way, Hyaluronic acid has the tremendous potential potential application in targeted cancer therapy [Amado et al. 2016; Fakhari et al., 2013; Bian et al., 2016]. It is also widely used as therapeutic agent carrier (Amado et al. 2016).

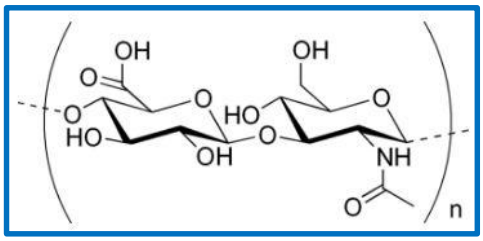


Figure 1.6: Structure of Hyaluronic acid [Amado et al., 2016].

1.3.6 Alginate

Alginate is a linear polysaccharide extracted from brown seaweed. It is composed of alternating blocks of 1-4 linked α-L-guluronic (G) and β-D-mannuronic (M) acid residues [Lee et al. 2012]. **Figure 1.7** shows the chemical structure of Alginate bonded through mannuronic and guluronic acid residues. Alginate can be treated as a true block copolymer composed of homopolymeric regions of M and G, called M- and G-blocks, respectively [Lee et al. 2012].It is water soluble and biodegradable. It is used in

pharmaceutics industry because of their *in-situ* gelation property, particularly in ocular drug delivery [Liu et al., 2006; Miyazaki et al., 2000]. It is also used as matrix for oral administration, specially, for paracetamol or cimetidine delivery [Liu et al., 2006; Miyazaki et al., 2000].

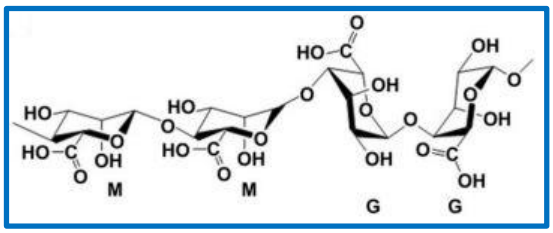


Figure 1.7: Chemical structure of Alginate [Lee et al. 2012].

1.3.7 Pullulan

Pullulan is polysaccharide containing α-(1→6) linked maltotriose units through α-(1→4) glycosidic bond [Aydogdu et al., 2016; Li et al., 2015]. It is derived through the fermentation of black yeast (e.g., Aureobasidium pullulans) [Li et al., 2015]. Pullulan is an ideal material for tissue engineering because of its non-toxicity, lack of immunogenicity, biocompatibility and biodegradability [Aydogdu et al., 2016]. It can be functionalize in various way as it contains several hydroxyl groups in its back bone. Pullulan and its functionalize derivatives are used in different way e.g., for drug delivery [Mocanu et al., 2014], for scaffold formation for bone tissue engineering [Fricain et al., 2013], and for vascular reconstruction [Shi et al. 2012]. The chemical structure of pullulan is shown in **Figure 1.8**.

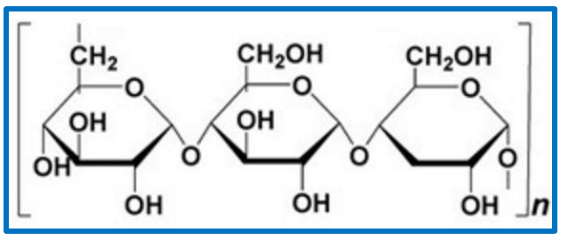


Figure 1.8: Structure of Pullulan [Aydogdu et al., 2016].

1.3.8 Chondroitin sulphate

Chondroitin sulphate is an anionic polyelectrolyte and structural constituent in connective tissues and cartilage [Young et al., 2016]. It contains repeating disaccharide units of β-1→3- linked d-glucuronic acid and β-1,4-linked N-acetyl galactosamine (**Figure 1.9**) [Young et al., 2016]. It is named as chondroitin sulphate A or C due to presence of sulphated groups at 4- or 6- position of *N*-acetyl galactosamine.These compounds are formed intracellularly from glucose or glucosamine precursors and are secreted at the cell surface by the chondrocytes [Young et al., 2016 ; Mikami et al., 2013). It shows anti-oxidation and anti-inflammation properties. It also acts as immune regulator (Young et al., 2016). In Europe, chondroitin sulphate is used for symptomatic, slow-acting drug treatment for osteoarthritis patient. It is also used to relieve pain in arthritic diseases [Young et al., 2016]. In United States, it is also prescribed as a dietary supplement [Young et al., 2016]. Chondroitin sulphate helps to reduce the inflammation at injury sites by accelerating the metabolism of cells keeping a normal microenvironment for cell growth [Young et al., 2016]. It can also be served for cartilage repair and tissue engineering purpose [Young et al., 2016].

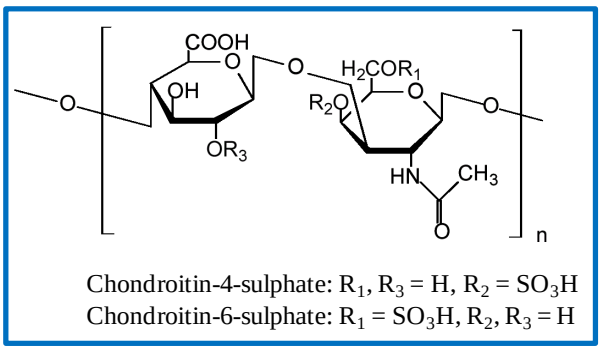


Figure 1.9: Chemical structure of Chondroitin sulphate [Young et al., 2016].

1.3.9 Problems with the existing polymers as drug delivery vehicle

All the above stated biopolymers are exclusively used in biomedical applications including drug delivery. Still their remain some limitations of with these polymers which restrict them for their use in wide-range applications. Hydrophobic and hydrophilic balance is an important criterion for the development of controlled drug delivery systems. Some polymers are highly hydrophobic and some are highly hydrophibic. So, an optimized hydrophilic and hydrophobic balance is required. Therefore, original biopolymers need some modifications. Mechanical stability also an important factor for designing drug delivery systems. Low mechanical strength of the native polymer is also a great disadvantage for their wide-range applications in biomedical applications. Henceforth, sufficient mechanical strength with suitable hydrophilic and hydrophibic balanced biopolymers are desirable for their possible use in drug delivery applications. Modification of the biopolymers via appropriate functionalization, grafting and developing nanocompoistes may enhance their different physicochemical properties.

1.3.9.1 Functionalization of polymer to improve the property

Different polymerization techniques are explored for the modification of the polymers. Here, some popular polymerization techniques are given which are adopted for the modification of the native polymers for their possible biomedical applications with their contribution in biologically active molecules delivery.

