CHAPTER 1

Introduction

1.1 Amphiphilic Block Copolymers

The word amphiphilic is derived from Greek A*mphis* means "of both kinds" and *philic* means "having an affinity for". So, the amphiphilic block copolymers are those macromolecules which are consist of both the hydrophobic and hydrophilic polymer blocks bonded with each other covalently. These are very important class of materials in terms of their wide field of industrial applications. Basically, amphiphilic block copolymers have the ability to act as compatibilisers, dispersion agents, modifiers and solubilizers [Hamley *et al.* (2004), Maric *et al.* (2002), Li *et al.* (2004]. These are similar with low molecular weight amphiphiles (lipids, surfactants) in nature. Amphiphilic block copolymers form micelles in solvent which is selective for one of the blocks. For example, in water, micelles are formed with hydrophobic blocks as core and hydrophilic block as shell, but, in oil, they form reverse micelle [**Figure 1.1**].

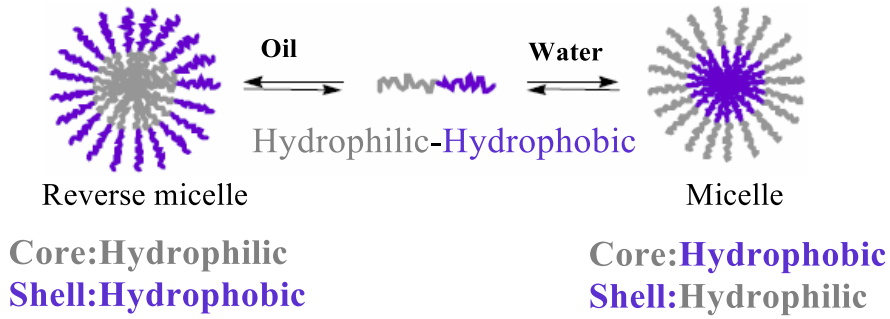


Figure 1.1 Self-aggregation in different solvents and formation of micelles.

The spontaneous formation of well-defined structures from the component of a system by non-covalent forces is known as self-assembly [Forster *et al.* (2002), Muthukumar *et al.* (1997)]. Due to the self-assembly phenomenon, micelles have definite morphologies and exhibit long-range order at higher concentrations. In block-selective solvents, the amphiphilic block copolymers self-assemble into a variety of structures *viz.*, micelles,

compound micelles, vesicles, tubes, and lyotropic liquid-crystal phases [**Figure 1.2**]. [Hamley *et al.* (2004)]

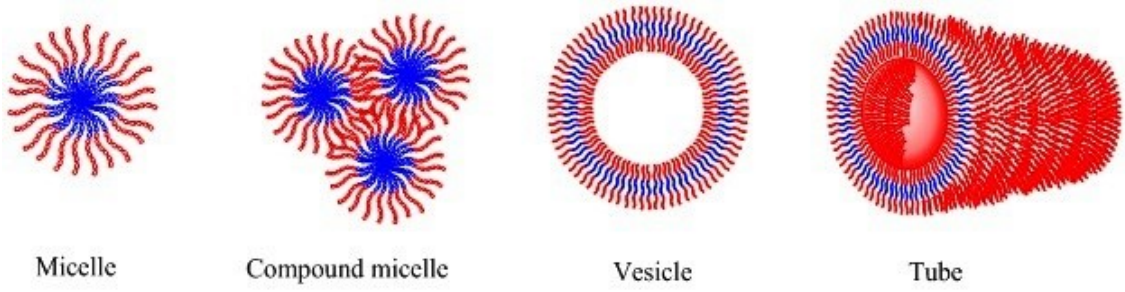


Figure 1.2 Examples of amphiphilic block copolymer's self-assemblies.

The morphologies can be controlled *via* the amount of water present in the medium, the initial copolymer concentration in the solution, the nature of the common solvent, the temperature, and the variations in the copolymer composition, the presence of additives such as ions, homopolymers, or, surfactants and the polydispersity of the copolymer chains. [Soo *et al*. (2004)]

1.2 Advantages of Amphiphilic Block Copolymers

The advantages of polymer self-assembly over the low molecular weight compounds such as lipids and surfactants, are the possibility of introducing additional mechanisms for colloidal stabilization, control over the polymer critical micelle concentration (*cmc*) [Forster *et al.* (1998)], lower permeability and improved stability of the amphiphilic polymer membranes [Mecke *et al*. (2006), Discher *et al*. (1999)]. These advantages might be useful in different technological applications. It is very important to mention that biological systems utilize biomaterials such as proteins and polysaccharides to

