CHAPTER I

INTRODUCTION

This chapter deals with the recent developments of micro/nano particulates for drug delivery applications. viz. Conventional Pharmacotherapy, Controlled release methods, Controlled release mechanism, various synthetic strategies for the preparation of micro/nano particulates as controlled drug delivery systems viz., polymeric particulates, smart, pH sensitive and thermo responsive properties etc. Different methods of drug loading and in-vitro drug release kinetics details along with a brief survey of literature pertaining to the present study including the aim of the work is also included in this chapter.

I. INTRODUCTION

Recently, drug-loaded polymers in different matrices like hydrogels, Interpenetrating polymer network systems (IPNS), microspheres, nanoparticles in different forms of release devices have become very popular in medicine [1-7]. These systems consist of a drug in the polymer matrix in the form of a microcapsule or a granule. The advantages of these systems are that they exhibit controlled or slow release of the core active ingredient (AI) leading to longer application intervals, reduction in dosage, stabilization of the core AI against environmental degradation (light, air, humidity, and microorganisms), reduction in mammalian toxicity or fish toxicity and human mucous-membrane, irritation, phytotoxicity or fish toxicity, reduction in evaporation loses and leaching, environmental, pollution and drift, increase in the number of target organisms, and ease of handling of the toxic materials. However, a proper designing of the encapsulation system is important to achieve the desired release characteristics. In order to get the optimal performance of the micro encapsulated products, time-dependent or site-specific release is desirable depending up on the type of the matrix used. Thus, it is important
to develop various functional microcapsules/microspheres/IPNs/hydrogels that are specific to target organism/sites. To achieve the CR characteristics, some of the naturally and cheaply available, biodegradable and environmentally friendlier polymeric matrices have been used [8-10]. However, hydrogel based formulations involve cross-linking of the matrix in the presence of active agents or emulsification followed by separation of microspheres without wasting solvents. In this chapter, various methods used to prepare the polymeric controlled release formulations are discussed in addition to polymers used in drug delivery [10-12].

One of the most attractive areas of micro and nano research is drug delivery. This includes the design of micro and nano carriers, synthesis of nanomedicines and production of nano systems that are able to deliver therapeutic drugs to the specific organs or tissues in the body for appropriate periods. For drug delivery vehicles it is very important that these systems have good blood and biocompatibility properties. They themselves or the degradation products should not have any toxic, allergic or inflammatory effects. The systems should also protect the activity of the drugs and improve their transport through the biological barriers. If some specific functionality is added on the system, it would also be possible to deliver the drug to the target site where the system is stimulated by an appropriate signal. In the design and formulation of delivery systems, the key parameters are the size of the device, entrapment method, stability of the drug, degradation parameters of the matrix and release kinetics of drugs.

1.1. Conventional Pharmacotherapy

Human health is threatened by autoimmune, neurodegenerative, metabolic and cancer diseases, just to mention a few, which are difficult to treat systemically with delivered drugs. Conventional pharmacotherapy involves the use of drugs whose absorption and therefore bioavailability depends on many factors, such as solubility, pKa, molecular weight, number of bonds per hydrogen atom of the molecule, and chemical stability, all of which can hinder the achievement of a therapeutic response.

In general, the nature of conventional therapeutics, especially their low molecular weight, confers them the capacity to cross various body
compartments and access numerous cell types and sub cellular organ cells. Thus these drugs are suitable for the treatment of diseases. However, this form of indiscriminate distribution leads to the occurrence of side effects and to the need for higher doses of the drug to elicit a satisfactory pharmacological response. Rapid renal clearance as a result of the low molecular weight of these compounds, among other factors such as protein binding, lipophilicity, ionizability, etc., implies frequent administration and/or a high dose to achieve a therapeutic effect.

Research is being conducted into new formulations that ensure a greater pharmacological response, which in turn would lead to lower doses and therefore the minimization of side effects. Thus, it is necessary to improve the bioavailability of drugs. Bioavailability is affected by several factors, including the physical and chemical characteristics of the drug, the dose and concentration, the frequency of dosing, and the administration route. Therefore, research into drug delivery systems seeks to improve the pharmacological activity of drugs by enhancing pharmacokinetics (absorption, distribution, metabolism and excretion) and also by amending pharmacodynamic properties, such as the mechanism of action, pharmacological response, and affinity to the site of action.

Active pharmaceutical ingredients (APIs) are almost never administered alone but in dosage forms that generally include other substances called excipients. The latter are added to formulations in order to improve the bioavailability and the acceptance of the drug on the part of patients. Excipients come in numerous forms, such as emulsifiers, dyes, lubricants, diluents, supporters, and chemical stabilizers. These substances were initially considered inert because they do not exert therapeutic action either on person or do they modify the biological action of a drug. However, it is currently upheld that excipients influence the speed and extent of drug absorption, and therefore the pharmaceutical form of these substances affects drug bioavailability.

In this regard, recent years have witnessed intense research on the modification of drug release and absorption. The development of new drug delivery systems will offer additional advantages to those mentioned above and
may facilitate the launch of poorly soluble drugs. They may also allow an extension of patent protection for an API. And finally, and possibly most importantly, these systems will facilitate more patient friendly administration, thus resulting in patient increased compliance and satisfaction. The integral components of drug delivery systems are usually high molecular weight carriers, such as nano and micro-particles, nano and micro capsules, capsosomes, micelles, and dendrimers, in which the drug is embedded or covalently bound. Hence, the main function of polymeric carriers is to transport drugs to the site of action. Drugs are protected from interacting with other molecules which could cause a change in the chemical structure of the active ingredient causing it to loose its pharmaceutical action. Moreover, polymeric carriers avoid the interaction of the drug with macromolecules such as proteins, which could sequester the active ingredient preventing its arrival at the action place.

If a polymeric carrier is to be used, the next step is to design a type of polymeric structure that will permit obtaining the desired release conditions. Therefore, the polymeric structure should be: i) biodegradable, because the chemical bonds that make up its chemical structure break; ii) disassemblable, because the various pieces forming the polymer disassemble but the chemical bonds do not break; and iii) undisassemblable, because the chemical bonds do not disassemble or break, that is, the polymer remains unchanged. In the first two cases, micro sized polymeric carriers could be used. However, if the polymeric structure of the polymer is not biodegradable or disassemblable, then nano-sized polymers must be used for renal elimination. A crucial feature of these polymers is the mechanism by which they are removed from the body. They may be excreted directly via kidneys (renal clearance) or biodegraded (metabolic clearance) into smaller molecules, which are then excreted. Passage through the renal glomerular membrane is limited to substances with a molecular weight under 50 KDa, although this value varies depending on the chemical structure of the molecule [13-15]. Molecular weight is especially relevant for substances that are not biodegradable, and macro- molecules with a molecular weight lower than the glomerular limit can be safely removed from the body by preventing their accumulation and therefore their potential toxicity.
In the case of biodegradable polymers, another option to consider in their design is the chemical structure of the polymer (degree of hydrophobicity, covalent bonds between monomers, etc.), since the speed and degradation condition, and therefore, the rate and site of drug release, can be modulated depending on the chemical structure of the polymer used. If the polymer is not biodegradable, the drug can be covalently attached to the polymeric structure by a linker which can be degraded under different conditions such as in an acidic medium or by different enzymes. On the other hand, targets can be bound covalently to the surface which will help the directionality of the vector to the site of action.

Another strategy to follow would be to use smart polymers. These are polymers that release drugs when they are induced by a stimulation which causes a change in their structure. Therefore, the drug is released at the appropriate time and place. Therefore, these new formulations seek to improve the pharmaceutical profile and stability of a drug, ensure its correct concentration, achieve maximum biocompatibility, minimize side effects, stabilize the drug in vivo and in-vitro, facilitate the accumulation of the drug at a specific site of action, and increase exposure time in the target cell. Hence, the later resources concentration on controlled release of the drug and their different methods of preparations.

I.2. Controlled Release Methods

Controlled release systems aim to improve the effectiveness of drug therapy [16, 17]. These systems modify several parameters of the drug: the release profile and capacity to cross biological carriers (depending on the size of the particle), biodistribution, clearance, and stability (metabolism), among others. In other words, the pharmacokinetics and the pharmacodynamics of the drug are modified by these formulations. Controlled release offers numerous advantages over conventional dosage forms. This approach increases therapeutic activity and decreases side effects, thus reducing the number of drug dosages required during treatment. Controlled release methods offer an appropriate tool for site-specific and time-controlled drug delivery.
There are two main situations in which the distribution and time-controlled delivery of a drug can be beneficial. The first is when the natural distribution of the drug causes major side effects due to its interaction with other tissues, while the second is when the natural distribution of the drug does not allow it to reach its molecular site of action due to degradation. Many different kinds of drugs can be benefited from distribution or time-controlled delivery, such as anti-inflammatory agents [18-21], antibiotics [22], chemotherapeutic drugs [23], immunosuppressant [24], anesthetics [25] and vaccines [26].

**I.2.1. Time-Controlled: Modified-Release Formulation**

This formulation refers to the release of the drug through a system that protects and retains it. Through controlled release over time, this formulation allows the drug to reach a therapeutic concentration in tissues. The usual dose administration of a drug can achieve immediate (short-term) therapeutic concentrations of the active ingredient [27] (Fig. I.1)

![Figure I.1. Curve of drug concentration in plasma over time after a single dose](image)

**Figure I.1.** Curve of drug concentration in plasma over time after a single dose [28-31].

The figure shows that drug absorption [32] does not usually stop abruptly when it reaches the maximum concentration, but may continue for some time along the downstream portion of the curve. Drug absorption ceases with time once the bioavailable dose has been absorbed. Therefore, the elimination phase of plasma drug concentration depends on the rate of removal by metabolism or excretion.
The effective (therapeutic) concentration is the minimal drug concentration required to achieve the desired therapeutic effect. In contrast, the maximum safe concentration is drug concentration in plasma above which toxic or side effects occur. The interval between these two concentrations is the therapeutic window. The concentrations that yield the desired new therapeutic effect in the absence of toxic effects fall within this window. In a chronic treatment, doses are administered at regular intervals (multiple doses, shown in Fig.(1.2)). This approach can lead to variations in API concentrations, reaching levels that are lower or higher than therapeutic concentrations, in other words, concentrations that are neither effective nor toxic, respectively.