1.3.9.2 Atom Transfer Radical Polymerization (ATRP)

ATRP is one of the most sucessful polymerization tehcniques to polymerize styrenes, (meth)acrylates and various monomers in a controlled fashion with predetermined molecular weight and low polydispersity. ATRP is helpful to prepare polymers with functionalities along the chain because of its radical nature. The initiator used in ATRP

determines the end groups of the polymers. By using suitable functional initiator having functionalities such as vinyl, epoxide, hydroxyl, cyano and other groups have been tagged at one end, while other end of the chain contains an alkyl halide. Halogens present in the polymer can be dehalogenated or the halogen can be transformed into other functional groups using nuleophilic substitution reaction or electrophilic addition reactions [Coessens et al., 2001]. The ATRP mechanism has been shown in **Figure 1.10**.

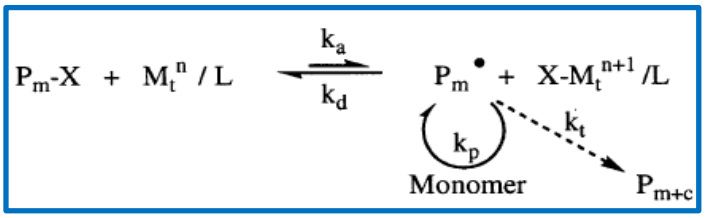


Figure 1.10: Mechanism of ATRP [Coessens et al., 2001].

By using ATRP method both functionality and architecture can be combined to develop multifunctional polymers of different composition and shapes such as block copolymers, multiarmed stars or hyperbranched polymers. ATRP methods have been applied for modification polymer for their possible use in biomedical field. Huang et al. have done surface modification of the mesoporous silica nanoparticels using visible light induced atom transfer radical polymerization (ATRP) and developed copolymers composites with Itanoic acid and poly(ethylene glycol)methyl acrylate as the monomers and 10-phenylphenothiazine as the organic catalyst [Huanga et al., 2017]. They have found that the such type of polymer nanocomposite delivers the anticancer drug (cisplatin) in a controlled way with pH responsive behavior. Liua et al. have functionalized lignin through ATRP grafting of poly(2-dimethylaminoethyl methacrylate) for gene delivery [Liua et al., 2015]. Huang et al. have designed pH-sensitive micelles from miktoarm star block copolymers by ATRP for their application as drug nanocarrier [Huang at al., 2016].

1.3.9.3 Reversible Addition-Fragmentation chain-Transfer (RAFT) Polymerization

RAFT polymerization is one kind of living radical polymerization was discovered at CSIRO I 1988. This method provide great advantage for synthetic tailoring of macromolecules with complex architectures including graft, block, comb and star structures with predetermined molecular weight. RAFT polymerization techniques can be applied for a wide range of monomers under various experimental conditions, including the preparation of water-solublematerials.The RAFT polymerization method follows

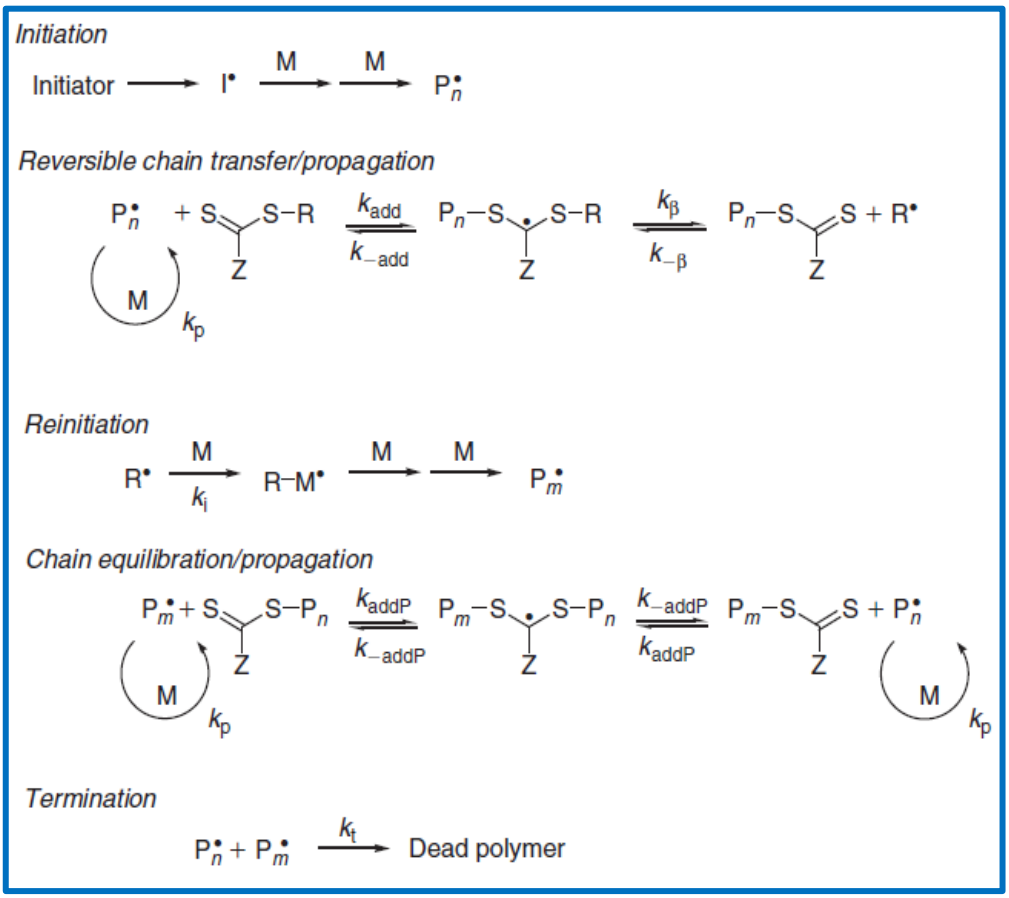


Figure 1.11: RAFT polymerization mechanism

the conventional free radical polymerization of a substituted monomer in the presence of a suitable RAFT agent. Most commonly used RAFT agents are thiocarbonylthio compound such as dithiocarbamates, dithiester, trithiocarbonate and Xanthates. The