solve problems of hetero-phase stabilization. Such biomaterials have the same macromolecular architecture as the amphiphilic copolymers and their assembly at different length scales, time scales and levels of interaction which make these compounds very attractive for the uses in biological purpose. The most interesting examples of their potential applications are in medical diagnostics [Najafi *et. al* (2003) Kwon *et al*. (1998)] and re-establishment of biological molecules [Nardin *et. al* (2000), Nardin *et. al* (2001), Graff *et. al* (2002)]. The different such polymers are mostly used in biotechnology up to now. For example, block copolymers used as carriers of hydrophobic drug molecules in the hydrophobic core as well as hydrophilic compounds in its hydrophilic shell [Rijcken *et al*. (2007)]. The use of polymer micelles as drug delivery systems was established by the group of Ringsdorf in 1984 [Bader *et al*. (1984)]. From this time polymeric micelles are extensively studied as a capable for nanoscopic drug carrier because of their attractive features to implement for selective drug delivery [Allen *et al*. (1999), Adams *et al*. (2003), Lavasanifar *et al*. (2002), Jones *et al*. (1999), Torchilin *et al*. (2001). Compared to other drug-carriers, the advantage of polymeric micelles is their relative ease of fabrication because of their inherent self-assembly property. The hydrophobic micellar core has large capacity to accommodate hydrophobic drugs [Yokoyama *et al*. (1992), Rapoport *et al*. (2004), Liggins *et al*. (2002), Shuai *et al*. (2004)]. Polymer micelles have shown tolerance for many kinds of therapeutic agents and studied for the delivery of several kinds of drugs [Nishiyama *et al*. (2001), Nishiyama *et al*. (2003), Lin *et al*. (2003), Rösler *et al*. (2001)]. Drug loaded polymeric micelles also can be used in various ways of administration like oral drug delivery [Mathot *et al*. (2006), Sant *et al*. (2005)], intravenous administration etc.

[Rijcken *et al*. (2007)]. The vesicular type of self-assemblies is also used as drug carriers [Cerritelli *et al*. (2007)].

Another application of amphiphilic block copolymers is the synthesis of nanoparticles with a combination of traditional, colloidal synthetic techniques and the self-assembly process by amphiphilic block copolymers. A variety of metallic nanoparticles, like pure metals (Au, Ag, Pd, Pt, Cu, Co, Ni, and Ru), semiconductors (GaAs, CdTe, CdSe, CdS. ZnSe, AgBr), and metal oxides (Al_2O_3, TiO_2, CeO_2, Fe_3O_4, ZrO_2, ZnO, SnO_2) [Lee *et al*. (2008)] have been prepared by these methods.

1.3 Synthetic Approaches for Amphiphilic Block Copolymers

The well-defined block copolymers generally synthesized by living polymerization technique involving sequential block growth. The living polymerization techniques generally used are living anionic polymerization, living cationic polymerization [Kennedy *et al*. (1999)], living ring-opening polymerization (ROP) [Brunelle *et al*. (1993)], ring-opening metathesis polymerization (ROMP) [Bielawski *et al*. (2007)], group-transfer polymerization (GTP) [Webster *et al*. (1983), Webster *et al*. (2003)] and living radical polymerization (LRP) [Zard *et al*. (1997)]. Each of these methods is restricted to limited classes of monomers and functional groups involved. Another very convenient technique to synthesize block copolymer is click chemistry method [Kolb *et al.* (2001), Huisgen *et al.* (1984)]. Click chemistry is a very promising tool to synthesize amphiphilic block copolymer.

1.3.1 Living radical polymerization (LRP)

Free radical polymerization has gained much attention in the field of industrial polymer synthesis due to its simplicity, compatibility and convenience [Wang *et al*. (2000)]. The major limitation of conventional radical polymerization is its characteristically broad molecular weight distribution of the resulting polymers. This limitation is mainly due to the termination process between two propagating radicals. Until recently, ionic polymerization was the only practical route towards block copolymers with controlled molecular weight and architecture [Pyun *et al*. (2003)]. Since ionic synthesis techniques cannot be applied to many functional monomers and require rigorous exclusion of water and oxygen, LRP techniques have been utilized to synthesize many copolymers with various controlled architectures [Wang *et al*. (1999)]. The general feature of these techniques is the use of reagents which convert chain propagating radicals into a "dormant" form in equilibrium with the "active" form. Among the LRP techniques have been developed, atom transfer radical polymerization (ATRP) [Matyjaszewski *et al.* (2001)], reversible addition fragmentation transfer (RAFT) polymerization [Chiefari *et al*. (1998)], and nitroxide mediated polymerization (NMP) [Hawker *et al*. (2001), Solomon *et al*. (1985)] are the most common. In these techniques, the main feature is the dynamic equilibrium between actively propagating radicals and dormant polymer chains. Further, the reaction conditions must be selected such that the dormant species is favored in the equilibrium which results in persistent, low concentrations of propagating radicals. The normal radical termination reactions are effectively suppressed by the low concentration of propagating radical species. Each technique differs primarily in the chemistry of the cap on the dormant polymer chain.

1.3.2 Ring Opening Polymerization (ROP)

Polylactones and polylactides can be prepared by two different approaches, one is polycondensation of hydroxycarboxylic acids and the other is ring-opening polymerization (ROP) of cyclic esters. Polycondensation technique is less luxurious than ROP and also it is difficult to obtain well defined high molecular weight polymers with specific end groups, whereas with ROP we could achieve well defined copolymers with specific functional end groups. The ROP of lactones and lactides has been thoroughly investigated for the last 40 years, due to its versatility in producing a variety of biomedical polymers in controlled manner. Carothers and coworkers first extensively explored the ROP technique for lactones, anhydrides, and carbonates [Carothers *et al*. (1932), van *et al*. (1934), Carothers *et al*. (1930)]. Since then the method has been applied to diverse monomers to produce many types of polymers with different types of initiator and catalyst systems.