![Graph showing drug concentration over time](image)

**Figure 1.2.** Drug concentrations in plasma after delivery by conventional injection in a chronic disease. Representation of the therapeutic window between the minimal therapeutic concentration (therapeutic level, dotted line) and the maximum therapeutic concentration (toxic level, solid line). [28-31]

Chronic treatment calls for adherence to a pattern of drug administration because an incorrect number of dosages may exceed the limit of therapeutic concentration and thus result in a toxic response. In general, in chronic treatments drug doses are separated by two times the half-life of the drug in blood. The time required for a compound that has reached the systemic circulation of the body to reduce the maximum concentration by half is called the half-life. In contrast, a modified-release formulation is designed to deliver the active ingredient at a predetermined speed or at a site other than that of administration. These formulations can be classified on the basis of the systems that regulate
I.2.1.1. Extended-Release Formulation

The release of the active ingredient in these formulations is initially produced in a sufficient amount to produce a therapeutic effect and then to continue with a release, which is not necessarily constant but extends the therapeutic action. There are two types of extended-release formulations. One is a polymeric matrix that contains the active ingredient, the delivery of which is controlled by diffusion through the polymeric system network. These formulations are presented in the form of hydrogels and nano and micro-particles. The other type of extended-release formulation comprises API capsules with polymer coatings, forming a reserve deposit. The permeability or the degradation of the polymer coating regulates the release of the drug. There are some commercial products (tablets) such as Plenidil Er or Kapanol, which provide extended release of felopine or morphine surfate, respectively. Both tablets are administered orally and the drug is released by diffusion through the system [33, 34].

There are other commercially available products in which the degradation of the polymer coating regulates the release of the drug. An example of this formulation is Sinemet CR. The newest is a polymeric-based drug delivery system that controls the release of carbidopa and levodopa by slowly eroding the polymer’s coating, making the system particularly suitable in the treatment of Parkinson’s disease and syndrome [35]. Finally, it is important to make a few comments about the Drug-Eluting Stent (DES), which consists of supports coated with drug-loaded polymers. Generally, a stent is a metal support wire whose function is to maintain arteries, blood vessels or other anatomical ducts open. One interesting example is the cardiovascular stent, which is a device that is inserted into the coronary arteries when they are blocked to keep the arteries open and normal blood flow is resumed. Sometimes, this stent can be coated with a drug or polymer which controls the release of the drug, thus preventing the arteries from reclosing. Nowadays, several DES platforms have been developed and evaluated for clinical use. The difference between them has to do with the type of stent, anti-proliferative drug and polymer used to control drug release.
Depending on the type of polymer used, different drug release kinetics are obtained. San Juan et al. [36] functionalized a biocompatible polymer for stent coating for local arterial therapy. Therefore, metal stents were coated with a cationized pullulan hydrogel which was loaded with small interfering RNA for gene silencing in vascular cells. It was observed that the release of siRNA from the polymer was modulated by the presence of the cationic groups. Consequently, the release of the drug can be modulated depending on the type of polymer used. There are some DES which have been approved for clinical use such as Cypher and Taxus containing sirolimus and paclitaxel, respectively (Fig. I.3 and I.4).

**Figure I.3.** A) Nano or micro capsules whose degradation allows the release of the drug. B) Nano or micro particles of hydrogels whose swelling structure allows the diffusion of the drug through the pores of the structure. Taken from reference [28].
Figure I.4. Representation of the concentration of active ingredient versus time for a prolonged release formulation. The graph shows the therapeutic window between the minimal therapeutic concentration (therapeutic level, dotted line) and the maximum therapeutic concentration (toxic level, solid line). Taken from reference [28].

I.2.1.2. Sustained-Release Formulation

The active substance is steadily released with a kinetic profile of zero-order. The therapeutic effect is maintained over a long period of time Fig. (1.5). The closest pharmaceutical formulations to this type of system are osmotic pumps. These devices control the outflow of drug solutions through osmotic potential gradients across semi-permeable polymer barriers. The pressurized chambers contain the aqueous solution of the drug and the polymeric osmotic system. Upon immersion in the water, the osmotic system is hydrated and swollen, thus causing an increase in pressure, which is relieved by the flow of the solution out of the delivery device through an orifice in the upper part [37].

Figure I.5. Osmotic pumps. Taken from reference [29].
Drug delivery through osmotic systems is affected by a number of factors such as solubility, osmotic pressure, size of the delivery orifice and membrane type and characteristics. An example of this type of product is Adalat OROS. The OROS is a 24-hour controlled release oral drug delivery system in the form of a tablet, which acts as an osmotic pump releasing nifedipine [38]. The tablet has a rigid water permeable jacket with one or more laser drilled small holes. As the tablet passes through the body, the osmotic pressure of water entering the tablet pushes the active drug through the opening in the tablet. Once the active ingredient in the Oros tablet has been released, the remnant is excreted in the feces (Fig.I.6).

![Graph](image)

**Figure I.6.** Representation of the concentration of active ingredient versus time for a sustained release formulation. The graph represents the therapeutic window between the minimal therapeutic concentration (therapeutic level, dotted line) and the maximum therapeutic concentration (toxic level, solid line). Taken from reference [28].

1.2.1.3. **Pulsatile-Release Formulation**

Another form of time-controlled release is a responsive delivery system in which drugs are released in a pulsatile manner only when required by the body. Thus, the drug is released after a well-defined lag time. In general, these drug delivery systems comprise two components, namely a sensor that detects the environmental parameter that stimulates drug release, and a delivery device that releases the drug. Pulsatile drug delivery systems release the drug at the right time...
Design and Development of polymeric micro/nano drug formulations for drug delivery applications

and place and in accurate amounts, and they are convenient for patients suffering from chronic problems such as diabetes, arthritis, asthma, and hypertension [39]. The pulsatile release formulation implies the release of the drug only when required by the body, thus preventing it from being continuously in the biophase.

Insulin formulations currently require repeated injections daily, and hence responsive drug delivery systems that release insulin in response to increased blood glucose levels have been designed. For the treatment of diabetes, a pulsatile release formulation that uses the enzyme glucose oxidase as a sensor has been proposed [40]. When blood sugar levels rise, glucose oxidase converts glucose to gluconic acid, thereby lowering the pH. This decrease in pH causes insulin release because the pH-sensitive polymers (smart polymers) either swell or degrade in acidic environments. Another example of the above is the formulation developed and studied by Sadaphal et al. [41]. This formulation is constituted by an impermeable anionic polymer called Eudragit S100 which encapsulates theophylline. This system attempts to mimic the circadian rhythm of the disease by releasing the drug at the appropriate times (Fig.1.7).

![Figure 1.7](image)

**Figure 1.7.** Representation of the concentration of the active ingredient versus time for a sustained release formulation. The graph shows the therapeutic window between the minimal therapeutic concentration (therapeutic level, dotted line) and the maximum therapeutic concentration (toxic level, solid line). Taken from reference [28]
I.2.1.4. Delayed-Release Formulation

In this case, the release of the active ingredient does not coincide with the time of administration and its therapeutic action is not extended. Normally, these formulations are enteric coatings that protect the active ingredient against adverse physiological media or protect certain parts of the body against the hostile action of the active ingredient. Typically, these coatings are sensitive to pH and allow drug release into the small intestine, thus preventing its delivery to the stomach. Therefore, modified release formulations provide alternatives to the routine administration of the drug for improving its management and optimizing its therapeutic action. Thus, controlled release is highly beneficial for drugs that show poor bioavailability, in other words, those that are rapidly metabolized and eliminated from the body after administration and thus have a short life, or those with a narrow therapeutic window. Some drugs are unstable in certain environments, such as in plasma or in acidic conditions. Therefore, in these conditions, it is of interest to use a polymer to protect the drug. These formulations are also used in pathologies in which the degree of compliance is low because of difficult administration or unpleasant taste of the medicine.

I.2.2. Controlled Distribution

The treatment of most diseases requires the distribution of the drug at a specific site. It is therefore important to use drug carriers with a controlled distribution system. Several approaches are currently available to overcome the obstacles posed by nonspecific drug delivery. One method is to functionalize the surface of nano and micro particles with receptor recognition elements that are present in diseased tissue. Thanks to molecular biology, a comprehensive database of molecular targets has been built and it is now known that some specific receptors are over expressed in malignant tissue. Therefore, the identification of these over expressed targets is used to functionalize polymers with tracking devices, such as antibodies [42, 43], carbohydrates [44], or simply an electrically charged species, which interact with these targets [45]. Consequently, these tracking devices help the polymer reach the tissues and regions of the body where the drug is to be released [46-48] (Fig.I.8).
Another example of a controlled distribution system is drug release by diffusion through a hydrogel. To this end, the drug is encapsulated or absorbed in the hydrogel and released in response to an external stimulus. The stimuli that can be used in various systems to trigger drug release have been reviewed by Qiu et al. and Gupta et al.[49, 50].

- One interesting model is called the pH-dependent system, which is characterized by ionizable groups in its structure. These groups are ionized depending on the pH, and therefore an attractive interaction may occur between them through hydrogen bonding, or a repulsive interaction may be induced because of ionic groups with the same charge. Thus, a change in the structure of these groups opens or closes the route through which the drug is released.

