In recent years, interest in controlled and sustained release of drugs has increased as the pharmaceutical companies seek improved methods of delivering therapeutic dosages of medicines. Benefits to patients would be enhanced if dosages could be sustained for extended periods. Tunable, sustained delivery platforms are therefore necessary to achieve optimal and functional delivery of drugs. Micro particulate drug formulations are one of the widely researched candidates in such localized delivery platforms. This chapter deals with the recent developments of microencapsulating of bioactive agents into Interpenetrating Polymeric Networks based micro particulates for drug delivery characteristics. Various synthetic strategies for the preparation of micro particulates as controlled drug delivery systems viz., pH sensitive and thermo responsive properties have been included in this chapter. Different methods of drug loading and in-vitro drug release details are also included in this chapter. This chapter also includes a brief survey of literature pertaining to the present study including the aim of the work.

The development and application biodegradable, biocompatible natural or synthetic polymeric materials are widely used in the development of micro particulate drug formulations for drug and food delivery systems. Because enhance the material stability, and to reduce the adverse or toxic effects, or extend material release. The use of micro particulate drug formulations for drug delivery is not limited to any specific illness, rather they can widely applied in many situations where continuous/controlled/targeted drug administration is essential. So, there is need to develop new type of polymeric materials for making micro particulate drug formulations for controlled drug delivery applications.

1.1. Concept of Controlled drug delivery

Controlled drug delivery systems (CDDSSs) are designed to deliver drugs at predetermined rates for predefined periods of time. CDDSSs may be classified into two general concepts; one is targeting and other is controlled release. Systems delivering active agent to the desired tissues and organs are called as “targeted CDDSSs” and systems controlling the release rate of active agent are called as “CDDSSs” [1].

Controlled release (CR) systems were first developed in the 1950s and were originally used to administer nonmedical agents, such as antifouling substances and pesticides. They were
first used in medical research in 1960s and in the 1970s systems for slow release of large molecules were developed. The earliest drug delivery systems, first introduced in the 1970s, were based on polymers formed from lactic acid. Today, polymeric materials still provide the most important parameters for drug delivery research, primarily because of their ease of processing and the ability of researchers to readily control their chemical and physical properties via molecular synthesis [2]. In 1980s, several polymer drug conjugate systems became available in clinical use [3].

CR formulations are mentioned under different terminologies which differs slightly from each other, such as (1) delayed release, (2) prolonged release, (3) sustained release, and (4) repeat action dosage forms.

- In delayed release products; release of active substance is delayed for a finite “lag time”, after which release is, unhindered.
- In prolonged release products; the rate of release of active substance from the formulation after administration has been reduced, in order to maintain therapeutic activity, to reduce toxic effects, or for some other therapeutic purposes [4].
- Sustained release products are designed to release loaded dose to produce an immediate response, followed by a constant dose (maintenance dose) required to maintain a therapeutically effective level for some desirable period. Generally sustained release systems emit drugs in less than a day, and environmental conditions influence release rates, which leads to patient to patient variations.
- A repeat action dosage form is designed to release initially the equivalent of a usual single dose of drug. And then after a certain period another single dose of the drug is released [5].

I.2. Conventional drug therapy versus controlled release

Providing control over the drug delivery can be the most important factor with time when traditional oral or injectable drug formulations cannot be used. These situations requiring the slow release of water-soluble drugs, the fast release of low-solubility drugs, drug delivery to specific sites. The ideal drug delivery system should be inert, biocompatible, mechanically
strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize.

CR systems aim to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. The traditional formulations, the drug level in the blood follows the profile shown in Fig I.1.a, in which the drug level rises after each administration of the drug and then decreases until the next administration. With traditional drug administration the blood level of the drug exceeds toxic level immediately after drug administration, and falls down below effective level after some time. Controlled drug delivery systems are designed for long-term administration where the drug level in the blood follows the profile shown in figure I.1.b, [6].

Mechanism for controlled release 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 semipermeable membrane with an orifice, surrounding an osmotic drug core. When the device is introduced into 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

Figure I.1: Drug levels in the blood plasma (a) traditional drug dosing and (b) controlled-delivery dosing.

Mechanism for controlled release 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 semipermeable membrane with an orifice, surrounding an osmotic drug core. When the device is introduced into 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 as shown in (Fig I.2.a, I.2.b). Chemically controlled systems may release drugs via polymer degradation or cleavage of drug from a polymer chain [10].