The literature [Duda *et al*. (2000), Kowalski *et al*. (1998), Kowalski *et al*. (2000)] in ROP described the influence of reaction conditions on the rate of polymerization and showed the livingness of the polymerization process. It was also proved that the concentration of the growing species remained constant throughout the process. Addition of carboxylic acid concentration temporarily converts the growing species into dormant molecules more, resulting in decrease of the polymerization rate whereas addition of alcohol in reaction medium increases the number of active sites, resulting in a higher polymerization rate.

In ROP, first step is the production of the active species *via* the reaction of alcohol with the catalyst, which starts the polymerization [**Figure 1.3**].

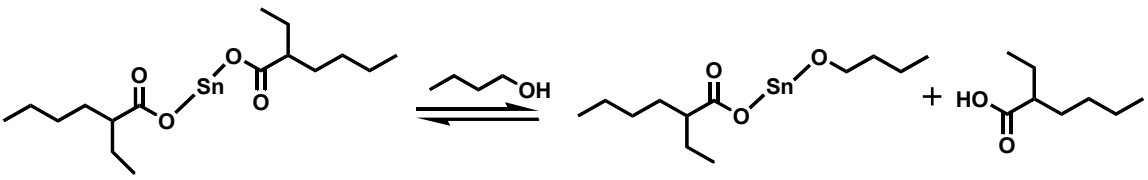


Figure 1.3 Formation of the active species for the ROP of ε-CL using tin(II) octoate as a catalyst.

On addition of more amount of alcohol, the equilibrium will be shifted towards the right and the more active species will be formed whereas with increasing carboxylic acid concentration, the equilibrium shifts to the left and active species concentration decreases in the medium. And this equilibrium exists throughout the polymerization [**Figure 1.4**].

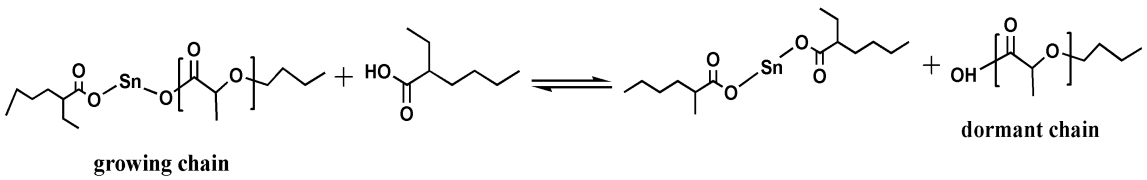


Figure 1.4 Formation of a dormant chain during the polymerization of D,L-lactide catalyzed by tin octoate.

Their formation is explained by the initial transformation of catalyst into an alkoxide. Subsequently, the polymeric chain is grown by the insertion of the monomers into the alkoxide bond [**Figure 1.5**]. Moreover, the concentration of initiator and the polymerization time, etc. influence the amounts of each compounds present in the medium. [Libiszowski *et al*. (2002)]

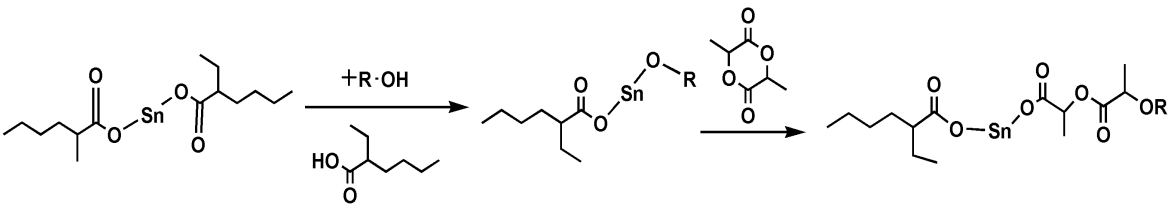


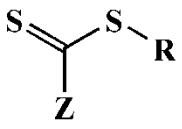
Figure 1.5 Initiation steps of the ROP of D,L-lactide initiated by an alcohol and catalyzed by tin(II) octoate. [Kowalski *et al*. (1998)]

Moreover, the primary alcohol compound, having a methyl group, is not a good initiator. Almost complete conversion can be reached and polymers with a minimum polydispersity can be obtained by the use of the more soluble secondary alcohols. However, the dimerization of the initiator through quadruple hydrogen bonding may occur which potentially increases the measured polydispersity. This is because, the dimer form cannot interact with the catalyst and as a consequence, formation of the polymeric chains is delayed until the initiator gets transformed into its monomeric form.

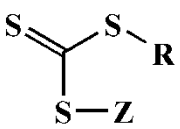
1.3.3 Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization

Among the existing LRP techniques, RAFT polymerization is perhaps the most versatile method, as it is tolerant to a wide variety of reaction conditions and functionalities, and can be performed in existing conventional free-radical polymerization set-ups [Chiefari *et al*.(1998), Le *et al*. (1998)]. RAFT proceeds via degenerative transfer process and relies on the use of compounds employed as chain-transfer (RAFT) agents. These agents are organic compounds possessing a thiocarbonylthio moiety, [Mayadunne *et al.* (1999), Destarac *et al.* (2000), Schilli *et al.*

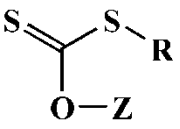
(2002)] for example, compound **1a**, **1b** and **1c**. The R group initiates the growth of polymeric chains and the Z group activates the thiocarbonyl bond towards radical addition and stabilizes the resultant adduct radical. [Charmot *et al.* (2000), Destarac *et al.* (2002), Pound *et al.* (2008)]