- Other systems are controlled by electrical signals. This stimulus can change features in the structure of the formulation, such as pore size and permeability.

- Another approach is the use of hydrogels, the behavior of which differs in response to temperature. Also of note are those systems that are triggered by external stimuli in the form of variations in the concentration of certain substances, such as glucose, urea and morphine.
The controlled release of an active ingredient can also be achieved by connecting the drug to the polymer through a specific linker [51] that can be degraded in an acidic environment or by a specific enzyme. Thus, the drug is released from the polymer when the entire system is exposed to either of these two conditions, such as in tumor tissues where the pH is lower than in healthy tissues, in (a) an endosomal acidic environment, and in (b) a lysosomal environment, which contains proteolytic and hydrolytic enzymes.

The introduction of macromolecules into cells by endocytosis [52] involves the invagination of the plasmatic membrane to form a small vesicle called the endosome. This early endosome matures and finally fuses with the lysosome, which contains hydrolytic and proteolytic enzymes in an acidic environment, thus forming what is known as a secondary lysosome. Therefore, in this environment within the lysosome, the linkers that are labile to acids or digestive enzymes are cleaved, causing drug release [53]. It is important to highlight that these linkers are stable in plasma and are degraded only in tumor tissues or in lysosomal or endosomal [54] microenvironments. Tumor tissues or early endosomes have a slightly acidic pH, where labile acid linkers such as cisaconityl or hydrazone can be degraded [55]. For example, the proteins carried by the polymer and conjugated in it with an acid-labile linker are released into the endosome because peptide bonds are degraded in the lysosome. Once the linker has been biodegraded, the drug is released from the endosome or lysosome by diffusion to the cytosol (Fig.I.9).
The linkers most frequently used are peptides and oligosaccharides. Linker libraries have been established by VectraMed, based on enzyme cleavage specificity of a selected disease. One example is the enzyme family of Cathepsin, which includes lysosomal endoproteases, Cathepsin B being among these [56], a molecule localized at the invasive edges of human tumors and therefore over expressed in many types of cancer. Other examples of proteins include Prostate Specific Antigen (PSA), which is expressed by prostate tumors, and Proline peptidases, which hydrolyze proline sites. These peptidases are over expressed in pulmonary hypertension and during inflammation.

**1.2.3. Diffusion-Controlled Systems**

Diffusion controlled systems have been developed by incorporating a drug into the core surrounded by a polymer membrane (reservoir systems) and/or by distributing the drug throughout the inert polymeric matrix (matrix devices). Common methods used to develop the reservoir type devices are: (1) preparation of microencapsulated drug particles and then palletizing to a tablet or putting into the capsules [57, 58] and (2) coating of the drug containing tablets with suitable polymers [58-61]. In this case, the release rates depend on coating additives [62, 63] and thickness of the coating material [64]. In the membrane coated systems, water

![Figure I.9](image-url)
diffuses into the membrane or the matrix, the drug dissolves in it, and then the dissolved drug diffuses out of the polymer where diffusion of water through the polymer becomes rate determining step [65]. If we use a proper polymer as an encapsulating material, the release will be diffusion controlled or otherwise it will be a combination of dissolution and diffusion.

In case of matrix systems, the solid drug dissolves from the surface layer of the matrix and when this layer gets reduced to the minimum of the drug, then the next layer begins to deplete by dissolution so that drug diffusion takes place through the matrix to the external medium. Hence, the rate of release depends upon the amount of the drug present at a particular time and the release rate becomes time-dependent [66, 67]. Several acrylic resins, synthetic [61, 68, 69] and natural [70, 71] polymers have been used to develop such diffusion controlled systems.

**I.2.4. Dissolution-Controlled Systems**

Dissolution-controlled systems are those, which are prepared by coating the drug with the slowly soluble materials (encapsulated dissolution systems) or by incorporating it into a tablet with a slowly soluble carrier (matrix dissolution systems). Encapsulated systems can be prepared by coacervation. Such dissolution matrix systems exhibit bulk degradation [66, 72]. Thus, degradation occurs throughout the polymer structure in a random fashion so that all the active materials will be available immediately for dissolution and absorption, which in turn depends upon the volume/thickness ratio of the matrix undergoing erosion. Hence, the release can be controlled by compressing the coated materials of different coating thickness into a tablet or placed into a capsule. But, the release rate is unpredictable, and the entire dose dumping can occur [73]. These problems have been eliminated by choosing a matrix system that displays surface rather than bulk degradation, in which, drug release rate is proportional to polymer degradation rate [74].

The matrix dissolution systems can be prepared by dispersing the drug in a polymer matrix. These matrix systems can be easily modified due to therapeutic requirements with minimum manufacturing technology. Release of the active ingredients from these systems is due to dissolution of the drug-dispersed matrix that is in immediate contact with the external dissolution media. In case of swell able
matrix systems, the release rate depends on the rate of water uptake, the rate of drug dissolution and the rate of matrix erosion, associated with the movements of the swelling front, diffusion front and erosion front, resulting to difficulty in achieving a constant release rate. On the other hand, in case of dissolution matrix systems, swelling and surface erosion are occurring simultaneously, which is the cause for drug release.

I.2.5. Controlled release mechanisms

Mechanisms for CR of drugs involve: (1) solvent activation, (2) diffusion, and (3) chemical reaction. Solvent activated systems may be either: osmotic or swelling controlled. A simple osmotic device (for water soluble agents) consists of a semi permeable membrane with an orifice, surrounding an osmotic drug core. When the device is introduced to an aqueous environment, water is absorbed at a controlled rate and a volume of saturated drug solution is released. The rate of drug release is constant as long as excess solid is present in the osmotic drug core. In swelling controlled systems release rate of the active agent is controlled by swelling rate of the polymer matrix. In diffusion controlled systems, release rate of the active agent is controlled by diffusion of active agent from an insoluble polymer. Diffusion controlled and swelling controlled systems may be either reservoir-type devices in which a drug formulation is present as a core surrounded by a polymer membrane, or monolithic devices where a dispersed or dissolved drug is uniformly distributed through a polymer matrix (Fig I.10.a, I.10.b). Chemically controlled systems may release drugs via polymer degradation or cleavage of drug from a polymer chain [75].
**Figure I.10.** Drug release from (a) reservoir and (b) monolithic swelling controlled release systems [76]

**I.2.6. Controlled drug delivery routes**

Drugs are introduced into the body by several routes. They may be taken by mouth (orally); given by injection (parenteral route); or implanted beneath the skin (subcutaneously); placed under tongue (sublingually); instilled in the eye (ocular route) for local (topical) or body wide (systemic) effects [77]. Drug delivery through the oral route has been the most popular method in pharmaceutical applications. In peroral administration drug is delivered to mouth, stomach, small intestine and colon. Drug carrier systems may be designed to release drugs in a controlled manner at the desired site. Gastrointestinal (GI) tract with its large surface area for systemic absorption is an attractive site of targeting drugs whereas colon-specific delivery has significance for the delivery of peptide and proteins [78].
Drug delivery to the skin (transdermal route) has been conducted for topical use of dermatological drugs. It has generated much interest, beginning with the introduction of scopolamine (a sedative drug depressing nervous system) patch in 1983. Skin is also considered as a possible site for systemic delivery of drugs. However, only eight drugs, which follow transdermal delivery, have been commercialized until the year of 2000 [79, 80]. Ocular route is rather difficult subject due to blinking and low permeability of cornea. Conventional formulations fail because of being rapidly eliminated from the eye. Therefore controlled release systems are preferred since they offer prolonged ocular residence time [81].

Parenteral administration has advanced greatly in recent years for systemic and local drug delivery. Systems for parenteral route have evolved from simple polymers to colloidal systems which provide extended time for the circulation of drugs in the blood stream. Nasal mucosa is considered to be the most permeable one of all the mucosal tissues, offering tremendous scope for peptide delivery particularly for vaccines. Several peptide formulations are on the market, but, high dose requirements and low efficiency of deposition on alveolar surfaces limits the use of nasal route [82].

1.2.7. Colon delivery

The development of drug delivery systems (DDSs) capable of selective release of drugs in the colon has received much attention in the last decades [83]. In addition to providing more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn’s disease and ulcerative colitis, colon specific delivery has an importance in oral delivery of macromolecular drugs. The colon may be the preferred absorption site for oral administration of protein and peptide drugs, because of the relatively low proteolytic enzyme activities in it. A colon-specifc drug delivery system should minimize drug release in the stomach and small intestine, and release the drug abruptly in the colon. This requires a system that can respond to physiological changes in the colon. However, the physiological factors along the gastrointestinal (GI) tract changes gradually. Enzymatic activity, motility, and fluid content decrease while pH increases. Since there is not a sudden change in the properties of the tract, colon-specific delivery becomes somewhat difficult. However, the presence of
specific bacteria in the colon and an increasing pH gradient may be utilized as triggering components in colon-specific drug release. Several approaches have been proposed for targeted colon delivery, namely, prodrugs, coating of pH-sensitive polymers, use of colon-specific biodegradable polymers, timed released systems, osmotic systems, bioadhesive systems, pressure controlled drug delivery systems, and micro flora activated systems [84, 85]. pH sensitive polymers have been used in delivery of low molecular weight drugs as well as high molecular weight protein drugs. pH sensitive hydrogels have potential in formulating site-specific drug delivery systems to GI tract due to pH gradient throughout the tract [86]. For example, treatment of colon cancer has been aimed by approaches of oral drug administration. A pH-sensitive polymer Eudragit P-4135F (methacrylic acid, methyl acrylate and methyl methacrylate copolymer) was used to prepare microspheres, and relatively high drug load for 5-Fluorouracil was achieved [87].