![Figure I.2: Drug release from (a) reservoir and (b) monolithic swelling controlled release systems [7].](image)

The idea of controlled release from polymers dates back to the 1960s through the employment of silicone rubber and polyethylene polymer microcapsules as delivery systems [7]. However, the requirement of eventual surgical removal because of nondegradability of these systems limited their applicability and urged the need to prepare systems which would be eventually eliminated from the body. Many new delivery systems like liposomes, hydrogels, etc. were designed thereafter and investigated but none emerged as perfect system due to issues with immunogenicity, stability, site and rate of administration, dosage, and control over release rates,
pharmacokinetics and pharmacodynamics. In terms of release kinetics, delivery of most drugs, whether by oral administration or through injection, follows what is known as “first-order kinetics” characterized by initial high blood levels of the drugs followed by exponential fall in blood concentration. This is problematic because once blood concentrations fall below certain levels, no therapeutic effect will be achieved. Furthermore, some drugs are toxic at high blood level concentrations. It is difficult to achieve a balance between effective levels and toxic levels when blood concentrations fall off so rapidly. Ideal delivery of drugs would follow “zero-order kinetics”, wherein blood levels of drugs would remain constant throughout the delivery period.

Consequently, scientists have been searching for methods to deliver drugs with zero-order kinetics. An unmet challenge has been to select the best controlled release technology. Microspheres, with many advantages described below, are on the forefront of this selection. Advantages of microspheres as controlled drug delivery devices are: a decrease in single dosage size, a continuous drug release, decrease in systemic side effects, and reduced possibility of dose dumping, reduced frequency of administration, and, therefore, increased patient compliance. Non-invasive placement and localized release of desired amount of therapeutic agents, circumvents the deleterious side effects of systemic administration [12]. This enables administration of larger, effective dosages. Furthermore, the size and size distribution of microspheres can be controlled to achieve a better predicted response. Moreover, microspheres can be manufactured with biodegradable materials or stimuli responsive materials which eliminate the need for device recovery. Because of their ability to act as a device for controlled release drug delivery, microspheres have been used to encapsulate many types of drugs, including small molecules, proteins, and nucleic acids.

Microspheres are defined as spherical microscopic particles that range from 1- 1000 µm in diameter [7, 8]. They are homogeneous structures made up of a continuous phase of one or more miscible polymers in which particulate drug is dispersed throughout the matrix unlike microcapsules, which have an inner core surrounded by a distinct outer shell [9]. A wide range of core materials have been encapsulated in microspheres, including adhesives, agrochemicals, live cells, active enzymes, flavours, fragrances [8], pharmaceuticals, and ink [10]. Other than drug encapsulation, microspheres have been used as fillers and bulking agents and even for

I.3. Interpenetrating Polymeric Networks

An interpenetrating polymer network is formed when a second hydrogel network is polymerized within a pre-polymerized hydrogel. This is typically done by immersing a pre-polymerized hydrogel into a solution of monomers and a polymerization initiator. IPNs can be formed either in the presence of a cross-linker to produce a fully interpenetrating polymer network (full IPN) or in the absence of a cross-linking mechanism to generate a network of embedded linear polymers entrapped within the original hydrogel (semi IPN), as illustrated in Fig I.4. The main advantages of IPNs are that relatively dense hydrogel matrices can be produced which feature stiffer and tougher mechanical properties, more widely controllable physical properties, and (frequently) more efficient drug loading compared to conventional hydrogels. Drug loading is often performed in conjunction with the polymerization of the interpenetrating hydrogel phase [13].

![Formation and structure of semi- and full interpenetrating polymer networks (IPN).](image)

IPN pore sizes and surface chemistry can also be controlled to tune the drug release kinetics, the interactions between the hydrogel and the tissues, and the mechanical properties of the gel [14]. Interpenetrating phases with different degradation profiles and/or different swelling responses to physiological conditions can be used to provide multiple controls over the swelling
responses of hydrogels and thus the potential drug release kinetics [15]. IPNs can also moderate the effect of environmental changes on hydrogel responses and burst drug release because of their ability to restrict the equilibrium swelling of either or both of the interpenetrating phases according to the elasticity (i.e. cross-linking density) of either or both gel phases. For example, a highly cross-linked interpenetrating network of a pH-sensitive hydrogel and a hydrolysable hydrogel restricts the typically rapid swelling response of a pH-swelling hydrogel to facilitate linear swelling profiles following an abrupt pH change from pH 7.4 to 1.2 [16]. Such responsivity is particularly suitable for minimizing burst release of drugs in oral drug delivery applications. As another example, a lightly cross-linked chitosan PNIPAM interpenetrating network significantly increased the loading capacity of diclofenac compared to a pure PNIPAM hydrogel while maintaining the sharp thermo sensitivity of the PNIPAM phase to regulate the release kinetics [17].