mechanism of RAFT polymerization has been shown in **Figure 1.11:** RAFT polymerization mechanism. Recently, great attentions have been paid to the RAFT polymerization method for the development of functionalized polymers for biomedical applications. Song et al. have designed a novel tri-block copolymer poly(oxopentanoate ethyl methacrylate)-block-poly(pyridyl disulfide ethyl acrylate)-block-poly(ethyl glycol acrylate) via RAFT polymerization for dual controlled drug delivery via pH stimulation and biodegradation . Davaran et al. have designed novel pH- and thermo-responsive ABC triblock copolymer {poly[(2-succinyloxyethyl methacrylate)-b-(N-isopropylacrylamide)-b-[(N-4-vinylbenzyl),N,N-diethylamine]]} via RAFT polymerization technique and check their performance in stimuli responsive anticancer drug delivery. Heng et al. fabricate luminescent hydroxyapatite nanorods through surface initiated RAFT polymerization for biological imaging and controlled drug delivery applications. Song et al. have developed thermo-responsive graft copolymer consisting of poly(methyl methacrylate-co-hydroxyethyl methacrylate) backbone and hydrophilic poly(N-isopropyl acrylamide) using a novel macro-RAFT agent. The developed amphiphilic graft copolymer forms micelle via self-assembly and capable of delivering guest molecules (drug).

1.3.9.4 Nitroxide-mediated Polymerization (NMP)

Nitroxide-mediated polymerization technique is one of the finest tools among the controlled radical polymerization. NMP is of great interest because of its simplicity as the polymerization procees thermally inititated in the absence of an external radical source or a metal catalyst. Stable free radicals, scuh as nitroxide, serve as reversible terminating agents to controll the polymerization process in NMP method. Reversible deactivation of the growing chains through covalent bond formation helps to generate dormant chains. Active growing chain and the nitroxide radical are formed through the homolytic

cleavage of the bonds at high temperature. Activation of is followed by the deactivation so that few monomer units can incorporate in the propagating chain [Grubbs et al., 2011]. The mechanism of NMP method is shown in **Figure 1.12**. Controlled radical

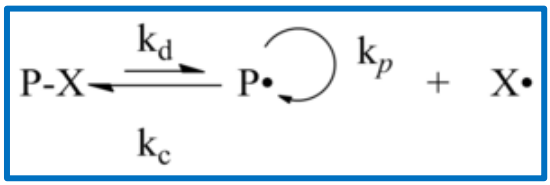


Figure 1.12: Mechanism of NMP [Grubbs et al., 2011].

polymerization techniques have been used in the design of glycopolymers and polymer-peptide bioconjugates because high robustness, flexibility and mild experimental conditions [Boyer et al., 2009; Heredia et al., 2007; Nicomlas et al., 2007; Opsteen et al., 2007]. Several examples in this field also found for NMP [Ohno et al., 1998; Narumi et al., 2001; Narumi et al., 2002; Wulf et al., 2001].

1.3.9.5 Grafting: A versatile approach to modify polymers

The modification of the polymers has drawn great attentions to change the properties of the polymers. Among different modification techniques, grafting is one of the promising one. In principal, a variety of functional groups can be incorporated into polymer chains through graft co-polymerization. Various approaches have been adopted to synthesize graft copolymers. Normally, some popular polymerization techniques are employed to such as, ring-opening metathesis polymerization, atom transfer radical polymerization, cationic and anionic polymerization, and free radical polymerizations. Synthesis of graft polymers are schematically presented in **Figure 1.13**.

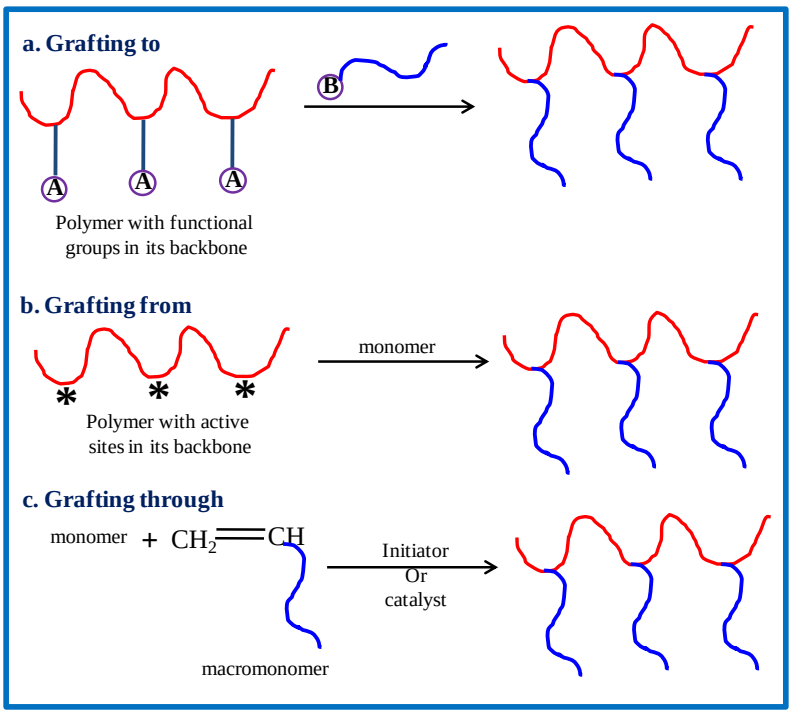


Figure 1.13: Schematic representation of different grafting method.

1.4 Mechanism of drug release from polymeric network

Drug delivery system can be categorized according to the drug release mechanism as follows [Peppas et al., 1993]:

A. Diffusion-controlled systems

(i) Reservoir (membrane systems)

(ii) Matrix (monolithic systems)

B. Chemically controlled systems

(i) Bioerodible and biodegradable systems

(ii) Pendent chain systems

C. Solvent-activated system

(i) Osmotically controlled systems

(ii) Swelling-controlled system

D. Modulated-release systems

Each type of delivery system can give different type of drug release depending on mode of mechanism, type of polymer and type of drug [Bajpai et al., 2008]. In our study, we have mainly focused on the swelling controlled systems and we prepared the matrix which follows such type of mechanism. The mechanism of drug release based on swelling is described below:

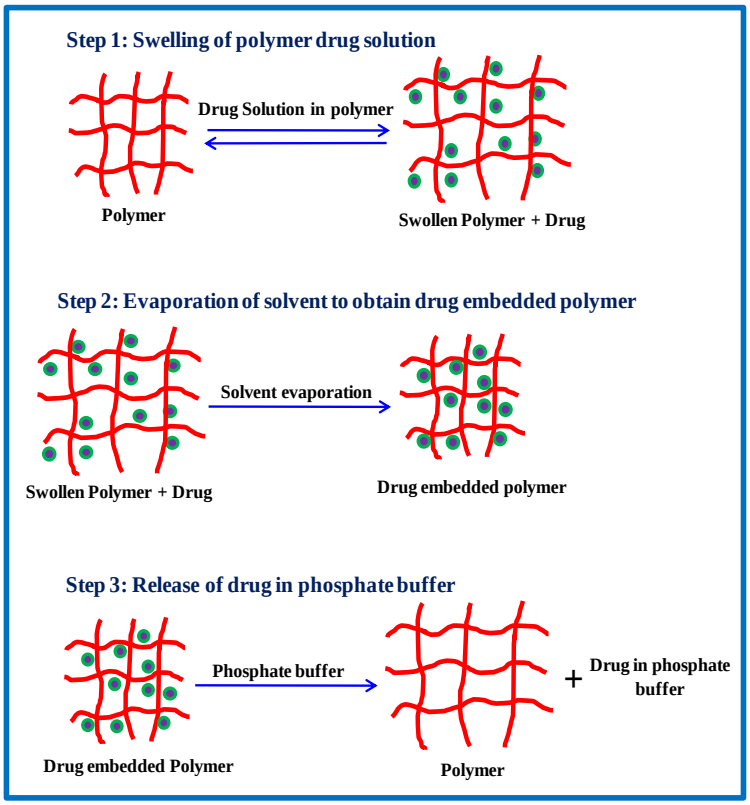


Figure 1.14: Swelling controlled drug release mechanism [Bajpai et al., 2008].

The crosslinked polymers with entangled mesh structure are capable for drug entrapment. The polymer chains of crosslinked polymers are become relaxed when come in contact with thermodynamically compatible solvent leading to the entrapment of solvent molecules inside the polymer matrix and get swelled. When these polymers are

immerged in saturated drug solution of such type of solvent, the drug molecules along with solvent are entrapped into the matrix (**Figure 1.14**). Drug molecules are entrapped in the polymer matrix after evaporation the solvent. The drug embedded polymers are kept in the release media in order to get drug release. Swelling of the polymer in release media leads results the release of the drug depending on the swelling kinetics of the polymer in the release media.

1.5 Polymeric Drug delivery systems

Polymers are extensively used in biomedical applications in several forms. Different polymeric drug delivery systems are discussed in details with their contribution in drug delivery.

1.5.1 Micelles

Micelles are known as colloidal nanoscale systems having spherical or globular form. They are formed by self assembly of amphiphilic block copolymers in aqueous solution forming hydrophobic core and hydrophilic shell. The block copolymers aggregate to form entropically favored, supra-molecular assembly in aqueous media under certain concentration (critical micelle concentration; CMC) and temperature. The hydrophobic core serves as a reservoir for hydrophobic molecules, such as therapeutics, and imaging agent improving their solubility and stability in the biological system. The hydrophilic shell helps to stabilize the hydrophobic core and thereby protects the loaded drugs from interaction with blood component. Therefore, micelles are found to be an appropriate vehicle for i.v. administration. Therapeutics are incorporated in polymeric micelles by physical, chemical or electrostatic interactions [Park et al., 2008]. The first micelles formulation with drug was prepared using paclitaxel, Genexol-PM (PEG-poly(D,L-lactide)-paclitaxel) which as administered without hypersensitivity reactions (HSRs) and a favorable toxic profile was observed [Kim et al., 2004]. A multifunctional star-shaped

polymeric micelles have been developed based on four-arm disulfide linked poly(e-caprolactone)-poly(ethelene glycol) amphiphilic copolymer having folate ligand. It was observed that such type of micelles show high stability, high drug loading efficiency and sustained release in physiological enviorment while fast release was noticed in acidic environment [Shi et al., 2014]. Wei et al. have developed a supramolecular nanomicellar system based on the amphiphilic dendrimer (AmDM) to deliver the clinical anticancer drug DOX enhancing anticancer activity [Wei et al., 2015]. Recently, lipid- and polyion complex based micelles have been used for rapid generation of multivalent agonists targeting necrosis factor receptor (TNFR) exhibiting promising therapeutic potential [Gilbreth et al., 2016].

1.5.2 Dendrimers

Dendrimers are highly branched molecules having 3D globular structure with high degree of surface functionality and versatility. They have been introduced in the mid-1980s as promising polymeric materials because of their unique properties such as uniform size, water solubility, narrow polydispersity, well-defined molecular weight, multivalency, and nanoscale size. Dendrimers contain internal cavities having specific species for the encapsulation of guest molecules and external periphery with multiple functional moieties for solubilization and conjugation of bioactive compounds. Dendrimers are very much attractive for biological and drug delivery application for their special structural features [Nanjwade et al., 2009; Mignani et al., 2013]. Two different types of synthetic approach have been developed for the preparation of dendrimers: the divergent approach where the core construction starts first and goes to the periphery with successive formation of new generations and another one is convergent approach developed by Hawker and Frechet in which the branches of the dendrimer known as Dendrons are formed first and the so called dendrons are attached to the central core

[Tomalia et al., 1985; Newkome et al., 1985; Vogtle et al., 1978; Hawker et al., 1990]. Several dendrimers are investigated for drug delivery application such as polyamidoamine (PAMAM). Melamine, polypropyleneimine (PPI), poly (glycerol-co-succine acid), poly(glycerol), poly-L-lysine(PLL), triazine, poly(ethylene glycol) (PEG) and poly[2,2-bis(hydroxymethyl)-propionic acid] as well as citric-acid-based and carbohydrate based ones. Among several dendrimers, PAMAM and PPI have drawn great attention because of their compact, globular structure, multiple surface functional groups and availability of interior cavity spaces, where drug molecules can be encapsulated both in the interior of dendrimer (physical encapsulation) as well as attached to the surface functional groups through covalent conjugations [Kesharwani et al., 2015; Singh et al., 2016]. Internal cavities of the dendrimers are hydrophobic in nature that can be used for encapsulation of poorly water soluble drug through hydrophobic interaction [Liu et al., 2016]. Pan et al. have designed a range of amphiphilic Janus dendrimers, which consisted of acidic amino acid and naproxen molecules as the peripheral groups, for potential bone-targeting dendritic drug delivery. They have shown that the solubility of naproxen remarkably enhanced by the dendritic drug delivery system [Pan et al., 2012]. Kurtoglu et al. have developed dendrimer-drug conjugate to improve in vivo efficacy of the drug. They have synthesized poly(amidoamine) dendrimer-NAC (N-Acetyl cysteine). NAC is an anti-inflammatory agent which is used in the treatment of neuroinflammation, stroke and cerebral palsy. Release kinetics indicate that dendrimer-drug conjugate can deliver 60% of its NAC payload within 1 h at intracellular GSH concentrations at physiological pH, whereas the dendrimer-drug conjugate did not release any drug at plasma GSH levels. They have observed that the drug conjugate show higher antioxidant activity compared to free drug [Kurtoglu et al., 2009]. Vembu et al. have synthesized piperazine core 1,3,5-trazine