1.3. POLYMERIC MATRICES AS CONTROLLED DRUG DELIVERY DEVICES

The design and preparation of polymeric matrices have attracted a great deal of interest in bio-medical engineering, pharmaceutical applications and biomaterial science because of their tunable chemical and three dimensional physical structures, good mechanical properties and biocompatibility. These unique properties offer great potential for the utilization of polymeric matrices for drug delivery applications. Among many factors affecting therapeutic effectiveness and safety therapeutic agents are the ultimate fate and the clearance of the administered drugs. Perhaps, the most feasible approach to overcome unfavorable biodistribution or clearance is to develop a drug delivery system that can protect the drug from degradation and deliver it in accessible target cells in a controlled manner. A wide range of polymeric materials have been used in biomedical applications. The number of materials that are approved for human use, however, is small. Much attention has recently been paid to materials used in controlled drug delivery over a prolonged period of time for targeting drugs to the specific sites in cancer chemotherapy [88] and intracellular parasitic diseases [89]. If drug delivery devices are resistant to biodegradation, upon repeated treatment they can accumulate in the liver of the patient, which could eventually result in adverse effects. As a
consequence, many hydrophilic polymers have been banned from human use. Therefore, biodegradable polymers are required in these applications [90]. Biodegradable systems have the advantages because there is no need of surgical removal of the drug-depleted device, which can always be a source of infection [91]. Potentially, biodegradable matrix systems have several other advantages such as simplicity of design and predictability of release, if the release is controlled solely by matrix degradation [92]. For many medical applications, it is desirable to have a biodegradable polymer in the form of micro particles [93] with diameters ranging from below 1 µm to over 100 µm and nanoparticles [94] with their sizes ranging between 50 nm and 1000 nm. Their spherical geometry offers distinct advantages over the more conventional film or fiber geometrics, since micro particles can not only control delivery of the incorporated drug, but also protect the agents like proteins and peptides from photolytic degradation. Moreover, they can be subcutaneously, intravenously and intramuscularly injected.

Nanoparticles can have matrix type structure or can be nanocapsules with a reservoir-like structure, consisting of a solid shell and an inner liquid core. Various other particulate systems have been described, including emulsions [95,96], phospholipids vesicles [97, 98], microcapsules [99,100] and microspheres prepared from a variety of materials, such as polysaccharides [101-103] alkylcynoacrylates [104,105], lactic acid, related polymers [106, 107] and proteins [108-110]. All these forms truly represent the membrane applications of polymers. The drug release from biodegradable micro particles is governed by various properties of the polymer, drug and the carrier system [111]. Polymer dependent factors include molecular weight and molecular weight distribution, the copolymer ratio and distribution as well as crystallinity. Effective ways to increase drug release from microspheres are to increase drug loading or to decrease molecular weight of the polymer or to prefer amorphous to crystalline polymer. Important drug dependent parameters are the solubility of the drug in dissolution or biological fluids, the molecular weight and possible polymer-drug interactions. These can be influenced through changes in pH of the medium. Carrier dependent factors comprise the type of micro particle, drug loading, physical state of the drug in the polymer matrix, particle size and particle size distribution, porosity and internal structure of the micro particle [112]. An
increase in matrix porosity enhances the drug release because of the easier accessibility of the drug by dissolution fluids.

Porous microspheres are also essential to deliver high molecular weight substances, which cannot diffuse out of a nonporous matrix and to deliver substances, which have high affinity for polymer and are not released unless the matrix is eroded. In recent years, polymeric micro/nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release (CR) of drugs, drug targeting to particular organs/tissues, as carriers of DNA in gene therapy in the delivery of proteins and peptides through the peroral route of administration [113-124]. Polymeric matrices are useful in developing the controlled release devices for the effective delivery of drugs in order to improve the patient compliance by maintaining the desired drug concentration in plasma, which helps to achieve a better therapeutic effect. In case of conventional drug therapy, drug is rapidly released from its dosage form, reaching a maximum level, which may be a toxic level, and then decays exponentially to a minimum level, below which the drug is no longer effective until the next administration. In order to maintain the therapeutic level of the drug for longer periods and to decrease its toxic levels, many efforts have been made to use polymers as membrane devices [125-129]. Polymers have been used as coated membranes or as matrices to extend the release rates of the drug. In these systems, drug can be released from a device to the outer medium by diffusion or dissolution mechanisms.

I.4. TECHNIQUES OF PREPARATION OF MICRO/NANO POLYMERIC MATRICES

The choice of the technique mainly depends on the nature of the polymer used, the drug, the intended use, and the duration of therapy. Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors as mentioned below:

Preparation of microspheres should satisfy certain criteria:

a) The ability to incorporate reasonably high concentrations of the drug.

b) Stability of the preparation after synthesis with a clinically acceptable shelf life.

c) Controlled particle size and dispersibility in aqueous vehicles for injection.
d) Release of active reagent with a good control over a wide time scale.
e) Biocompatibility with a controllable biodegradability and
f) Susceptibility to chemical modification [130].
g) Reproducibility of the release profile and the method.
h) There should be no toxic product(s) associated with the final product.

The properties of polymer matrices have to be optimized depending on the particular application. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly advantageous to have preparation techniques at hand to obtain polymer micro/nano matrices with the desired properties for a particular application.

**I.4. Preparation of micro/ nanoparticles may be achieved by any of the following methods:**

**I.4.1. Methods for preparation of micro/ nanoparticles from dispersion of preformed polymer**

A) Solvent evaporation  
B) Nano precipitation  
C) Emulsification/solvent diffusion  
D) Salting out  
E) Dialysis  
F) Supercritical fluid technology (SCF)  
G) Spry drying method.

**A) Solvent evaporation**

Solvent evaporation was the first method developed to prepare PNPs. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform preformed polymer [131] were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. The emulsion is converted into a nano particle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional
methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods utilize high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized [131,132]. Lemoine et al [133] prepared PLGA nanoparticles of about 200nm by utilizing dichloromethane 1.0% (w/v) as the solvent and PVA or Span 40 as the stabilizing agent. Song et al.[134] prepared nanoparticles of PLGA with a typical particle size of 60–200nm by employing dichloromethane and acetone (8:2, v/v) as the solvent system and PVA as the stabilizing agent. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

![Schematic representation of the solvent-evaporation technique](image)

**Figure I.11.** Schematic representation of the solvent-evaporation technique [132].
B) Nanoprecipitation

Nanoprecipitation is also called solvent displacement method. It involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant [135-138]. The polymer generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension [139]. To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a non solvent of the polymer [140]. The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. The usefulness of this simple technique [139], is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvent produce a certain instability when mixed in water, spontaneous emulsification is not observed if the coalescence rate of the formed droplets is sufficiently high [141]. Although, acetone/dichloromethane are used to dissolve and increase the entrapment of drugs, the dichloromethane increases the mean particle size [142] and is considered toxic. This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water-soluble drugs. This method has been applied to various polymeric materials such as PLGA [136], PLA [143], PCL [144], and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA) [145,146]. This technique was well adapted for the incorporation of cyclosporin A, because entrapment efficiencies as high as 98% were obtained [147]. Highly loaded nanoparticulate systems based on amphiphilic cyclodextrins to facilitate the parenteral administration of the poorly soluble antifungal drugs Bifonazole and Clotrimazole were prepared according to the solvent displacement method [148].
C) Emulsification/solvent diffusion (ESD)

This is a modified version of solvent evaporation method [149]. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent in the opposite case. Subsequently, the polymer water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. This technique presents several advantages, such as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water soluble drug into the saturated aqueous external phase during emulsification, reducing encapsulation efficiency [132]. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs [139]. Several drug-loaded nanoparticles were produced by the ESD.
technique, including mesotetra(hydroxyphenyl)porphyrin loaded PLGA (p-THPP) nanoparticles [150,151], doxorubicin-loaded PLGA nanoparticles [152] plasmid DNA-loaded PLA nanoparticles [153] coumarin loaded PLA nanoparticles [154] indocyanine [155], cyclosporine (Cy-A)- loaded gelatin and cyclosporin (Cy-A)- loaded sodium glycolate nanoparticles [156].

![Schematic representation of the emulsification/solvent diffusion technique.](image)

**Figure 1.13.** Schematic representation of the emulsification/solvent diffusion technique.

**D) Salting out**

Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. The salting out procedure can be considered as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as poly (vinylpyrrolidone) or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the salting out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting out agent are then eliminated by cross-flow filtration. This technique used in the preparation of PLA, poly (methacrylic) acid, nanospheres leads to high efficiency and is easily scaled up. The
main advantage of salting out is that it minimizes stress to protein encapsulants [157]. Salting out does not require an increase of temperature and therefore, may be useful when heat sensitive substances have to be processed [158]. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticle washing steps[159].

**Figure I.14.** Schematic representation of the salting out technique

**D) Dialysis**

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PN [131, 135, 160-162]. Polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cut off. Dialysis is performed against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles. The mechanism of PNP formation by dialysis method is not fully understood at present. It is thought that it may be based on a mechanism similar to that of nanoprecipitation proposed by the Fessi et al. [135]. A number of polymer and copolymer nanoparticles[163-172] were obtained by this technique. Poly(benzyl-l-glutamate)-b-poly(ethylene oxide), Poly(lactide)-b-poly(ethylene oxide) nanoparticles were prepared using DMF as the solvent[173,174]. The solvent used in the preparation of the polymer solution affects the morphology and particle size distribution of the nanoparticles.
Chronopoulou et al.[175] reported a novel osmosis based method (Fig.I.15.) for the preparation of various natural and synthetic PNP. It is based on the use of a physical barrier, specifically dialysis membrane or common semi permeable membranes that allow the passive transport of solvents to slow down the mixing of the polymer solution with a non solvent; the dialysis membrane contains the solution of the polymer.