1a



1b



1c

The general accepted mechanism of RAFT polymerization is shown in [**Figure 1.6**]. The first step is the initiation, where a radical is created [step (i)]. The oligomeric radicals produced in the initiation step react with the RAFT agent (**1**) [step (ii)]. There is convincing evidence in the literature that all of the RAFT agents (if appropriately selected) are consumed in this step before any propagation commences [McLeary *et al*. (2005), Semsarilar *et al*. (2010)]. This is due to highly reactive C=S bond of the RAFT agent. It means that radical addition to the RAFT agent is favoured over the addition to any of the double bonds that are present on the monomer. The radical intermediate (**2**) can fragment back to the original RAFT agent (**1**) and the oligomeric radical or, fragment to yield an oligomeric RAFT agent (**3**) and a reinitiating R radical. The structure of R should be such that it is a good reinitiating group. It should also fragment as quickly as the initiator or polymer chains from the stabilized radical intermediate (**2**). Following re-initiation, polymer chains grow by adding monomer [step (iii)], and they rapidly exchange between existing growing radicals (as in the propagation step) and the species capped with a thiocarbonylthio group [step (iv)]. The rapid interchange in the

chain-transfer step *via* the formation of intermediate limits the termination reactions. But still limited termination reactions occur *via* combination or, disproportionation mechanisms (step v).

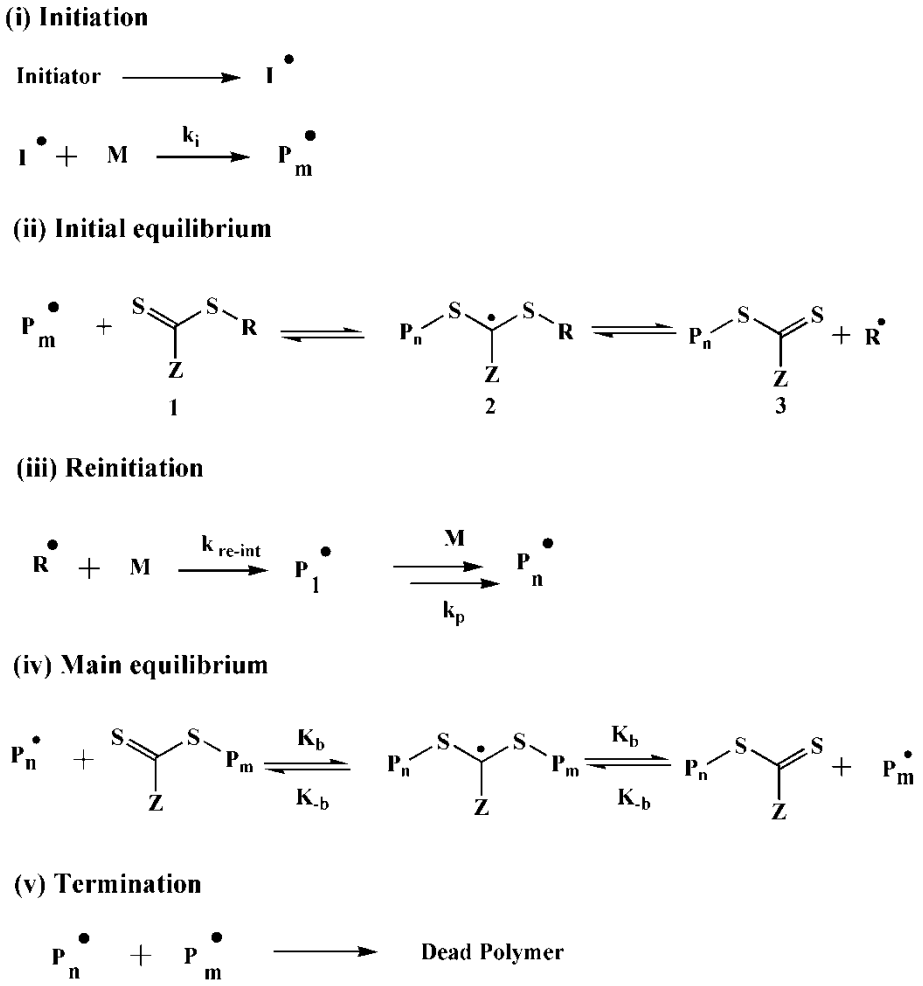


Figure 1.6 General accepted mechanism of RAFT polymerization.

The structures of the R and Z groups (see compound **1a**, **1b** and **1c**) are of critical importance for a successful RAFT polymerization. The R group of a RAFT agent is important in the pre-equilibrium stage of the polymerization. The R group should be a better leaving group than the propagating radical and must efficiently reinitiate

monomer as an expelled radical. Most of the monomers that are polymerized via conventional free-radical polymerization can also be prepared using the RAFT methodology. This opens up the route to a wide range of functionality and makes the RAFT process to be the choice to produce functional polymeric architectures. Styrene derivatives, acrylate and acrylamides, methacrylates, and methacrylamides [Vasilieva *et al*. (2004), Donovan *et al*. (2002)] and vinyl esters [Chiefari *et al.* (1998), Le *et al.* (1998), Convertine *et al.* (2003), Vasilieva *et al.* (2004),] are typical classes of monomer used in RAFT polymerization.