dendrimer with eight molecules of ciprofloxacin drug as a surface moiety [Vembu et al., 2015]. Drug encapsulated dendrimer shows significant antibacterial activity against gram-positive and gram-negative bacteria because of hydrophobic and hydrophilic balance per repeat unit of this dendrimer. It is observed that such type of dendrimer exhibits five times higher activity when compared with ciprofloxacin as standard.Siriviriyanun et al. have designed hybrid carriers by chemically binding OH-terminated fourth generation poly(amido amine) dendrimer and folic acid on grapheme oxide(GO). They have used GO of different size (100 and 1500 nm) and check their drug carrier ability. They have found that the small hybrid carriers possesses 1.3 times lower loading capacity for doxorubicin than the larger one but the small hybrid carriers release the drug 1.14 times faster way than the larger hybrids [Siriviriyanun et al., 2015].

1.5.3 Liposomes

Liposomes are small, spherical, self-closed, synthetic vesicles having one concentric lipid bilayer in which an aqueous phase remain in the center. Bargham et al. had discovered them in 1965 and explored them as a deliveryvehicle for therapeutic molecules. They are biodegradable and biocompatible in nature. It has the ability to encapsulate the hydrophilic agents (hydrophilic drug, DNA, RNA etc.) in their inner aqueous core and hydrophobic agents within their lamellae. These unique characteristics make them excellent therapeutic carrier. Amphiphilic drug can also be encapsulated in the inner aqueous core of the liposome using remote loading methods like the ammonium sulphate method for doxorubicin [Bolotin et al., 1994] or using pH gradient method for vincristine [Boman et al., 1994]. However, rapid clearance of liposome from blood stream restricts its practical application. The development of Stealth® liposomes enhance the circulation half-life of liposomes from less than a few minutes (conventional liposomes) to several hours (Stealth liposomes). In such type of liposomes have a surface coating of

hydrophilic polymer, usually a lipid derivative of polyethylene glycol (PEG) is used [Sapra et al., 2003]. Lopes et al. have synthesized nisin encapsulated nanoliposomes from soy phosphattidylcholine (PC) and pectin or polygalacturonic acid [Lopes et al., 2017]. They have found that encapsulation efficiency is 87.9% to PC-pectin and 84% to PC-polygalacturonic acid nanoliposomes. The nicin release profile shows that the nicin release rate of PC-pectin and PC-polygalacturonic acid liposomes is lower than the PC liposomes suggesting sustained releasing potential for food applications. Li et al. have developed low molecular weight chitosan coated liposomes for ocular drug delivery. They have observed that after coating liposome display a prolonged invitro release of dicofenac sodium when compared with non-coated liposome or drug solution [Li et al., 2009]. Kaldybekov et al. have designed maleimide-funstionalised PEGyalated (PEG-Mal) for drug delivery to the urinary bladder. In vitro release kinetics reveals that fluorescein sodium salt loaded PEG-Mal liposomes show longer drug release as compared to the conventional PEGyalated liposome [Kaldybekov et al., 2018].

1.5.4 Nanoparticle

Now a days, polymeric nanoparticles are heavily used as biomaterial because of their interesting characteristics interms of good biocompatibility, structural variety and easy to design. PNPs are used as smart drug delivery system as they have a marked role to bring the therapeutics to the right position in human body with excellent efficiency. PNPs are considered as an ideal candidate for cancer therapy, vaccines delivery and targeted antibiotics delivery with suitable choice of polymer and capacity to adjust drug release from PNPs.**A** lipid-polymer hybrid nanoparticle has been synthesized for topical and site targeting delivery of Norfloxacin [Dave et al., 2017]. Lipid –polymer hybrid nanoparticle exhibits potential activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Norfloxacin loaded lipid-polymer hybrid nanoparticle show 89.72% drug release of the

total drug. Lipid-polymer hybrid nanoparticles Norfloxacin has the potential to be used as a topical antibiotic drug carriers. Most of the nanopartciles are fabricated from biocompatible and biodegradable polymers for application in drug oral delivery science which is permitted by the US Food and Drug Administration (FDA) to be safe for human use [Hrkach et al., 2012]. Polymeric nanopartciles provide the special advantage by fixing antibodies, peptide, or small molecular targeting ligand on its surface for hastening and permitting specific interactions with cellular receptors or tissue component [Yu et al., 2012]. Nanoparticles can shield the drugs from degradation and deliver them to the intended sites with more efficient and sustained way [Ensign et al., 2012]. Polymeric nanoparticles have small size and large surface area for interaction with epithelial surfaces [He et al., 2012; Pridgen et al., 2007].

1.5.5 Hydrogel

Hydrogels are three-dimensional polymeric cross-linked network prepared from water soluble polymers. Hydrogels can be made from any kind of water-soluble polymer with wide range of chemical composition and bulk physical properties. Hydrogels can be obtained in different physical forms including slabs, coatings, microparticles, nanoparticles and films. Hydrogels are exclusively used in clinical practice and experimental medicine, including tissue engineering and regenerative medicine, cell immobilization, diagonostics, separation of biomlolecules or cells [Lee et al., 2001; Linden 2003, Jen et al., 1996; Wang et al. 1993; Bennett 2003]. Some unique physical properties of hydrogel like highly porous structure and the affinity of the hydrogel for aqueous environment make them of particular interest in their use in drug delivery purpose. Hydrogels are highly biocompatible in nature because of their high water content and physicochemical similarities to the native extracellular matrix, both compositionally and mechanically.Hydrogels are deformable and adopt the shape of the

surface to which they are applied. Muco- or bioadhesive properties of some hydrogel help them to immobilize at the site of application or in applying them on surface which are not horizontal. Jalalvandi et al. have designed a new type of hydrogel network from dextran aldehyde and polyhydrazidevia Schiff-base reaction to form hydrozone linkages as cross-linking sites for hydrophilic drug delivery [Jalalvandi et al., 2018]. They have encapsulated 5-flurouracil (5-FLU), a hydrophilic anticancer drug in the gel and observe that the drug has released almost in two days. Release behavior also found to be pH dependent. A novel type pH-responsive controlled release carrier has been developed from ethylene glycol dimethacrylate (EGDMA) cross-linked guar gum oleate-*graft*-poly(methacrylic acid) (GGO-*g*-PMAc) hydrogel for colon-specific drug delivery [Seeli., 2017]. They have found that release rate is higher at pH 7.4 than that at pH 1.2. Ghorpade et al. have developed Citric acid crosslinked β-cyclodextrin–carboxymethylcellulose (βCD-CMC) hydrogel film for ketoconazole [Ghorpade et al., 2017]. βCD helps to suppress the burst effect and retard the release rate of ketoconazole. Kong et al. have prepared a hydrogel system using hyaluronic acid-hydroxyethyl cellulose for effective transdermal delivery of isoliquiritigenin [Kong et al., 2016].