![Figure I.15. Schematic representation of osmosis based method for preparation of polymer nanoparticles [131].](image)

F) **Supercritical fluid technology**

The need to develop environmentally safer methods for the production of PNP has motivated research on the utility of supercritical fluids as more environmental friendly solvents, with the potential to produce PNP with high purity and without any trace of organic solvent[131,176,177]. Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods. Two principles have been developed for the production of nanoparticles using supercritical fluids

1. Rapid expansion of supercritical solution (RESS)
2. Rapid expansion of supercritical solution into liquid solvent (RESOLV).
**F.1. Rapid expansion of supercritical solution**

In traditional RESS, the solute is dissolved in a supercritical fluid to form a solution, followed by the rapid expansion of the solution across an orifice or a capillary nozzle into ambient air. The high degree of super saturation, accompanied by the rapid pressure reduction in the expansion, results in homogenous nucleation and thereby, the formation of well-dispersed particles. Results from mechanistic studies of different model solutes for the RESS process indicate that both nanometer and micrometer-sized particles are present in the expansion jet [178]. A few studies were carried out on the production of PNPs using RESS. Poly (perfluoropolyetherdiamide) droplets produced from the rapid expansion of CO$_2$ solutions. The RESS experimental apparatus consists of three major units: a high-pressure stainless steel mixing cell, a syringe pump, and a pre-expansion unit. A solution of polymer in CO$_2$ is prepared at ambient temperature. Before the solution leaves the nozzle, using syringe pump, it is pumped to the pre-expansion unit and is heated isobarically to the pre-expansion temperature. The supercritical solution is now allowed to expand through the nozzle, at ambient pressure. The concentration and degree of saturation of the polymer have a considerable effect on the particle size and morphology of the particles for RESS [179-182].

![Figure I.16. Experimental set-up for preparation of polymer nanoparticles by rapid expansion of supercritical fluid solution [131].](image-url)
F.2. Rapid expansion of supercritical solution into liquid solvent

A simple, but significant modification to RESS involves expansion of the supercritical solution into a liquid solvent instead of ambient air, termed as RESOLV [131,183]. Meziani et.al [184] reported the preparation of Poly (heptadecafluorodecyl acrylate) nanoparticles having an average size of less than 50 nm. Even though in RESS technique no organic solvents used for the formation of PNPs, the prime products obtained using this technique are micro scaled rather than nanoscaled, which is the main drawback of RESS. In order to overcome this drawback a new supercritical fluid technology known as RESOLV has been developed. In RESOLV the liquid solvent apparently suppresses the particle growth in the expansion jet, thus making it possible to obtain primarily nano sized particles [184-186].

Figure I.17. Experimental set-up for the rapid expansion of supercritical fluid solution into liquid solvent process [131].

G) Spray drying method

Spray drying is widely used in pharmaceutical and materials science to prepare capsules, granules, fine powders, and agglomerates. This method involves the use of a spray dryer, mainly consisting of atomizer and drying chamber. Solutions and suspensions of drugs, polymers, and particles are atomized to fine droplets. A stream of hot air induces quick evaporation of solvent from the droplets.
in a drying chamber, resulting in the formation of microspheres or microgels. The obtained particles settle into a bottom collector, which are further dried in a vacuum chamber or modified in separated experiments. The size of the resulting microspheres is determined by nozzle size, spray flow rate, atomization speed, and extent of crosslinking. This method has been explored to prepare drug loaded microspheres of CS [187-191] and HA [192] as biodegradable drug delivery carriers. An aqueous solution of these biopolymers containing crosslinkers was spray-dried with various drugs, such as cimetidine, sodium diclofenac, vitamin D2, ampicillin, betamethasone disodium phosphate, and cromolyn sodium salt. The resulting spheres had a size of 1–10 mm in diameter. Further encapsulation of the resultant bio-microspheres was achieved. As an example, CS-based microspheres with a size of 1.8–2.9 mm were prepared by spray drying method, and then encapsulated into Eudragits microspheres with a size ranging from 152 to 223 mm using an oil-in-oil solvent evaporation method [193]. The spray drying technique has also been used to prepare submicron-sized nanoparticles of silica/poly (L-lysine)/alginate [194]. In-situ reduction of Co$^{2+}$ ions resulted in the formation of magnetic cobalt silicate nanoparticles. The resulting nanoparticles could enter cells through endocytosis and degrade in fibroblast cells, suggesting that they may be suitable for targeted drug delivery applications.

1.4.2. Preparation of micro/ nanoparticles from polymerization of a monomer

To attain the desired properties for a particular application, suitable polymer nanoparticles must be designed, which can be done during the polymerization of monomers. Processes for the production of PNPs through the polymerization of monomers are discussed below

A) Emulsion polymerization

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable. The method is classified into two categories, based on the use of an organic or aqueous continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse micro emulsion, or into a material in which the monomer is not soluble.
Polyacrylamide nanospheres were produced by this method [195, 196]. As one of the first methods for production of nanoparticles, surfactants or protective soluble polymers were used to prevent aggregation in the early stages of polymerization. This procedure has become less important, because it requires toxic organic solvents, surfactants, monomers and initiator, which are subsequently eliminated from the formed particles. As a result of the non biodegradable nature of this polymer as well as the difficult procedure, alternative approaches are of greater interest. Later, poly (methylmethacrylate) (PMMA), poly (ethylcyanoacrylate) (PECA), and poly (butylcyanoacrylate) nanoparticles were produced by dispersion via surfactants into solvents such as cyclohexane, n-pentane, and toluene as the organic phase. In the aqueous continuous phase the monomer is dissolved in a continuous phase that is usually an aqueous solution, and the surfactants or emulsifiers are not needed. The polymerization process can be initiated by different mechanisms. Initiation occurs when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that might be an ion or a free radical. Alternatively, the monomer molecule can be transformed into an initiating radical by high-energy radiation, including $\gamma$-radiation, or ultraviolet or strong visible light. Chain growth starts when initiated monomer ions or monomer radicals collide with other monomer molecules according to an anionic polymerization mechanism. Phase separation and formation of solid particles can take place before or after termination of the polymerization reaction [132, 140, 197].

**B) Mini-emulsion polymerization**

Publications on the mini-emulsion polymerization and the development of a wide range of useful polymer materials have recently increased substantially. A typical formulation used in mini-emulsion polymerization consists of water, monomer mixture, co-stabilizer, surfactant, and initiator. The key difference between emulsion polymerization and mini-emulsion polymerization is the utilization of a low molecular mass compound as the co-stabilizer and also the use of a high-shear device (ultrasound, etc.). Mini-emulsions are critically stabilized, require a high-shear to reach a steady state and have an interfacial tension much greater than zero [131]. The various polymer nanoparticles were
C) Micro-emulsion polymerization

Micro-emulsion polymerization is a new and effective approach for preparing nanosized polymer particles and has attracted significant attention. Although emulsion and micro-emulsion polymerization appear similar because both methods can produce colloidal polymer particles of high molar mass, they are entirely different when compared kinetically. Both particle size and the average number of chains per particles are considerably smaller in micro-emulsion polymerization. In micro-emulsion polymerization, an initiator, typically water soluble, is added to the aqueous phase of a thermodynamically stable micro-emulsion containing swollen micelles. The polymerization starts from this thermodynamically stable, spontaneously formed state and relies on high quantities of surfactant systems, which possess an interfacial tension at the oil/water interface close to zero. Further more, the particles are completely covered with surfactant because of the utilization of a high amount of surfactant. Initially, polymer chains are formed only in some droplets, as the initiation cannot be attained simultaneously in all micro droplets. Later, the osmotic and elastic influence of the chains destabilize the fragile micro-emulsions and typically lead to an increase in the particle size, the formation of empty micelles, and secondary nucleation. Very small latexes, 5–50nm in size, coexist with a majority of empty micelles in the final product. The types of initiator and concentration, surfactant, monomer and reaction temperature are some of the critical factors affecting the micro emulsion polymerization kinetics and the properties of PNP [135, 192].

D) Interfacial polymerization

It is one of the well-established methods used for the preparation of polymer nanoparticles. It involves step polymerization of two reactive monomers or agents, which are dissolved respectively in two phases (i.e., continuous- and dispersed-phase), and the reaction takes place at the interface of the two liquids [198]. Nanometer-sized hollow polymer particles were synthesized by employing interfacial cross-linking reactions as polyaddition and polycondensation [199-201] or radical polymerization [202,203]. Oil-containing nanocapsules were obtained by...
the polymerization of monomers at the oil/water interface of a very fine oil-in-water micro emulsion [204]. The organic solvent, which was completely miscible with water, served as a monomer vehicle and the interfacial polymerization of the monomer was believed to occur at the surface of the oil droplets that formed during emulsification [140,205,206]. To promote nanocapsule formation, the use of aprotic solvents, such as acetone and acetonitrile was recommended. Protic solvents, such as ethanol, n-butanol and isopropanol, were found to induce the formation of nanospheres in addition to nanocapsules [207]. Alternatively, water-containing nanocapsules can be obtained by the interfacial polymerization of monomers in water-in-oil micro-emulsions. In these systems, the polymer formed locally at the water-oil interface and precipitated to produce the nanocapsule shell [208,209].