1.3.4 Click Chemistry

Barry Sharpless has introduced "Click" chemistry in 2001. He defined Click chemistry as the generation of complex substances by bringing together smaller units *via* hetero atoms. This is inspired by the fact that nature also generates substances by joining small modular units. While a range of chemical reactions can fulfil these criteria. Good examples often originate from five broad classes of reactions that appear to fit the framework of "Click" chemistry exceptionally well:

1. Addition to carbon–carbon multiple bonds.
2. Carbonyl reactions of the non-aldol type.
3. Cycloaddition of unsaturated species: 1, 3-dipolar cycloaddition.
4. Cycloaddition of unsaturated species: [4+2]-cycloaddition (Diels–Alder).
5. Nucleophilic substitution/ring-opening reactions.

Therefore, the term "Click" refers to energetically favoured, specific, a versatile chemical transformations, which lead to a single reaction product. The essence of click chemistry is its efficiency and simplicity. Yet, the last few years saw the emergence of

Click toolbox, which includes, for example, Diels–Alder cycloadditions, thiol–ene additions, oxime formation and copper(I)-catalysed Huisgen azide–alkyne cycloadditions (CuAAC).[Lutz *et al*. (2008)] However, CuAAC has rapidly become the most popular Click reaction to date and in recent literature, the term Click chemistry has been used almost exclusively to denote these reactions.[Kolbe *et al*. (2003)] Dimroth in the early 1900's reported first time the formation of triazoles *via* the cycloaddition of azide and acetylene. But the generality, scope and mechanism of these cycloadditions was not fully realised until in the 1960's developed by Huisgen. [Huisgen *et al*. (1961)] In the absence of a transition-metal catalyst, these reactions are not regioselective, relatively slow, and require high temperatures to reach acceptable yields. The reaction generates a mixture of 1, 4- and 1, 5-disubstituted triazoles, [**Figure 1.7**]

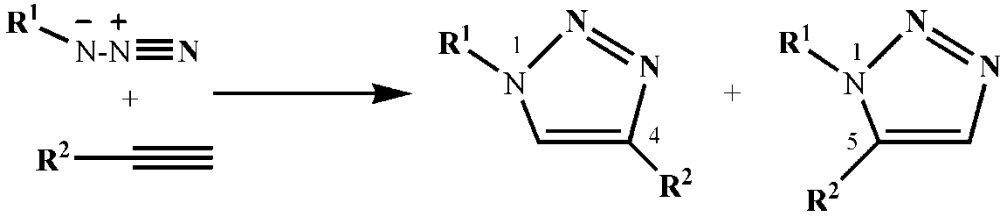


Figure 1.7 Huisgen 1, 3-dipolar azide–alkyne cycloaddition

Various attempts to control the regioselectivity have been reported without much success until the discovery of the copper (I)-catalysed reaction in 2002 by Sharpless and Meldal, [Rostovtsev *et al.*(2002), Tornoe *et al.* (2002)] which exclusively yields the 1, 4-disubstituted 1, 2, 3-triazole. This type of Click reactions is highly efficient and specific. Moreover, these are experimentally simple needing no protection from

oxygen, requiring only stoichiometric amounts of starting materials and generating virtually no by-products. Furthermore, click chemistry is a benign chemistry. Sharpless and Fokin have demonstrated that CuAAC can be successfully performed in polar media, such as tert-butyl alcohol, ethanol or pure water. Numerous authors collectively demonstrated that CuAAC is a true example of efficient and versatile Click reaction. [Collman *et al*. (2004), Helms *et al*. (2004), Lutz *et al*. (2004), Tsarevsky *et al*. (2005)]

1.4 Polymer Segments Employed for the Synthesis of Amphiphilic Block Copolymers

1.4.1 Poly(D,L-lactide) (PDLLA)

Polylactide (PDLLA) or, poly(lactic acid) (PLA) is a bioabsorbable, biodegradable, biocompatible and renewably derived thermoplastic polyester extensively investigated over the last several decades.[Gottschalk *et al*. (2006), Okada *et al*. (2002), Rasal *et al*. (2009), Ray *et al*. (2002), Zhang*et al*. (2006), Auras *et al*. (2004)] Lactic acid can be produced by converting sugar or, starch obtained from vegetable sources (e.g., corn, wheat, rice etc.) using either bacterial fermentation or, a petrochemical route. Lactic acid exists in two optical isomers, L- and D-lactic acid. Lactic acid produced by petrochemical routes is an optically inactive 50/50 mixture of the D and L forms.