1.5.6 Scaffold

Porous materials having the ability to deliver hydrosoluble drugs, cells, or active compounds have been heavily used in biomedical field [Nair et al., 2007]. Materials used for scaffold designing can be natural and synthetic polymers, ceramics, metals and glasses [Habraken et al., 2007; Misra et al., 2006; Lee et al., 2007]. The most common features for all these materials are must be biocompatible and have porous microstructure which is the essential criteria for loading and releasing of chemical compounds [Tyson et al., 2011; Martins et al., 2007; Vallet-Regi et al., 2010; Vallet-Regi et al., 2011]. In general, the release of the therapeutic agent from the scaffold is controlled by their

diffusion through the matrix and their solubility in external environment. Sometimes, chemical agents are used to promote crosslinking in the scaffold to form a reticular structure of interconnected pores which encapsulate the drug in the matrix and ensure their progressive release.Samandari et al. have designed cytocompatible and non-toxic nanocomposite scaffolds using chitosan-graft-poly(acrylic acid-co-acrylamide)/hydroxyapatite for drug carrier [Samandari et al., 2013]. They have used celecoxib as a model drug and observed biphasic drug release pattern with a low initial burst and a sustained release of up to 14 days. Kumar et al. have developed a nanocomposite scaffold using a mixture of pectin, chitin and nano $CaCO_3$for their possible use in biomedical application such as tissue engineering and drug delivery [Kumar et al., 2013]. In vitro drug release kinetics has been carried out using a bisphosphonate called Fosamax and sustained release of the drug form the scaffold is observed and the release of the drug increases with increase in drug concentration.Dubnika et al. have developed novel composite scaffold using sodium alginate and chitosan incorporating silver ion and local anesthetic agent lidocaine hydrochloride to prevent bacterial infection in bone tissue regeneration [Dubnika et al., 2017]. The results suggest that the scaffold possess the antibacterial activity up to one year and simultaneously release the anesthetic agent in controlled way up to weeks. Ji et al. have designed porous structure scaffold with excellent biocompatibility by freeze-drying the mixture of nanosized Ibuprofen-loaded halloysite nanotubes (HNTs) and gelatin [Ji et al., 2017]. The mechanical property of the composite scaffold is significantly increased by HNTs to > 300% as compared to the pure gelatin scaffold. Ibuprofen-loaded gelatin scaffold shows 8 h drug release, where as ibuprofen-loaded HNTs incorporated in scaffold extend the release over 100 h. The novel HNTs/gelatin

scaffolds with high mechanical property and controllable drug release behavior make them a promising artificial bone grafting material.

1.6 Chitosan as a drug delivery vehicle: A brief overview

Chitosan was discovered and discussed by Rouget in 1859 as a deacetylated form of a natural polymer chitin [Raafat et al., 2009]. In the starting of 1990s chitosan has got its position in pharmaceutical field and attracted the researchers and industrialist to develop more effective and novel therapeutic system based on it. The presence of active amino groups on its backbone makes it versatile in nature as it behave as a reactive site for a variety of new group attachment under mild reaction conditions. Initially chitosan was used as a medical stuff such as wound dressing, slimming and tissue engineering but with time chitosan become a promising and demanding candidate for drug delivery system. Chitosan offers some exceptional biological properties for which it has gained much importance in biomaterial formulation and has been explored in drug delivery application. Chitosan is well known for its biocompatibility and biodegradability. Some other important properties of chitosan are bacteriostatic, haemostatic, anticholestermic, fungistatic and anticarcinogenic [Saikiaet et al., 2015].Chitosan has been used in different forms according to the function and application of the carrier. Various formulation of chitosan in drug delivery application has been summarized here with their contribution in drug delivery.

1.6.1 Tablets

Gels forming hydrophilic polymers are drawing much attention for their usage as a matrix for oral extended release dosage because of their simple preparation procedure, low cost and biocompatibility [Miyazaki et al., 2000]. Chitosan have been used as matrix for tablet preparation because of their biocompatibility and non-toxicity. Tablets are

prepared in simple way either by weight granulation or simply direct compression technique [Ofori-Kwakye et al., 2016]. Miyazaki et al. have prepared oral mucosal adhesive tablets containing chitosan and alginate as matrix encapsulating drug (diltiazem) by direct compression technique [Ofori-Kwakye et al., 2016]. They have found that in vitro adhesion properties of the developed tablets are comparable to the commercially available tablets. The release rate is quite high but it can be controlled by changing the mixing ratio of chitosan and alginate. Bioavailability of the drug is found 69.6% when administered in rabbit. Shao et al. have investigated the effect of other anionic polymer on release rate when combined with chitosan [Shao et al., 2015]. In vitro drug release study in simulated gastric juice reveal that the combination of chitosan with other anionic polymer slows down the release rate as compared to single polymer. The chitosan-xanthan gum is found to be the best combination to extend the release rate upto 24 hours.Khlibsuwan et al. have prepared matrix tablets using chitosan-clay (Magnesium aluminium silicate (MAS)) microparticles with various molecular weights of chitosan for the release of propranolol [Khlibsuwan et al., 2016]. They have showed that chitosan-MAS tablets exhibits sustained release patterns in both acid and neutral media and the drug release of the tablets in neutral media decreases with high molecular weight chitosan.

1.6.2 Beads

Chitosan can be modified in different forms in order to control release rate and efficiency of the bioactive agent. Crosslinked chitosan beads are another important form of chitosan explored as delivery system [Aral et al., 1998; Shu et al., 2000;]. Chitosan which is a cationic polysaccharide forms gels bead in presence of counter ions. Polyphosphates are mostly used as counter ions but other counter ions like molybdate is also reported, where as multivalent ions act as a coagulating agent [Aral et al., 1998; Shu et al., 2000].

Kulkarni et al. have prepared glutaraldehyde crosslinked chitosan beads and investigated the release of diclofenac sodium, a drug used in the treatment of chronic inflammatory disease [Kulkarni et al., 2007]. They have reported that the release rate is dependent on the number of factors such as temperature, pH, beads characteristics and stirring rate. Release rate from beads becomes slower at high temperature. The most interesting finding of the investigation is multi layered chitosan-alginate beads offer controlled gastrointestinal passage of low molecular weight compounds like ampicillin [Anal et al., 2005].