E) Controlled/living radical polymerization (C/LRP)

The primary limitations of radical polymerization include the lack of control over the molar mass, the molar mass distribution, the end functionalities and the macromolecular architecture. The limitations are caused by the unavoidable fast radical–radical termination reactions. The recent emergence of many so called controlled or ‘living’ radical polymerization (C/LRP) processes has opened a new area using an old polymerization technique [131,210-212]. The most important factors contributing to this trend of the C/LRP process are increased environmental concern and a sharp growth of pharmaceutical and medical applications for hydrophilic polymers. These factors have given rise to “green chemistry” and created a demand for environmentally and chemically benign solvents such as water and supercritical carbon dioxide. Industrial radical polymerization is widely performed in aqueous dispersed systems and specifically in emulsion polymerization. The primary goal was to control the characteristics of the polymer in terms of molar mass, molar mass distribution, architecture and function. Implementation of C/LRP in the industrially important aqueous dispersed systems, resulting in the formation of polymeric nanoparticles with precise particle size and size distribution control, is crucial for future commercial success of C/LRP [213]. Among the available controlled/living radical polymerization methods [214,215], successful and extensively studied methods are
1) Nitrooxide mediated polymerization (NMP)[216-220].
2) Atom transfer radical polymerization (ATRP)[221-227] and
3) Reversible addition and fragmentation transfer chain polymerization (RAFT) [228-230].

The nature and concentration of the control agent, monomer, initiator and emulsion type (apart from temperature) are vital in determining the size of PNPs. Of these, the nature of the control agent is critical in determining the particle size of the final product.

**F) Ionic gelation or coacervation of hydrophilic polymers**

Polymeric nanoparticles are prepared by using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation [231,232]. Amir Dustgani et.al [233], prepared Dexamethasone Sodium Phosphate loaded chitosan nanoparticles by ionic gelation method. This method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly (anion sodium tripolyphosphate). In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.
I.5. Drug loading and drug release kinetics

The active components are loaded over the microspheres principally using two methods, i.e. during the preparation of the microspheres or after the formation of the microspheres by incubating them with the drug/protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface adsorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer (monomer if used). Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives (e.g. cross linking agent, surfactant stabilizers, etc.), heat of polymerization, agitation intensity, etc. Release of the active constituent is an important consideration in case of microspheres. The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug. The release of drug from both biodegradable as well as non-biodegradable microspheres is influenced by structure or micro-morphology of the carrier and the properties of the polymer itself. The drugs could be released through the microspheres by any of the three methods, (i) osmotically driven burst mechanism, (ii) pore diffusion mechanism, and (iii) erosion or the degradation of the polymer. In osmotically driven burst mechanism, water diffuse into the core through biodegradable or non-biodegradable coating, creating sufficient
pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecule/polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres. The pore diffusion method is named so because as penetrating water front continue to diffuse towards the core. The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix. Drug release from the non-biodegradable type of polymers can be understood by considering the geometry of the carrier. The geometry of the carrier, i.e. whether it is reservoir type where the drug is present as core, or matrix type in which drug is dispersed throughout the carrier, governs overall release profile of the drug or active ingredients.

### I.1.6. In-vitro Drug Release Studies

Drug release from micro/nanoparticles and subsequent biodegradation are important in developing the successful drug-loaded formulations. The release rates of micro/nanoparticles depend upon: (i) desorption of the surface-bound/adsorbed drug, (ii) diffusion through the matrix, (iii) diffusion (in case of micro/nanocapsules) through the polymer wall; in case of biodegradable polymers it is by (iv) matrix erosion and (v) combined erosion/diffusion processes. On the other hand, drug release from hydrogel microspheres or nanoparticles occurs by swelling of the delivery systems, which can be monitored by maintaining the extent of cross-linking.

Methods to study in vitro release are by: (i) side-by-side diffusion cells with artificial or biological membranes, (ii) dialysis bag diffusion, (iii) reverse dialysis, (iv) ultracentrifugation or (v) ultra filtration. Despite continuous efforts in this direction, there are still some technical difficulties to study the in vitro drug release from micron and submicron size particles [234,235]. In order to separate the particles and to avoid the tedious and time-consuming separation techniques, dialysis has been used; here, the suspension of micro/nanoparticles is added to the dialysis bags/tubes of different molecular mass cut-off. These bags are then incubated in the dissolution medium for the release study [236-238]. Release profiles of the drugs from spherical particles depend upon the nature of the delivery
system. In case of a matrix device, drug is uniformly distributed/dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. A biphasic release is observed for the micro/nanoparticles i.e., an initial rapid release followed by a delayed release phase; the rapid initial release is due to the release of the drug migrated to the surface of the particles. However, the later phase is due to the diffusion of the drug from the matrix.

Recently, Polakovic, et al. [239], theoretically studied the release from PLA particles loaded with varying amounts (7-32 % w/w) of lidocaine. Two models were used to study the drug release: (i) by crystal dissolution and (ii) by diffusion through the polymer matrix. When the drug loading is < 10 % (w/w) (the drug is molecularly dispersed), the release kinetics shows a better fit to the diffusion model. The existence of lidocaine crystals at higher concentration (>10 %) is observed. Since the drug should dissolve first from the crystals and then diffuse from the matrix, the overall release mechanism was described by a dissolution model. The most commonly used equation for diffusion-controlled matrix system is an empirical equation proposed by Ritger and Peppas[240], in which early time release data can be fitted to obtain the diffusion parameters using,

\[
\frac{M_t}{M_\infty} = kt^n
\]

Here, \( M_t/M_\infty \) represents the fractional drug release at time t, k is a constants characteristic of drug-polymer system and \( n \) is an empirical parameter characterizing the release mechanism. If \( n=0.5 \), the drug diffuses and release out of the polymer matrix following a Fickian diffusion. For \( n > 0.5 \), anomalous or non-Fickian type drug diffusion occurs. If \( n = 1 \), a completely non-Fickian or case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to anomalous type diffusive transport [240,241].

1.7. Factors Affecting Drug Delivery

1.7.A. Control of microsphere size

Microsphere size can be affected by the polymer concentration in the second emulsion, temperature, viscosity, the stirring rate in the second emulsion step, and the amount of emulsifier employed. Considering the effect of polymer concentration, it has often been reported that increasing the concentration of
polymer in the second emulsion increases sphere size [242-246]. In another study, [247] used scanning electron microscopy (SEM) to show that sphere size was temperature dependent; lower and higher temperatures produced larger spheres whereas intermediate temperatures produced smaller spheres. Once again, different mechanisms dominated microsphere formation at different temperatures. At lower temperatures, the solution’s higher viscosity resulted in the formation of larger spheres; this has also been confirmed by other researchers [248]. Larger spheres were obtained at higher temperatures due to the higher rate of solvent evaporation which resulted in higher solvent flow pressure moving more material from the sphere center outward. Jail and Nixon et.al [249] studied the variation of sphere size with respect to the stirring rate and the influence of the emulsifier in the second emulsion step. It was shown that microsphere size decreased with increasing stirring rate since increased stirring results in the formation of finer emulsions. The authors employed a sorbitan ester as an emulsifier and reported a sharp drop in diameter when the sorbitan ester concentration was increased from 1 to 2%. Little change in diameter size was reported by increasing emulsifier concentration beyond 2%. It is possible that in this particular case, emulsifier packing was optimum at 2% concentration and that no more emulsifier could be adsorbed at the sphere surface above this concentration.

I.7.B. Factors affecting drug release rate

Controlled release is an attainable and desirable characteristic for drug delivery systems. The factors affecting the drug release rate revolve around the structure of the matrix where the drug is contained and the chemical properties associated with both the polymer and the drug. Conventional oral delivery is not rate controlled. A drug encapsulated in a slowly degrading matrix provides the opportunity for slower release effects, but polymer degradation is not the only mechanism for the release of a drug. The drug release is also diffusion controlled as the drug can travel through the pores formed during sphere hardening. In some cases, drugs containing nucleophilic groups can cause increased chain scission of the polymer matrix, which also increases the rate of drug expulsion.

Polymer molecular weight, drug distribution, polymer blending, crystallinity, and other factors are important in manipulating release profiles. The most desirable
release profile would show a constant release rate with time. However, in many cases release profiles are more complicated and often contain two main expulsion processes: the first being an initial burst of expelled medication from the sphere surface; the second, a usually more constant stage with release rates dependent on diffusion and degradation [243, 250,251].

Some researchers have been able to achieve a relatively constant release after the initial burst, some have been able to achieve close to zero-order kinetics without a significant burst effect, and others have obtained even more complex but adjustable profiles depending on the desired application [243, 252-255]. In the following discussion, the factors responsible for different release profiles will be discussed in terms of physical and chemical properties of the microsphere.

1.7.C. Polymer molecular weight

Degradation of polymer microspheres shows a clear dependence on the molecular weight (MW) of the polymer. In a study by park et.al [256], it was found that polymer spheres initially released small molecular weight oligomers by rapid diffusion regardless of MW, following the initial period, low MW degradation products were released. In spheres initially containing lower MW chains, the quantity of degradation products increased with time and the polymers making up the microspheres decreased in molecular weight. However, for spheres made from high MW polymers, the quantity of degradation products and the polymer MW remained constant for longer periods of time. Park [256,257] provided evidence suggesting that the varying degradation profiles occur due to the differences in glass transition temperatures ($T_g$) and crystallinity associated with polymers of different MW. The rate of drug release from particles containing higher MW polymers was initially high, followed by a decrease which was then followed again by an increase. The two-stage release profile suggested the presence of two dominating release mechanisms in high MW polymers. Degradation is the main release mechanism for low MW polymers after the initial burst stage [256], spheres containing high MW polymers likely undergo initial slow drug release due to diffusion, followed by the main drug release due to degradation [253] showed this by correlating observed drug release with microscopic observation of the microspheres; the drug release was fastest for the degradation of swollen spheres.
I.7.D. Crystallinity

Crystallinity in microspheres has been usually investigated by DSC or X-ray diffraction (X-RD) studies. DSC can detect phase transitions including the melting of crystalline regions, whereas X-RD directly detects the crystallinity properties of a material. Le Corre et al. [257], observed the crystallinity of a lipophilic drug in polymer microspheres by DSC. Usually, drug has been found to be molecularly dispersed inside a polymer matrix and crystallinity is not observed [257, 258-260]. However, in their case, Lecorre et al. [257] found that the relatively highly loaded drug existed in a particulate dispersion instead of a molecular dispersion, which is possibly due to its lack of solubility in the polymer matrix. [250]

Yuksel et al. [261], Used X-RD and DSC to investigate crystallinity and drug-polymer interactions. They observed that although a physical mixture of the drug and polymer exhibited crystallinity, the drug was amorphous after dispersion in the microspheres. Attempts to crystallize the drug inside the microspheres by annealing above the polymer’s \( T_g \), and by heat–cool cycles were unsuccessful showing clear molecular dispersion of the drug. In the same study it was shown that molecular dispersions may be more favorable than particulate dispersions for drug delivery since the drug was released more readily from a microsphere system than from a particulate form at pH 7.4. The polymer matrix likely disturbs drug crystallinity and initiates rate-controlled delivery with higher drug delivery efficiencies.