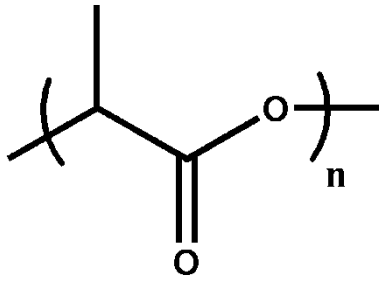


Figure 1.8 Structure of poly(D,L-lactide) (PDLLA)

Since the fermentation approach is more eco-friendly, it has been used more extensively. [Gupta *et al*. (2007)] Polymerisation of lactic acid to PLA can be achieved by a direct condensation process that involves solvents under high vacuum. Alternatively, in a solvent-free process, a cyclic dimer intermediate called lactide is formed followed by catalytic ROP of the cyclic lactide. Lactide can be found in three different versions, i.e., D,D-lactide, L,L-lactide, and D,L-lactide (meso-lactide). [Sodergard *et al*. (2002] The final properties of the polymer can be determined by the stereochemical composition of lactide monomers. [Sawyer *et al*. (2003] Due to the presence of water and impurities, with the direct condensation route, only low molecular weight ($M_w \sim 2 - 10$ kDa) polymers can be produced. [Garlotta *et al*. (2001)] Typically, low molecular weight PLA has substandard mechanical properties. Therefore, it suffers from the need for the use of solvents under high vacuum and temperature, water removal and increased colour and racemisation of PLA. Because of these disadvantages, the commercial manufacture of PLA commonly involves ring opening polymerization (ROP) of lactide. [Vink *et al*. (2003)] PLA degrades to form lactic acid, which is normally present in the body. The lactic acid decomposes to water

and carbon dioxide when it enters the tricarboxylic acid cycle. PLA degrades by hydrolysis and not by microbial attack. At higher temperatures and humidity high molecular weight PLA is not contaminated by microbes. [Bhardwaj *et al*. (2013)] This feature of PLA is good for applications where the polymer would be indirect contact with the human body or, foods and for this reason it has been approved by FDA. PLA can also be degraded by enzymes which accelerate hydrolysis of PLA as well as other biodegradable plastics and can be incorporated into the natural cycle of organic materials. [Masaki *et al*. (2005)] PLA takes only 3-4 weeks for complete degradation, if composted properly. The first stage of degradation is hydrolysis to water-soluble oligomers and lactic acid. PLA is clear, provides good gloss and clarity, but it is brittle and thermally unstable. In addition, the lack of reactive side-chain groups and the hydrophobic character of PLA limit the successful implementation of PLA without modifications in most practical applications. Therefore, it has been a challenging task to surface/bulk modify PLA. [Rasal *et al*. (2010)]

1.4.2 Poly(D,L-lactide-*co*-glycolide) (PLGA)

Poly(lactide-co-glycolide) (PLGA), from the ester family, has been widely used in the biomedical industry as a major components in biodegradable sutures, bone fixation nails and screws [Moghimi *et al*. (2001), Gombotz *et al*. (1995)]. Its degradation sub-products are nontoxic, it provides controlled drug release profiles by changing the PLGA copolymer ratio which affects the crystallinity (low crystallinity, more amorphous polymer means more fast degradation) of PLGA [Bala *et al*. (2004), Anderson *et al*. (1997)].

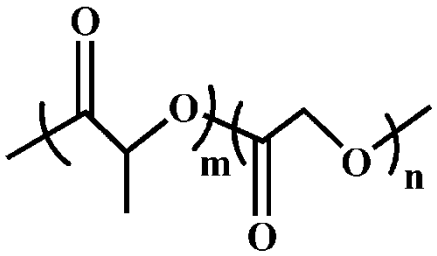


Figure 1.9 Structure of poly(lactide-co-glycolide) (PLGA)

It is a biocompatible, biodegradable synthetic polymer that is easy to fabricate into size-specific nanoparticles and has a well-documented ability for sustained therapeutic release.[Dinarvand *et al*. (2011), Kumari *et al*. (2010] PLGA nanoparticles still have significant limitations when it comes to using them to deliver therapeutics to a specific disease site.[Mahapatro *et al.* (2011), Soppimath *et al*. (2001)] One such limitation is that PLGA nanoparticles, when delivered intravenously, have no active targeting capabilities and are restricted to passive targeting *via* the enhanced permeability and retention (EPR) effect seen in cancerous and inflamed tissues.[Soppimath *et al*. (2001), Storm *et al*. (1995), Hans *et al*. (2002)]

1.4.3 Poly(*N*-vinylpyrrolidone) (PNVP)

In 1939, Fikentscher and Herrle prepared first PNVP via the free radical polymerization of *N*-vinylpyrrolidone. [Fikentscher *et al*. (1945)] During the Second World War its application was spread in a salt water solution as a synthetic blood plasma volume expander. Since that time the use of PNVP has been widely employed in medical science owing to its ease of manufacture, high biological activity, water solubility, zero toxicity and subsequent processing.[Kirsh *et al*. (1998)] PNVP is an industrially

important water soluble polymer that has many applications as a homo- or co-polymer ranging from use in drug delivery, [Lai *et al*. (1999), Rus *et al*. (2007)] cosmetics, [Vogel *et al*. (1989)] stabilization and clarification of beverages [McMurrough *et al*. (1998)] etc.

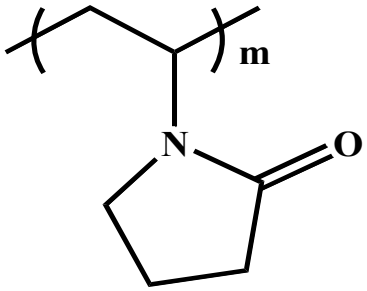


Figure 1.10 Structure of poly(*N*-vinylpyrrolidone)

It has also applications in adhesive sticks and water remoistenable adhesives, as a phase transfer catalyst, [Kondo *et al*. (1988)] a selective chelating agent for the separation of metals, [Del *et al*. (2006)] a food thickener. [Schwarz *et al*. (1990)] Unfortunately, NVP monomer is incompatible for use with living cationic or, living anionic polymerization due to the amide group present in it. Moreover, this monomer is incompatible with ATRP due to its tendency to form complexes with transition metal catalysts. It was not until recently that PNVP has been made in a controlled fashion using controlled radical polymerization techniques such as RAFT [Bindu *et al*. (2005), Ray *et al*. (2004), Wan *et al*. (2005)] and NMP [Bilalis *et al*. (2006)] polymerization methods which are far more tolerant towards impurities and functional groups. Before this, only traditional free radical polymerization technique has been employed. This technique has exhibited the same tolerances though offer little control over molecular weight distribution and only poorly defined PVP has been produced.