Semi-IPNs can more effectively preserve rapid kinetic response rates to pH or temperature (due to the absence of a restricting interpenetrating elastic network) while still providing most of the benefits of IPNs in drug delivery (e.g. modifying pore size, slowing drug release, etc.). For example, entrapping linear cationic polyallylammonium chloride in an acrylamide/ acrylic acid copolymer hydrogel imparted both higher mechanical strength and fully reversible pH switching of theophylline release [18].

I.4. Methods of preparation of polymeric microspheres

I.4.1. Emulsion polymerization method

Emulsion polymerization [19] is commonly used for water based preparation of polymer particles which is similar to suspension polymerization, except that the initiator is insoluble in the organic phase and soluble in the continuous phase. Anionic stabilizers are typically used as dispersing agents and can form micelles with a hydrophobic core containing the monomers. Above the critical micelle concentration free radical oligomers are formed in the continuous phase and diffusion into the micelles where they react with the entrapped monomer. Alternatively, the polymer may aggregate and grow in continuous phase. The drug can be either encapsulated in the resulting microspheres or adsorbed to their surface.
I.4.2. Water-in-Oil (W/O) emulsion method

The W/O emulsion has been investigated extensively during the past 25 years. The permeate for this method is emulsification of polymeric solution an aqueous continuous phase. The W/O emulsion is produced by the agitation to immiscible liquids. The drug substance is either dispersed in solution in the polymer/solvent system (or) is captured in dispersed phase of the emulsion. Agitation of the system is continued until solvent particle into the aqueous phase and is removed by evaporation. This process results in hindered microspheres which contain the active moiety. Several methods have been utilized to achieve dispersion of the oil phase in the continuous phase. The most common method is used of a propeller style blade attached to a variable speed motor and as the speed of the motor is increased the size of dispersed droplet decreased as a result of the high shear include by propeller. Homogenization is also produced on emulsion with this type of dispersion system a homogenizer equipped with a rotor and stirrer type blade attached to a variable high speed motor since high shear is used to produce the emulsion the resultant product has a much smaller particle size then the emulsion produced by conventional agitation other methods include the use of micro fluidizer [20], to produced micro emulsion, sanitation [21] and potentiometric dispersion [22].

I.4.3. Spray drying technique

In principle, the polymer is dissolved in a volatile organic solvent such as dichloromethane or acetone, the drug in solid form is then dispersed in polymer solution by high speed homogenization or it can be dissolved in a solvent then this solution is atomized in a stream of heated air. From the droplets formed, the solvent evaporates instantaneously yielding free micro particles [23] of typical size ranging from 1 to 100 µm depending upon the atomizing conditions like, size if the nozzle, spray flow rate and inlet air temperature. The microspheres are collected from air stream by cyclone separator. Residual solvents are removed by vacuum drying. The process can be operated under aseptic conditions and in closed loop configurations. Spray drying in nitrogen atmospheres is technically feasible. Important advantages of this technique over other encapsulation techniques are the proven reproducibility, well defined control of particle size, control of drug release properties of the resulting microspheres and the process is quite tolerant to small changes in polymer specifications.
I.4.4. Ionic Gelation

The use of complexation between oppositely charged macromolecules to prepare microspheres has attracted much attention, because the process is very simple and mild [24]. In addition, reversible physical crosslinking has been applied to avoid the possible toxicity of regents and other undesirable effects. Recently, many researchers have explored the ionic gelation technique for potential pharmaceutical usages [25-26]. Cationic polymers such as chitosan can undergo ionic gelation when reacted with polyanion such as tripolyphosphate (TPP), whereas the anionic polymers like sodium alginate and gellan gum undergo ionic gelation with bivalent cations such as calcium or zinc [27]. In this method, the polymer is dissolved in aqueous solution to get the charged species of the polymer. This aqueous solution of the polymer is then added drop wise under constant stirring condition to a solution containing the oppositely charged species. Due to the complexation between oppositely charged species, polymer undergoes ionic gelation and precipitates to from spherical particles.