1.6.3 Films

Chitosan have excellent film forming properties and therefore, it finds many application in drug delivery application as a carrier for bioactive agents from small moleculelike antibiotic to macromolecules like proteins and nucleic acid [Mengatto et al., 2012]. Chitosan enhance wound healing rate due to its bacteriostatic property and also possess haemostatic properties [Noel et al., 2008]. Generally solution casting methods have been applied for preparation of the film. Simple chitosan and crosslinked chitosan films are reported for various applications. Generally, crosslinking remarkably enhance both physical and mechanical properties of chitosan like tensile strength, thermal stability, moisture retaining capacity, and water resistance property etc. [Kavianinia et al., 2015; Jin et al., 2004; Remu˜nán-López et al., 1997; Kiuchi et al., 2008]. Chitosan and crosslinked chitosan films have been explored as drug delivery vehicles in several fields like oral mucosal delivery, transdermal delivery, buccal delivery, sublingual delivery and periodontal delivery [Tang et al., 2014; Senel et al., 2000; Thein-Han et al., 2004; Thein-Han et al., 2004 et al., Can et al., 2013; Ammar et al., 2008; Remu˜nán-López et al., 1998; S¸ enel et al., 2000; Abruzzo et al., 2012; Giovino et al., 2012; Patel et al., 2011; Perugini et al., 2003; Ahmed et al., 2009; São Pedro et al., 2009]. Tang et al. have

developed chitosan film loaded with ibuprofen by using super critical solution impregnation method for oral mucosal delivery [Tang et al., 2014]. They have done in vivo drug release study and confirms that 70% drug has been released from the film in 460 min across the rabbit mucosa.Varshosaz et al. have developed crosslinked chitosan film and studied the release behavior of local anesthetic agent, Lidocaine for oral mucosal delivery [Varshosaz et al., 2007]. They have used tripolyphosphate penta sodium salt as a crosslinker. They have observed that the flux rate of lidocaine is increased with high molecular weight chitosan and concentrated solution of chitosan, whereas with increasein the concentration of crosslinker the flux and release rate of the drug decreases.

1.6.4 Nanofibers

Preparation of ultrafine fibers of nano size from polymer solution through electrospinning is another simple and novel technique. Nanofibers have been paid great attention for drug delivery purpose because its processing variables are precisely controllable during fabrication [Pillay et al., 2013; Sridhar et al., 2015]. Nanofibers are heavily used in biomedical field and drug delivery field because of their integrally high surface to volume ratio and porosity which results enhanced drug loading capacity and cell attachment [Hu et al., 2014]. Surface functionalization of nanofibers further improves their efficacy in drug and gene delivery applications [Yoo et al., 2009]. Many researchers have reported preparation and application of the nanofibers of chitosan through electrospinning technique [Homayoni et al., 2009; Duan et al., 2004; Geng et al., 2005; Ojha et al., 2008]. Chitosan-polyethylene oxide (Cs-PEO) nanofibrous scaffold have been designed for controlled drug delivery and tissue remodeling [Bhattarai et al., 2005].These nanofibrous materials are highly biocompatible with chondrocytes and have the capacity to endorse the attachment of human osteoblast and chondrocytes without

changing their cell morphology [Subramanian et al., 2005]. Jiang et al. have prepared ibuprofen-loaded poly(lactide-co-glycolide)/poly(ethylene glycol)-g-chitosan (PLGA/PEG-g-Cs)electrospun membranes for controlled drug delivery [Jiang et al., 2004]. They have reported that PEG-g-Cs significantly slow down the burst release of the drug from PLGA electrospun membrane. Toshkova et al. have prepared Doxorubicin (antitumor drug) loaded electrospun of quaternary chitosan to check the antitumor efficacy [Toshkova et al., 2010]. Drug loaded electrospun show high cytotoxicity against Graffi tumorcells. The implants completely inhibit the tumor growth in rat. A hybrid nanofiber with average diameter 248-600 nm prepared from chitosan and phospholipids have been investigated for transdermal drug delivery by Mendes et al. [Mendes et al., 2016]. These nanofibers are found to be biocompatibleand stable for seven days in PBS buffer.Nanofibrousscaffolds of PEO/Cs/GO shows pH dependent Doxorubicin (antitumor drug) release and faster release rate is obtained in acidic medium [Ardeshirzadeh et al., 2015]. The faster release rate in acidic pH is attributed the instability of hydrogen bonding between GO and doxorubicin.

1.6.5 Hydrogels

Hydrogels are three dimensional polymeric networks having the capability to absorb large amount water in it. Hydrogels have the capability to transport small molecules like drugs. Fully swollen hydrogel possess some unique characteristics which is very common to living tissues andlow interfacial tension with water and biological fluids make them suitable to be used as biomaterials. Now a days, chitosan hydrogel as a drug delivery vehicles draw a great attention of many scientists. Chitosan hydrogel has recognized as an intelligent drug delivery system as it can deliver the drug under different environmental stimuli [Bhattarai et al., 2010; Ahmadi et al., 2015].Recently, Duan et al. have reported a new solvent system to dissolve chitosan consists of lithium hydroxide(4.5

wt.%), potassium hydroxide (7 wt.%) and aqueous urea solution (8 wt.%) [Duan et al., 2015].In this way alkaline solution hydrogel with high strength having unique nanofibrous architecturehas been developed. These hydrogels exhibits exceptional biocompatibility and smart controlled drug release behavior under acidic environment. Dai et al. have reported the swelling behavior and release characteristics of alginate-chitossan hydrogel beads [Dai et al., 2008]. Nifedipine, a drug used to treat hypertension was used as model drug. The release kinetics show pH dependency and the nifedipine released from the hydrogel increases withincreasing pH (42% at 1.5 pH and 99% at 6.8 pH).Thus such type of hydrogels has the ability to holds the drug at low pH and can acts as a promising candidate for intestinal tract delivery.Sami et al. have reported the formulation of hydrogel consisting chitosan and guar gum for sustained release of Paracetamol [Sami et al., 2018]. They have observed that the release rate follow zero order kinetics and Non-Fickian law of diffusion. They have proposed that formulated hydrogels could be safely used as a dermal patch for the sustained drug release of Paracetamol.A new type of physically crossllinked hydrogel has been developed for controlling the drug release pattern [Neufeld et al., 2017]. Sustained release of three model drugs (mesalamine, curcumin and progesterone) over a period of 24 h in physiological conditions is observed and suggested pectin–chitosan thermoreversible hydrogelsmay improve the life style of many patients by reducing the daily uptake of chronic medicines. Chitosan and polyvinyl alcohol (CS/PVA)-based hydrogel composite has been used for gallic acid delivery and the drug loaded composite hydrogel has shown high antioxidant properties [Thanyacharoen et al., 2017].