Edlund and bertsson et al. [262] suggested that degradation occurred first in the amorphous microsphere regions followed by a slower degradation in the crystalline regions. This suggests that the crystallinity in the polymer chains can affect the degradation rate. Furthermore, at the beginning of sphere degradation, the degree of crystallinity actually increased slightly. This was attributed to the crystallization of partly degraded chains and the preferential degradation of amorphous regions. Izumikawa et al. [263]. Studied polymer crystallinity and drug crystallinity employing PLA microspheres loaded with progesterone. At low drug loading (5%), X-RD and DSC showed that the polymer dominates the crystalline properties of the microsphere and no crystallinity arose from the drug; the drug was dispersed in the sphere. At high drug loading (30%), crystallinity was dependent on
the organic solvent removal process; at slow solvent removal rates sphere crystallinity was observed from both the drug and polymer but fast removal resulted in amorphous spheres. At high concentrations and slow solvent removal, the drug formed a particulate dispersion resulting in the presence of drug crystallinity. However, the faster solvent removal rate may have resulted in amorphous spheres by not giving the drug and polymer molecules adequate time to crystallize. Release profiles suggest that more amorphous spheres release the drug less rapidly than crystalline spheres. Therefore, the lack of polymer crystallinity suggests better drug dispersion and increased drug–polymer interactions. The drug release rate can be tailored by manipulating the degree of crystallinity; reduced crystallinity is favorable when slow release is desired.

**1.7.E. Effects of the loaded drug**

In some cases the drug employed can induce polymer chain scission through nucleophilic degradation. Typically this is observed in medications containing amines whose nitrogen atom is nucleophilic, just like the oxygen atom in water. Cha and Pitt [264] reported that satirically available amines increased the rate of polymer degradation. In the case of a less active, satirically hindered tertiary amine, polymer degradation was not significant nor was drug release, unless it was co-loaded with another drug capable of causing polymer chain scission. Other groups have also considered chain scission when reporting their results [257, 265, 266]. How the drug is distributed in the medium can also vary its release profile [255]. Drug release begins at the sphere surface followed by release from the inner layers of the sphere; therefore the diffusional distance between the initial drug locations inside the sphere affects the release profile [267, 268]. Drug uniformly dispersed in the sphere matrix can increase the initial burst effect.

Kakish *et al.* [255], have successfully modified the drug distribution in microspheres so as to obtain constant drug release. The microspheres were modified by stirring 10h dry spheres in an ethanol–water mixture followed by freeze-drying. The resulting distribution is believed to be characterized by an increase in drug concentration toward the center of the microspheres thus resulting in a more constant release rate [255], obtained a system with a relatively constant release rate over 10h. Their microsphere treatment methods significantly improved controlled
release when compared to untreated microspheres whose drug release rate decreases with time.

**I.7.F. Porosity**

The porosity in a system of spheres is determined during microsphere hardening as the organic solvent evaporates during preparation. As already mentioned above, sphere porosity can be controlled by changes in sphere preparation technique and differences in porosity do affect release kinetics [247,248]. This is noticeable in a study by Yanget *et al.* [247], where a highly porous matrix released a drug at a considerably higher rate than its non-porous counterpart. Other researchers also reported that sphere porosity affected the release profile in similar ways [269]. Therefore, when preparing microspheres, it should be kept in mind that increasing the number of pores should increase the release rate. Another factor related to sphere porosity is the already mentioned initial burst effect, which corresponds to a rapid initial release of drug and is normally followed by relatively-controlled linear release. This is attributed to the leaching which occurs at the outer wall of the sphere as it becomes hydrated [270]. This can be minimized by supporting the formation of a non-porous outer sphere skin which can be controlled by sphere fabrication temperature [247].

**I.7.G. Size distribution**

The release profiles are also dependent on the size of the microspheres; the rate of drug release was found to decrease with increasing sphere size [252,271-273]. Therefore, by mixing microspheres of different sizes is possible. Depiction of a core-shell microsphere containing a drug-loaded microsphere (in some cases only the drug) as the core and another polymer as the outer shell to obtain another degree of controlling release. More importantly, linear, zero-order kinetics is obtainable by combining the proper formulation of microsphere sizes. Narayani and Rao [252] have combined microspheres of different sizes to obtain linear release profiles. Employing gelatin microspheres with sizes of 1–3 mm, they successfully achieved good zero-order drug release. In a detailed study, [254] have also obtained a zero-order release by mixing microspheres of different sizes; sphere size was well controlled by fabricating spheres using the spray dry technique.
I.8. SMART POLYMERS

Recent years have witnessed increasing attention to smart polymers as drug carriers because their properties can be tailored to fit the requirements required for drug delivery. Hydrogels are a good choice for their use in controlled drug release because they are biocompatible and display satisfactory swelling properties in aqueous media. The main interest in studying these materials is to control the swelling by specific stimuli that will allow the controlled release of bioactive molecules. Stimuli such as pH, electrical signals, and temperature can change the features of the system, such as pore size and permeability. Therefore, once this stimulus causes polymer swelling, the drug is released through a diffusion mechanism. The volume of the hydrogel or the degree of swelling depends on the balance between the attractive and repulsive interactions present in the network. Thus, the combination of molecular interactions such as vander Waals forces, hydrogen bonds, hydrophobic interactions and electrostatic interactions, determine the degree of hydrogel swelling at equilibrium. Researchers modify these structural properties to design these intelligent polymers. The smart polymers that received most attention are those that respond to stimuli of temperature and pH because these are inherent variables of physiological systems.

I.8.1. pH-Sensitive property

These polymers are characterized by the presence of acid groups, such as carboxylic acids or sulfonic acids, or also basic groups, such as amines, in their structures. These groups can accept or donate protons depending on the pH of the medium, leading to ionization variations in their structure. In the case of polymers [274] that contain acidic groups, when the pH of the medium rises, the degree of polymer ionization increases. In contrast, in the case of polymers that has amino groups in their structure, when the pH of the medium rises, the degree of polymer ionization decreases. This change in the degree of ionization causes an alteration in the swelling as a result of electrostatic repulsion forces between the charges present. Therefore, a way to decrease the repulsion between charges is to ensure polymer swelling, thereby maintaining the charges as far apart as possible.
Classical monomers are acrylic acid (AAc), methacrylic acid (MAAc), maleic anhydride (MA), and N,N- Dimethylaminoethyl methacrylate (DMAEMA). But also polymers containing phosphoric acid derivatives have been reported [275, 276]. The pH responsive swelling and collapsing behaviour has been used to induce controlled release of model compounds like caffeine [277], drugs like indomethacin [278], or cationic proteins like lysozome [279, 280] and anti cancer drugs [281,282]. pH-sensitive hydrogels have been used to develop controlled release formulations for oral administration. Lowman et.al [283] studied the use of a hydrogel that responds to a change in pH for the transport of orally administered insulin. This drug is a peptide labile to proteolytic degradation in the acidic stomach. Therefore, the researchers examined a drug carrier that would protect the peptide in this organ and allow its release in the intestine where the pH is slightly alkaline. This drug carrier was poly(methacrylic-g-ethylene glycol) (P(MMA-g-EG)) and the peptide was absorbed in the preformed polymer. Thus this pH-responsive carrier protected insulin in the acidic environment of the stomach as a result of the intermolecular interaction that prevented the hydrogel from swelling. But once the microparticles reached alkaline and neutral environments, namely the intestine, the interactions that occurred previously were lost and the pore size of the hydrogel increased, thus allowing insulin release.

pH-sensitive hydrogels have also been used to produce biosensors. Generally, hydrogels are loaded with enzymes that change the local pH of the microenvironment inside the gels. For example, there is an enzyme that catalyzes the conversion of glucose to gluconic acid. The formation of gluconic acid leads to a decrease in local pH, thereby resulting in a change in the swelling of the hydrogel. This mechanism is widely used for the release of insulin [284].

The anionic pH-sensitive polymers were prepared by copolymerizing or blending with poly(acrylic acids). Peppas and coworkers [285, 286] reported the synthesis of anionic copolymers of methyl methacrylate (MMA) with hydroxyl ethyl methacrylate (HEMA) by bulk polymerization and cross-linked with tetra ethylene glycol dimethacrylate (TGDMA). These copolymers exhibit pH-dependent swelling. Since the copolymer of MAA has the pKₐ value ranging from 4.3 to 5.9, an increased
water uptake was observed at this pH range due to ionization of carboxylic acid group. In another study [287], copolymers of poly(acrylic acid) (PAA) and HEMA have shown that the release of oxprenolol was increased with an increase in pH of the dissolution media. For instance, diffusion coefficient at pH 2.9 was $0.3 \times 10^{-7}$ cm$^2$/sec. Also, the release of oxopronolol hydrochloride was relatively higher from the acrylic acid copolymer than the methacrylic acid polymer. The reason is that MAA containing polymers are more hydrophobic due to the presence of $\alpha$-methyl group [288].