I.4.5. Solvent evaporation method

In this method, the polymer is dissolved like organic solvent like dichloromethane, chloroform, or ethyl acetate. The drug is dissolved or dispersed into the preformed polymer solution, and this mixture is then emulsified into an aqueous solution to make an oil (O) in water (W) i.e., O/W emulsion by using a surfactant/ emulsifying agent like gelatin, Poly (vinyl alcohol), polysorbate-80, poloxamer-188, etc. After the formation of a stable emulsion, the organic solvent is evaporated either by increasing the temperature under pressure by continuous stirring. The effect of process variables on the properties of microspheres was discussed earlier [28-30]. The above methods use a high-speed homogenization or sonication. However, these procedures are good for a laboratory-scale operation, but for a large-scale pilot production, alternative methods using low-energy emulsification are required and it pursuit following have been attempted.

I.4.6. Spontaneous emulsification/solvent diffusion method

In a modified version of the solvent evaporation method [31-33], wherein the water-soluble like acetone or methanol along with the water insoluble organic solvents like dichloromethane or chloroform were used as an oil phase. Due to the spontaneous diffusion of
water-soluble solvents, an interfacial turbulence is created between two phases leading to formation of smaller particles. As the concentration of water-soluble solvent increases a considerable decrease in particle size can be achieved.

I.4.7. Salting out/emulsification-diffusion method

Methods discussed above require the use of organic solvents, which are hazardous to the environment as well as to the physiological system [34]. Since, the use USF/DA has specified the residual amount of organic solvents in injectable colloidal systems [35-36], in order to meet these requirements. Allemann and co-workers have developed two methods of preparing microspheres. The first one is a salting-out method [37-38] while the second one is the emulsification-solvent diffusion technique [39-40].

I.4.8. Reverse micellar method

Reverse micelles are thermodynamically stable liquid mixture of water oil and surfactant macroscopically, they are homogenous and isotropic, structured on a microscopic scale into aqueous and oil micro domains separated by surfactant rich films. One of the most important aspects of reverse micelle hosted systems is their dynamic behaviour. Nanoparticles prepared by the conventional emulsion polymerization methods are not only (>200 nm), but also have a broad size range. Preparation of ultra-fine polymeric nanoparticles with narrow size distribution could be achieved by using a reverse micellar medium [41]. Aqueous core of the reverse micellar droplets can be used as a non-reactor to prepare such practices. The size of the reverse micellar droplets usually lies between 1 and 10nm [42], and these droplets are highly monodispersed, the preparation of drug-loaded nanoparticles in reverse micelles would produce extremely fine particles with a narrow size distribution and the micellar droplets are in Brownian motion, they undergo continuous coalescence followed by re-separation on a time-scale that varies between millisecond and microsecond [43] the size, polydispersity and thermodynamic stability of these droplets are maintained in the system by a repaid dynamic equilibrium.

I.4.9. Dispersion polymerization method

Dispersion polymerization [44] is simpler when compared to the above techniques in that the reaction occurs in a single phase. Polymerization begins in a homogeneous mixture of
monomers, free-radical initiator and polymeric stabilizer in single solvent or solvent mixture. Once the reaction is initiated, the polymer chain grows in solution until it reaches a critical size at which the polymer precipitates to form nano-size nucleation particles. These particles aggregate into larger particles of a few hundred nano-meters and the stabilized by adsorption of stabilizer. The particles can continue to grow by capturing smaller nucleated particles and growing oligomers and by polymerizing monomers inside the particles. The resulting microparticles are generally in the range of 1-20µm in diameter and rather monodispersed. Moreover particles can be prepared from verity of monomers.