1.6.6 Nanoparticles

The uses of nanaoparticles in pharmaceutical fields are increasing day by day. The small size of nanoparticles provides them great advantage for moving through various

biological barriers to bring the drug to the target site enhancing its efficacy [Wang et al., 2011]. Chitosan nanoparticles (CsNPs) behave as an excellent drug carrier because of its non-toxicity, biocompatibiulity, biodegradability and some specific target specific triggered by its cationic character. Several methods have been adopted for the preparation of chitosan naoparticle, such as precipitation, emulsion, ionic gelation, nanoprecipitation, reverse micellar method and sieving etc. [Qi et al., 2004; Luque-Alcaraz et al., 2016; Agnihotri et al., 2004]. The enzymatic degradation of labile drugs in the gastrointestinal tract can be protected by incorporating the drug in the nanoparticle. Several research articles have been published on chitosan nanoparticle based drug carrier highlighting their advantages and advance properties of nano sized chitosan in drug delivery [Agarwal et al., 2015; Liu et al., 2007; Liu et al., 2008]. Mitra et al. have designed dextran-Doxorubicin conjugate loaded chitosan nanoparticle for tumor targeted drug delivery [Mitra et al., 2001]. Doxorubicin is an a anticancer drug having many side effects like cardio toxicity, but conjugation with dextran minimize its side effect and furthermore, encapsulation of this conjugate in chitosan hydrogel increase its efficacy. CsNPs loaded with paclitaxel have been investigated for tumor homing capacity after systematic administration into SCC7 tumor bearing mice. The excellent result will lead to an early stage cancer diagnosis, drug delivery and therapy, simultaneously [Kwon et al., 2008]. Janes et al. have reported the entrapment of DOX into CsNPs via ionic gelation method [Janes et al., 2001]. Release kinetics shows a minimal burst release along with the retention of the cytotoxicity activity of the drug in vitro. They have also proposed the endocytic mechanism for the cellular uptake of the nanoparticles. Chitosan nanoparticle have the potential to transfer various macromolecules across nasal tracheal oral and ocular epithelium and also support the enhance absorption of the drugs across nasal mucosa [Dyer et al., 2002; Janes et al., 2001].

1.7 Scope of the present work

The main focus of the ongoing research work is to develop polymeric controlled drug delivery systems. To solve this purpose, biompolymers are suitable than synthetic polymers as discussed in introduction part. In my thesis work, controlled drug delivery system is designed based on naturally occurring biopolymer, chitosan. Chitosan is well known in biomedical field because of its well documented biocompatible, biodegradable nature. Chitosan can be chemically modified in different way because of the presence of amino and hydroxyl groups in its structure. Several reports have been found on chemically modified chitosan derivative in drug delivery applications. Controlled drug delivery systems have been designed based on chitosan via chemical modification of chitosan and developing nanohybrids. Recently, much attention have been paid to functionalize synthetic polymers by natural ones, mainly polysaccharides for the development of biomaterials. In the present work, polyurethane has been functionalized using chitosan as chain extender for biomedical applications. Polyurethanes are the most adaptable synthetic polymers which have been used for different biomedical applications. Chitosan based polyurethanes are of great interest because they provide unusual flexibility in both mechanically and biologically for wide range of applications. It is believed that $NHCOCH_3$, NH_2 and NHCOO groups present in chitosan and polyurethane may impart bioactive properties for biomedical applications. Therefore, incorporation of polyurethane chain into chitosan moieties will enhance the biocompatibility and reduce the cytotoxicity of the derivatives. Chitosan based polyurethanes derivatives has been extensively used in biomedical application such as tissue engineering scaffolds, wound healing, stent coating, separation membrane, sensor and drug delivery systems.

Still chitosan-polyurethane chemistry is not extensively explored. The research work is mainly focused on the development of polyurethane based chitosan biomaterials for their

possible in controlled drug delivery systems. The research work is carried out keeping the following points in mind.

- The effect grafting of polyurethane chain on chitosan backbone on mechanical behavior is not well reported.
- The effect of grafting of polyurethane chain onto chitosan in drug delivery is not extensively studied.
- Biodistribution of the polyurethane grafted chitosan has not been studied yet.
- Polyurethane based chitosan hydrogel/injectable hydrogel for controlled drug delivery yet not reported.
- How the cells are growing on polyurethane based chitosan scaffolds has not been investigated.
- How does the Layer double hydroxide nanoparticle influence the different physicochemical properties and drug release kinetics in chitosan nanohybrid has been remain unexplored.

1.8 Objective of the present thesis

The objective of the thesis is to develop chitosan based drug delivery systems. Therefore, chitosan is chemically modified through grafting of polyurethane chain onto its backbone. The complete modification technique, characterization process and the efficiency of the developed chitosan derivatives as drug delivery vehicles have been evaluated in this thesis using the following outline:

a. Polyurethane-grafted-chitosan for controlled drug delivery

- Synthesis and characterization of the polyurethane grafted chitosan with different degree of substitution.
- Drug loading and in vitro release assay using graft copolymer film.
- Evaluation of bio-and hemocompatibility of the grafted materials using platelets.
- Biodistribution of the polyurethane graft chitosan in rat model.
- Histopathological investigation of the different organs to check the side effect of the graft copolymers.

b. Polyurethane-chitosan based brush copolymer as injectable hydrogel for controlled drug delivery.

- Synthesis and characterization of Polyurethane based chitosan brush.
- Fabrication of brush copolymer into hydrogel and scaffold.
- Drug loading and release study using both hydrogel and scaffold.
- Invivo gelation study of the brush copolymer in rat model for their possible use as injectable hydrogel.
- Cytotoxicity assay of the hydrogel and scaffold using NIH 3T3 fibroblast cell line.

C. Chitosan nanocomposite hydrogel and scaffold for controlled drug delivery.

- Development and characterization chitosan nanohybrid.
- Evaluation of the mechanical strength of the nanohybrid using both hydrogel and scaffold.
- Drug loading and in vitro release study using chitosan based nanohybrids (Hydrogel and Scaffold)
- Antibacterial activity of the drug loaded chitosan nanohybrid (hydrogel).
- Cytotoxicity assay of chitosan nanohybrids using both hydrogel and scaffold.