**1.8.2. Temperature-responsive property**

Temperature responsive polymers and hydrogels exhibit a volume phase transition at a certain temperature, which cause a sudden change in the solvent state. Polymers, which become insoluble upon heating, have so called lower critical solution temperature (LCST). Systems which become soluble upon heating have upper critical solution temperature (UCST). LCST and UCST systems are not restricted to an aqueous solvent environment, but only the aqueous systems are of interest for biomedical applications. The change in hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intra- and intermolecular hydrogen bonding of the polymer molecules are favoured compared to a solubilisation by water. Thermodynamics can explain this with a balance between entropic effects due to the dissolution process itself and due to the ordered state of water molecules in the vicinity of the polymer. Enthalphic effects are due to the balance between intra-and intermolecular forces and due to salvation e.g. hydrogen bonding and hydrophobic interaction. The transition is then accompanied by coil-to-globule transition. There are also systems, which exhibit both LCST and UCST behaviour, but that is usually not occurring within the setting of the intended biomedical applications. Typical LCST polymers are based on N-isopropyl acrylamide (NIPAM) [289, 290], $N,N$-diethyl acrylamide (DEAM) [291], methyl vinyl ether (MVE) [292, 293], and $N$-vinyl caprolactam (NVCL) [294, 295] as monomers. Of these temperature sensitive polymers, NIPAM is probably the most extensively used. It exhibits a sharp phase separation in water (LCST) around 32°C. Below the LCST, the hydrogen bonding between amide groups in polymer and water molecules leads to the dissolution of polymer chains. Above the LCST,
the hydrogen bonds are broken and water molecules are expelled from the polymer, resulting in precipitation of the polymer. Recently NVCL also possessed very interesting properties for medical and biotechnological applications, e.g. solubility in water and organic solvents, biocompatibility, high absorption ability, and a transition temperature (33°C) within the setting of these applications [296]. The combination of thermo responsive monomer like NIPAM and NVCL with one of a pH responsive monomer yields double responsive copolymers [297]. Most applications are the change from room temperature to body temperature in order to induce a change in the physical properties for e.g. gelation especially in topical applications and in injectable biodegradable scaffolds. *In-vitro* applications in cell culture are also using stimulated swelling and collapsing of hydrogels with their change in surface properties.

**Table I.1.** Temperature responsive behaviour of polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>LCSTs (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly((N)-isopropylacrylamide) (PNIPAM)</td>
<td>32</td>
</tr>
<tr>
<td>Poly((N,N)-diethylacrylamide) (PDEAM)</td>
<td>25</td>
</tr>
<tr>
<td>Poly((N)-ethylmethacrylamide) (PNEMAM)</td>
<td>58</td>
</tr>
<tr>
<td>Poly(methyl vinyl ether) (PMVE)</td>
<td>34</td>
</tr>
<tr>
<td>Poly(2-ethoxyethyl vinyl ether) (PEOVE)</td>
<td>20</td>
</tr>
<tr>
<td>Ploy((N)-vinylcaprolactam (PNVCa)</td>
<td>30-35</td>
</tr>
<tr>
<td>Poly((N)-vinylisobutyramide) (PNVIBAM)</td>
<td>39</td>
</tr>
<tr>
<td>Poly((N)-vinyl-(n)-butyramide) (PNVIBAM)</td>
<td>32</td>
</tr>
</tbody>
</table>
Rameshbabu et al. [298], developed 5-fluorouracil loaded poly(acryl amide-co-methylmethacrylate) novel core-shell microspheres: In vitro release studies. The core shell microspheres were prepared by free radical emulsion polymerization using varying amounts of acrylamide, methylmethacrylate and MBA. The microspheres were characterized by DSC, X-RD and SEM. Core shell microspheres with different copolymer compositions have been prepared in yields ranging 80-85%. DSC and X-RD techniques indicated a uniform distribution of 5-fluorouracil particles in core shell microspheres. SEM suggested the formation of well defined core shell structures. Prolonged and controlled release of 5-fluorouracil was observed.

Mallikarjuna et al. [299], developed temperature sensitive semi-IPN microspheres from sodium alginate and N-isopropylacrylamide for controlled release of 5-Flurouracil. The semi IPN microspheres were characterized by FTIR, DSC and SEM. Studies were done on the drug loaded microspheres to confirm the polymorphism of 5-FU and surface morphology of microspheres. Microspheres exhibited the release of 5-FU up to 12h.

Rokhade et al. [300], reported novel interpenetrating polymer network microspheres of chitosan and methylcellulose for controlled release of the ophylline. Theophylline an antiasthmatic drug was encapsulated into microspheres under varying ratios of MC, and CS, % drug loading and amount of GA added. DSC, X-RD studies were performed to understand the crystalline nature of drug after encapsulation into IPN microspheres. Theophylline encapsulation of up to 82% was achieved as measured by UV spectrometer equilibrium swelling was performed in distilled water. In-vitro release studies were performed in both 0.1N HCl and pH 7.4 buffer solutions and the release were extended up to 12 h.

D. Hernan Perez de la Ossa et al. [301], Poly-ε-caprolactone microspheres as a drug delivery system for cannabinoid administration: Development, characterization and in-vitro evaluation of their antitumoral efficacy. PCL microparticles could be an alternative delivery system for long term drug administration. Showing potential therapeutic advantages over free drug delivery.
Xudong Wan et al. [302]. Drug distribution a within poly(ε-caprolactone) microspheres and in-vitro release studies. Katia P et al. [303] Poly (ε-caprolactone), Eudragit RS100 and Poly(ε-caprolactone), Eudragit RS100 Blend submicron particles for the sustained release of the antiretroviral efavirenz. Balmayar et al. [304] Preparation and characterization of starch ER– poly-ε-caprolactone micro particles incorporating bioactive agents for drug delivery and tissue engineering applications. Kiran Chaturvedi et al. [305] have synthesis poly(3-hydroxybutyrate) and Cellulose acetate Phthalate blend microspheres. The microsphere was studied in-vitro release studies in both basic and acidic conditions. A pH-dependent swelling mechanism was proposed on the basis of the formation of intramolecular complexation by hydrogen-bonding. The pH-dependent release of 5– Fluorouracil was observed. Mallikarjuna et al. [306]. Development of Triprolidine – Hydrochloride loaded pH-Sensitive Poly (Acrylamide-co-Acrylamido glycolic Acid) Co-Polymer Microspheres: In- vitro Release Studies of Triprolidine hydrochloride.


K. Madhusudana Rao et al. [312] development of novel nanogels (NGs) with both pH and thermo responsive properties were synthesized by free radical emulsion polymerization of N-vinyl caprolactam (NVC) and acrylamidoglycolic acid (AGA). 5-flurouracil, an anti cancer drug, was successfully loaded into these nanogels via equilibrium swelling method. The % encapsulation efficiency of 5-FU. Here we observed the novel potential drug delivery system showing both pH and temperature sensitive release of 5-FU. Fourier transforms infrared spectroscopy
(FTIR), and differential scanning calorimetric (DSC) was used to examine the structure and morphology of the NGs. Transmission electron microscopy (TEM) indicates the diameter of the NGs. The size distribution of NGs was investigated using dynamic light scattering (DLS), the average diameter and polydispersity, interestingly, the in-vitro release studies of 5-FU demonstrated the dual nature (pH and temperature) of NGs. The cumulative release data were analyzed using an empirical equation to compute the diffusion exponent (n); whose values suggest Fickian diffusion was observed.

K.M.Rao et.al. [313] synthesis and characterization of pH sensitive poly (Hydoxyethylmethacrylate-co-Acrylamidoglycolic acid) polymer-solvent interaction parameter of hydrogels Differential scanning calorimetry and X-ray diffraction were performed to understand the crystalline nature of hydrogel and drug after encapsulation into hydrogels. In vitro release studies indicated the release of 5-Fluorouracil for more than 12 h. was observed.

I.10. AIM OF THE PRESENT STUDY

From the literature it is noticed that a few or strictly to say that no attempts were made to use the synthetic monomers (N-vinylcaprolactam) (NVC), Cellulose acetatephthalate (CAP), Hydroxy ethyl methacrylate (HEMA), and 2-dimethylaminoethylmethacrylate (DMAEMA), to prepare polymer matrices for drug delivery applications. Under these circumstances non bio-degradable polymer (PMMA) and bio-degradable polymers like aliphatic polyesters are used to develop the polymeric controlled drug delivery systems and their applications for drug delivery has become important basis for the present study. These polyesters are cheaply available and give wide scope for structural modifications. The biodegradable polyesters, monomer units can be easily assimilated or easily eliminated from the body. Chemical modifications of these polyesters are also possible. Some of the widely used polyesters are from poly(glycolide) (PGA), poly(caprolactone) (PCL), poly (lactide) (PLA), and their derivatives etc. Chemical modification followed by crosslinking coverts them into micro particle /hydrogels, which plays an important role in the present work for drug delivery applications.
The present thesis aims to Design and development of polymeric micro/nano drug formulations for drug delivery applications. By using the more efficient synthetic bio-degradable polymer (PCL, PEO etc.), non bio-degradable polymer (PMMA), and smart monomers (HEMA, DMAEMA, NVC, CAP, etc.) in developing successful formulations for their large-scale commercialization for drug release studies. Thus, the theme of the thesis is timely and presents the comprehensive approach to the above mentioned problem. Details of each of these systems for drug release applications will be covered in subsequent chapters.
I.11. References


Design and Development of polymeric micro/nano drug formulations for drug delivery applications


Design and Development of polymeric micro/nano drug formulations for drug delivery applications


Design and Development of polymeric micro/nano drug formulations for drug delivery applications

Catarina Pinto Reis, Ronald J. Neufeld, Antonio J. Ribeiro, Francisco Veiga. 


Design and Development of polymeric micro/nano drug formulations for drug delivery applications


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