I.4.10. Emulsion crosslinking

In this method, water in oil (W/O)emulsion is prepared by emulsifying the polymer aqueous solution in the oil phase. Aqueous droplets are stabilized using a suitable surfactant. The stable emulsion is cross linked by using an appropriate cross linking agent such as glutaraldehyde to harden the droplets. Microspheres were filtered and washed repeatedly washed with n-hexane followed by alcohol and then dried [45]. By this method, size of the particles can be controlled by controlling size of aqueous droplets. However, the particles size of the final product depends upon the extent of cross linking agent used, while hardening in addition to speed of stirring during the formation of emulsion. The emulsion cross linking method has few drawbacks, since it involves the tedious produces as well as use of harsh cross linking agents, which might possibly induce chemical reactions with may be difficult in this process. In addition micro particles were prepared by crosslinking the polymer into obtains a non-sticky glassy hydrogel followed by passing through a sieve [46].

I.4.11. Coacervate/precipitation

This method utilizes the physicochemical properties of the polymers. For instance, chitosan is insoluble in alkaline pH medium, but precipitates/ Coacervate upon contact with the alkaline solution. Particles are produced by blowing chitosan solution into the alkali solution like sodium hydroxide, NaOH-methanol or ethanolamine using a compressed air nozzle formed Coacervate droplets [47]. Separation and purification of particles was done by filtration/centrifugation followed by successive washing with hot and cold water followed by
successive washing with hot and cold water. Varying compressed air pressure or spray-nozzle diameter controlled the size of the particles and then by using the cross linking agent to harden the particles could control the drug release.

**I.4.12. Emulsion droplet coalescence method**

The novel emulsion-droplet coalescence method was developed by Tokuitsu et al. [48], which utilize the principles of both emulsion cross linking and precipitation. However, in this method, instead of cross-linking the stable droplets, precipitation is induced by allowing the coalescence of chitosan droplets with NaOH droplets. First, a stable emulsion containing aqueous solution of chitosan along with drug is produced in liquid paraffin oil and then, another stable emulsion containing chitosan aqueous solution of NaOH is produced in same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating the chitosan droplets to give small size particles.

**I.5. Drug loading into polymeric matrices**

Drug loading in polymeric micro particulate systems can be done by two methods i.e., during the preparation of particles (incorporation) and after the formation of particle (incubation). In these systems, the drug is physically embedded into the matrix or adsorbed on to the surface. Many methods of loading have been developed to improve the efficiency of loading, which largely depends upon the method of preparation as well as physicochemical properties of the drug. Maximum drug loading can be achieved by incorporating the drug during the formation of particles, but it may get affected by the process parameters such as method of preparation, presence of additives.

Both water-soluble and water insoluble drugs can be loaded into polymeric particulate systems. Water soluble drugs are mixed with polymer solution to form a homogeneous mixture and then particles can be produced by any of the methods discussed earlier. Water insoluble drugs can be loaded by the soaking method [49] or by using the multiple emulsion technique.
I.6. Mechanism of drug release from microspheres:

The drug release from biodegradable microparticles is governed by various properties of the polymer, drug and the carrier system [50]. 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 microparticle, drug loading, physical state of the drug in the polymer matrix, particle size and particle size distribution, porosity and internal structure of the microparticle [51]. An increase in matrix porosity enhances the drug release because of the easier accessibility of the drug by dissolution fluids.

![Figure I.4:Schematic representation of Matrix swelling-control release systems.](image)

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 eroding. 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 per oral route of administration [52-63]. 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 maximum 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 [64-68]. 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.

Figure I.5: Schematic representation of biodegradable drug delivery device.

I.6.1 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 [69, 70] and (2) coating of the drug containing
tablets with suitable polymers [70-73]. In this case, the release rates depend on coating additives [74, 75] and thickness of the coating material [76].

In the membrane-coated systems, water 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 [77]. 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.

![Figure I.6: Schematic representation of diffusion controlled drug delivery device.](image)

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 drug present at a particular time and the release rate becomes time-dependent [78, 79]. Several acrylic resins, synthetic [73, 80, 81] and natural [82, 83] polymers have been used to develop such diffusion-controlled systems.

### I.6.2 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 [78, 84]. Thus, degradation occurs throughout the polymer structure in a random fashion so that the entire active 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 [85]. 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 [86].

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 swellable matrix systems, the release rate depends on the rate of water uptake, the rate of drug dissolution and the rate of matrix 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. Sodium carboxymethylcellulose (NaCMC) is one of the commonly used matrix materials for developing such systems [87, 88]. Other matrices used in such applications include natural gums like lucost bean [89], xanthan gum [90], guar gum [91-97], sodium alginate, hydroxyl propyl methylcellulose, Chitosan etc. Synthetic polymers like, PVP, PVA, PEG etc can also be used as matrix for controlled drug release studies. Some of the above have been taken up in the present study.