1.5 Literature Review

There are various reports on the synthesis, characterization and study of the physical properties of amphiphilic biocompatible block copolymers containing different hydrophobic and hydrophilic blocks. [Keddie *et al*. (2008), Ouchi *et al*. (2009), Moad *et al*. (2008), Moad *et al*. (2012), Yamago *et al.* (2009)] Among these, very few reports are on the amphiphilic block copolymers containing poly(D,L-lactide) and PLGA as hydrophobic blocks and poly(*N*-vinylpyrrolidone) (PNVP) as hydrophilic block.

Hydrophobicity of PLA is a serious challenge when using them for the biomedical applications. This could be convincingly solved by making the PLA into amphiphillic block copolymer by adding some hydrophilic block into it. This approach has been facilitated by preparation of well-controlled PDLLA containing amphiphilic block copolymers (ABPs) and could be used for drug delivery applications. These ABPs self-assemble in water and forming core/shell micellar nanoparticles (NPs) where hydrophobic core is capable of carrying a variety of hydrophobic therapeutic agents and hydrophilic coronas ensure water-solubility and biocompatibility of the NPs. This approach could be used for the hydrophobic drug delivery, where the hydrophobicity of PLA and its copolymers enhances the uptake of drug-loaded NPs through mononuclear phagocyte system (MPS), resulting in their short residence time in circulation. In addition, PLA-based ABPs have been explored for the development of other biomaterials, including crosslinked hydrogels as tissue engineering scaffolds and self-assembled metal hybrid nanomaterials as imaging platforms.

1.5.1 PDLLA-*b*-PNVP Block Copolymers

Very few articles were found on synthesis of PDLLA and PNVP block copolymers. Benahmed *et al.* (2001) reported first the synthesis, characterization and self-assembly properties of PNVP-*b*-PDLLA block copolymers prepared by conventional radical polymerization of NVP using 2-isopropoxy ethanol chain-transfer agent followed by conventional anionic ring-opening polymerization. They loaded poorly water-soluble drug indomethacin into PVP-*b*-PDLLA micelles and demonstrated that the entrapment efficiency was higher than the control poly-(ethylene glycol)-*b*-PDLLA micelles. They hypothesized that specific interactions with the hydrophilic outer shell could contribute to the increase in drug loading.

Later, Luo *et al*. (2004) reported the synthesis, characterization and self-assembly properties of PNVP-*b*-PDLLA block copolymer prepared through the combination of conventional radical polymerization of NVP in the presence of 2-mercaptoethanol chain transfer agent, and the ROP of DLLA using anionic ring-opening polymerization. They observed a control over molecular weight (MW) profile of PNVP, leading to number average MW as low as 2500 Da and polydispersity indexes close to 1.5. MALDI-TOF mass spectrometry indicated that the insertion of hydroxyl group on one chain end was quantitative. Further, they used hydroxyl-terminated PNVP as macro initiator in ring-opening polymerization of D, L-lactide yielding amphiphilic poly(*N*-vinylpyrrolidone)-*b*-poly(D,L-lactide) (PNVP-*b*-PDLLA) diblock copolymer with polydispersity indexes as low as 1.14.

Kang *et al*. (2004) reported the synthesis and self-assembling properties in water of novel A-B-A type triblock and star-block amphiphilic copolymers of *N*-(2-

hydroxypropyl) methacrylamide (HPMA) or, *N*-vinyl-2-pyrrolidone (NVP) and D,L-lactide (DLLA). These uncontrolled polymers were prepared via free radical polymerization of HPMA or, NVP in the presence of novel thiol-terminated PDLLA chain transfer agents.

Recently, Xiong *et al*. (2009) have reported the synthesis, characterization, and degradation of PDLLA-*b*-PNVP-*b*-PDLLA triblock copolymer prepared through the ROP of DLLA using dihydroxy-terminated PNVP as macro-initiator and dibutyl tin dilaurate (DBTDL) as catalyst. The triblock copolymers exhibited apparently microphase separations between hydrophilic PNVP segment and hydrophobic PDLLA segments. By combining hydrophilic PNVP segment with PDLLA, the degradation rate of copolymers apparently increased as compared with that of PDLLA homopolymer, and increased with increasing PNVP content. The degradation generated polymeric fragments, which included the PDLLA oligomers, lactates and soluble chains composed of PNVP blocks attached with short PDLLA ones. The new kind of copolymers with controllable degradability and good biocompatibility can be expected to have potential biomedical applications such as drug delivery systems.