I.7. In-Vitro release studies

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 sac, (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 [98, 99]. 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 incubated in the dissolution medium for the release study [100-102].

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. [103] 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 the dissolution model.

The most commonly used equation for diffusion-controlled matrix system is an empirical equation proposed by Ritger and Peppas [104], in which early time release data can be fitted to obtain the diffusion parameters using,

\[
\frac{Mt}{M_\infty} = k t^n
\]

Here, \(Mt/M_\infty\) represents the fractional drug release at time \(t\), \(k\) is a constant 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 [104, 105].
I.8. Survey of literature relevant to the present study

Natural polymers are biodegradable and biocompatible and are mostly used for biomedical applications. Among the natural polymers chitosan, sodium carboxymethyl cellulose, guar gum, and sodium alginate are mostly used & sometimes their blends are used. Some synthetic polymers like, PVA, PVP, PEG, poly (N-isopropyl acrylamide), N-vinyl caprolactam, acrylamide, 2-(dimethyl amino) ethyl methacrylate and acrylamidoglycolic acid are also used for polymeric networks in the form of microparticles preparation.

Krishna Rao et al. [106], explains that the novel drug-loaded beads were prepared by different combinations of sodium alginate (NaAlg) and hydroxy ethyl cellulose (HEC) polymers, crosslinked with CaCl₂ to investigate the slow release of two representative drugs, viz., diclofenac sodium (DS) and ibuprofen (IB). The beads formed were smooth with nonporous surfaces as seen by SEM micrographs. Particle size varied slightly depending upon the formulation parameters like either by changing the blend ratio or drug content as well as crosslinking agent. The % encapsulation efficiency varied significantly with the polymer blend ratio and the amount of drug present in the beads. Greater encapsulation efficiency was observed for IB than DS.

Ramesh babu et al. [107] studied preparation of sodium alginate-methylcellulose blend microspheres for controlled release of Nifedipine (NFD). The microspheres were prepared by water-in-oil emulsion (w/o) method. The microspheres were characterized by differential scanning calorimetry to explain the molecular level of distribution of NFD in the polymer matrix. SEM picture of the microspheres suggested the formation of spherical particles. Drug was released in a controlled manner up to 12 h.

Raghavendra et al. [108] developed the interpenetrating network hydrogel membranes of sodium alginate and poly (vinyl alcohol) for controlled release of prazasin hydrochloride. Interpenetrating network hydrogel membranes were prepared by casting/solvent method for delivery of antihypertensive drug. The X-Ray diffraction studies indicated the amorphous dispersion of drug in the membranes. Differential scanning calorimetric analysis confirmed the IPN formation and suggests that the membrane stiffness increases with increased concentration.
of glutaraldehyde (GA) in the membrane. The IPN membranes extended the drug release up to 24 h.

Rokhade et al.[109] reported novel interpenetrating polymer network microspheres of chitosan and methylcellulose for controlled release of theophylline. Theophylline an anti-asthmatic drug was encapsulated into microspheres under varying ratios of MC, and CS, % drug loading and amount of GA added. DSC, XRD 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.

Ramesh babu et al. [110] developed 5-fluorouracil loaded poly (acrylamide-co-methyl methacrylate) novel core-shell microspheres and conducted the in-vitro release studies. The core shell microspheres were prepared by free radical emulsion polymerization using varying amounts of acrylamide, methyl methacrylate 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-flurouracil particles in core shell microspheres. SEM suggested the formation of well-defined core shell structures. Prolonged and controlled release of 5-fluorouracil was observed.

Recently Sunil Shah et al. developed the thermo response copolymeric microspheres [111]. Sarmila Sahoo et al. synthesised the chitosan-polycaprolactone blend for control delivery of ofloxacin drug [112]. Preparation and evaluation of alginate-chitosan microspheres for oral delivery of insulin was done by Yueling Zhang et al. [113]. Gheorghe Fundueanu, et al. published the study of pH and temperature-sensitive pullulan microspheres for controlled release of drugs and entrapment and release of drugs by strict “on-off” mechanism in pullulan microspheres with pendant thermo sensitive groups [114-115]. Preparation of sodium alginate/poly (vinyl alcohol) blend microspheres for controlled release applications was done by Subha et al. [116]. Reddy et al. developed the control release of chlorophenylamine maleate through IPN beads of sodium alginate-g-methyl methacrylate [117]. Preparation and
characterization of IPN beads for controlled release of acebutolol hydrochloride was done by Chowdoji Rao et al. [118].

Brahmaiah et al. [119] prepared and evaluated the mucoadhesive microspheres of Simvastatin; by orifice-ionotropic gelation method using polymers such as HPMC (K 100 M), carbopol 940P [120], sodium CMC [121], guar gum [122], sodium alginate [123], ethyl cellulose [124], methyl cellulose [125] and xanthan gum [126]. Totally 15 different formulations of Simvastatin were prepared by using the above polymers. The microspheres were characterized for drug content, entrapment efficiency, mucoadhesive property by in vitro wash-off test and in-vitro drug release. The in-vitro release data of all microsphere formulations were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release. Simvastatin release from these mucoadhesive microspheres was slow and extended up to 8h. Hence these microspheres were suitable for oral controlled release of Simvastatin.

Neelam jain and Arunabha banik [127] prepared Gg-PVA IPN mucoadhesive microspheres by the emulsion cross-linking method using glutaraldehyde as cross-linking agent for the effective encapsulation and controlled release of anti-ulcer drug, ranitidine HCl. Drug encapsulation of up to 87.80% was achieved as measured by UV method. FT-IR, DSC and X-RD techniques indicated a uniform distribution of 5-flurouracil particles in core shell microspheres. SEM suggested the formation of well-defined structures. Both equilibrium swelling studies and in vitro release studies were performed in pH 1.2 media. Based on the results of in vitro studies it was concluded that these IPN mucoadhesive microspheres provided oral controlled release of ranitidine HCl. Microspheres were able to provide drug release for an extended period of time (8 h or more) in 0.1 N HCl solution (pH 1.2). The amount of cross-linking agent and the ratio of Gg: PVA influences the drug entrapment efficiency and release of ranitidine HCl from microspheres.

Kuldeep et al. [128] established new polysaccharide for the colon targeted drug delivery system, its formulation and in vitro and in vivo evaluation. They prepared pectin/bora rice microspheres crosslinking with zinc acetate, and glipizide was loaded into the microspheres and measured the releasing parameters. And employed to study the effect of independent variables, polymer to drug ratio, and concentration of cross linking agent on dependent variables, particle
size, swelling index, drug entrapment efficiency and percentage drug release. Spheres were
discrete, spherical and free flowing. Beads exhibited small particle size and showed higher
percentage of drug entrapment efficiency. Drug entrapment efficiency 68% and drug release was
also controlled for more than 24 hours.

Madhusudana Rao et al. [129] Prepared microgels with new type of graft copolymer from
sodium alginate and N-Vinyl caprolactam by free radical graft copolymerization. The grafting
was confirmed by FTIR and DSC techniques, and used Ca$^{+2}$ as a cross linker. They measured
swelling studies of microgels, and exhibited excellent pH and temperature behaviour. SEM
studies indicated the microgels were spherical in nature and good compatibility between graft
chains and NaAlg. 5-FU an anti-cancer drug was loaded (E.E. = 84 %) in to the microgels and
performed releasing studies at different pH and temperatures. In-vitro release studies of 5-FU
indicated that these responsive microgels can be used effectively for colon cancer drug delivery
for more than 12 h.

Narayana Reddy et al. [130] CS-HPMC microspheres were prepared by water-in-oil
emulsion technique and were loaded with an anti-cancer drug 5-fluorouracil (5-FU). CS-HPMC
microspheres were characterized by Fourier transform infrared spectroscopy to confirm the
cross-linking reaction. And encapsulation efficiency was found to be 42.2 to 59.0%, depending
upon the blend composition. Surface morphology and size of particles were measured from SEM
analysis. The in vitro dissolution studies performed in pH 7.4 buffer medium have shown that
release of 5-fluorouracil is dependent upon the amount of drug loaded, polymer composition and
crosslinking. 5-fluorouracil is released in a sustained and controlled release manner from the
blend microspheres up to 10 h.