Very recently, Shin *et al*. (2014) reported the amphiphilic, biocompatible poly(*N*-vinylpyrrolidone)-*b*-poly(L-lactide) (PVP-b-PLLA) block polymers using a hydroxyl-functionalized *N,N*-diphenyldithiocarbamate reversible addition–fragmentation chain transfer (RAFT) agent, 2-hydroxyethyl 2-(*N,N*-diphenylcarbamothioylthio) propanoate (HDPCP), as a dual initiator for RAFT polymerization and ring-opening polymerization (ROP) in a one-step procedure.

1.5.2 PLGA- based Block Copolymers

Few papers have been reported on synthesis of amphiphilic block copolymers with PLGA as a hydrophobic block. Nam *et al*.(2003) reported the synthesis of amphiphilic block copolymers composed of oligomeric polyethylenimine (PEI) and poly(D,L-lactide-*co*-glycolide) (PEI-*b*-PLGA) *via* direct coupling of PLGA having a carboxyl terminal group with PEI. They showed that the aggregated micelles of these amphiphilic block copolymers exhibited enhanced cellular uptake within cells presumably *via* endocytosis without cytotoxicity.

Qian *et al*. (2011) reported the controlled random copolymerization of glycolide and a racemic mixture of D- and L-lactide using poly(ethylene glycol) monomethyl ether (mPEG-OH) as a macro initiator. The resulting amphiphilic mPEG-*b*-PLGA block copolymers possessed well-controlled MWs with low polydispersities.

Later, Lin *et al*. (2011) reported the synthesis of PLGA-*b*-PEG-*b*-PLGA with similar chemical compositions and chain lengths but having different sequencing of D, L-lactide and glycolide in PLGA block. They showed that the macromolecular sequenced structure influences the hydrophobic/hydrophilic balance of these amphiphilic copolymers and thus alters their mesoscopic micellization and the forthcoming macroscopic physical gelation in water.

Freichels *et al*. (2012) reported synthesis of mannosylated PLGA-*g*-PEO copolymer and studied the interaction of their micelles with concanavalin A, a glycoreceptor, using quartz crystal microbalance.

Kun *et al*. (2014) reported the synthesis of amphiphillic diblock copolymers PEG-*b*-PLGA conjugated with folate at PLGA chain end and capecitabine (CAP) at

PEG chain-end. They showed that these block copolymers form self-assembled nanoparticles in aqueous solution upon blending with CAP and tetramethoxysilane.

Zhang *et al*. (2014) reported that PEG-*b*-PLGA copolymers have great potential in drug delivery systems as tumor-targeting carriers. The low critical aggregation concentration and small particle size of PEG-*b*-PLGA micelles increase the stability and prolong the circulation time.

Recently, Chen *et al*. (2014) reported the synthesis of PLGA-*b*-PEG-*b*-PLGA triblock copolymers *via* ring-opening polymerization of D,L-lactide and glycolide in the presence of poly(ethylene glycol) (PEG). They reported that molar-mass dispersity serves as a regulator of the condensed state of amphiphilic block copolymers in a selective solvent.

1.6 Aim of This Work

Above background of literature survey clearly shows that there is no report of synthesis of well-defined amphiphilic block copolymers containing a hydrophobic, biocompatible, and biodegradable segment as poly(D,L-lactide) (PDLLA) or poly(D,L-lactide-co-glucolide) (PLGA) and hydrophilic and biocompatible segment poly(*N*-vinylpyrrolidone) (PNVP) by the combination of the controlled ROP and the controlled metal-free xanthate-mediated RAFT polymerization and click chemistry. The main object of this work is to the (i) synthesis and characterization of controlled molecular weight, variable chain length, biocompatible amphiphilic block copolymers like linear (AB type), double hydrophilic (ABA type) and star shaped amphiphilic block copolymers [$(AB)_4$]; (ii) study of their self-assembly properties, thermal and crystalline properties of these block copolymers; and (iii) study of the application of the self-

assembly properties of such amphiphilic block copolymers as nano-carrier for drug delivery. Specifically, the aims of my work are as follows-

- Synthesis, characterization of Methotrexate-loaded poly(D,L-lactide)-*b*-poly(*N*-vinylpyrrolidone) amphiphilic block copolymers *via* the combination of ROP and xanthate mediated RAFT polymerization and study of their usefulness in *in vitro* drug delivery, cell viability, cytotoxicity, cellular growth inhibition, and apoptosis. **(Chapter 2)**
- Synthesis, characterization of Methotrexate-loaded well-defined four-arm star poly(D,L-lactide)-*b*-poly(*N*-vinylpyrrolidone) amphiphilic block copolymers and study of their usefulness in *in vitro* drug delivery, cell viability, cytotoxicity, cellular growth inhibition, and apoptosis. **(Chapter 3)**
- Synthesis, characterization of Doxorubicin-loaded well-defined poly(*N*-vinylpyrrolidone)-*b*-poly(D,L-lactide)-*b*-poly(*N*-vinylpyrrolidone) amphiphilic tri-block copolymers and study of their usefulness in *in vitro* drug delivery. **(Chapter 4**)
- Synthesis and characterization of novel well-defined poly(D,L-lactide-*co*-glycolide)-*b*-poly(*N*-vinylpyrrolidone) (PLGA-*b*-PNVP) amphiphilic diblock copolymers and study of their self-assembling properties and usefulness in *in vitro* drug delivery, cell viability, cytotoxicity, and apoptosis. **(Chapter 5)**