Recently Ana M. Puga et al., [131] developed Pectin-coated chitosan microspheres
loading with 5-FU and measured the controlled releasing parameters. An encapsulation
efficiency of 5-FU into the microspheres were showed 100% and diameters with 200–600 µm
range. The microspheres showed pH-dependent release rate, being slower at acid pH than at pH
7.4. Pectin coating improved the controlled release of 5-FU in acid medium. Cytotoxicity studies
performed with epithelial adenocarcinoma of cervix and malignant melanoma cells revealed that
the spheres promoted the cytotoxic effect of 5-FU, in case of pectin-coated micospheres, for the site specific delivery of antitumor agents to colon cancer cells.

Jingquan Li et. al., [132] evaluated the slow-release 5-fluorouracil PLGA microsphere, spray-drying method was used for preparation of 5-FU loaded microspheres, in vitro released profile and pharmacokinetic characteristics were carried out through high-performance liquid chromatography. The size of the microsphere was less than 100 µm, drug loading was 20 % and drug release time lasted as long as 30 days. 5-FU loaded microsphere were significantly restrained tumor growth and this effect correlated with decreased expression of vascular endothelial growth factor in tumor cells.

Kuntal Ganguly et. al., [133] focused on the Colon targeting of 5-FU using PEGlycol Cross-linked CS microspheres. Microspheres were prepared and enteric coated with cellulose acetate phthalate to regulate 5-FU loading in both the coated and uncoated PEG-cross-linked CS microspheres on their release profiles. The encapsulation efficiency of the microspheres varied from 22 to 30%. The absence of drug-polymer interactions between individual polymers, polymer blends and 5-FUloaded PEG-cross-linked CS microspheres have been confirmed by FTIR. Higher controlled release values of 5-FU occurred in alkaline pH than in acidic pH. The developed devices may be useful as the pH-independent swelling matrices and particularly well-suited for colon delivery of 5-FU.

Krishna Rao et al. [134] synthesized graft polymeric networks by using chitosan/acryl amido glycolic acid, and prepared polymeric micro networks cross-linked with UF in acidic media at room temperature and loaded with 5-FU. The remarkable advantage of this hydrophilic polymeric system is that it is solely made of chitosan and its copolymers with AGA, which are non-toxic, and biodegradable. The preliminary results of 5-FU loading and release experiments indicate that this system seems to be a very promising vehicle for the administration of hydrophilic drugs. The release of drug was controlled by penetration of external medium into the matrix or by drug diffusion into the matrix pores or by both. SEM, DSC and XRD studies of 5-FU loaded CS-PMNs have shown molecular dispersive level of the drug in the matrices.

Poly (3-hydroxybutyrate) and Cellulose acetate phthalate blend microspheres were developed by solvent evaporation technique [135] for Colon Delivery of 5-Fluorouracil.
Encapsulation efficiencies of microspheres 5-FU was found 42-57%. These pH-sensitive polymers were utilized to investigate the colon delivery, an anticancer drug. The surface morphology of the microspheres was studied by SEM with the range of 29 to 67 µm, as well as spherical and pore structures before and after drug loading. FTIR spectroscopy was used to confirm the polymer blend compatibility and to confirm the absence of drug-polymer interactions. In vitro release experiments were performed at 37 °C in PB medium of the stomach (pH 1.2) for 2 h, followed by intestinal medium (pH 7.4). It was found that the release of 5-FU from blend microspheres followed pH-dependent release as compared to that of plain PHB microspheres.

Ashlee et al., [136] revealed that the study of PLGA microspheres for drug delivery applications. They described about degradation, erosion and drug release from the bulk polymeric micro particulates. And these microspheres were used as transports of drugs for controlled release applications. Numerous mathematical models have been published for predicting degradation, erosion, and drug transport and overall drug release from PLGA microspheres. The more sophisticated models that treated the coupled interactions between phenomena brought predictive capability to the regimes where autocatalysis plays a significant role in drug release dynamics. Predictive, high accuracy models that rigorously include autocatalytic effects could decrease the number of experimental trials needed to explore release from different microsphere distributions by optimizing controlled drug release in silicon.

Poly lactic-co-glycolic acid (PLGA) micro particles have been most attractive polymeric candidates used for drug delivery, tissue engineering and biomedical applications [137]. Hirenkumar et. al., tells that PLGA micro particles are biocompatible and biodegradable, exhibits a wide range of erosion times, has tuneable mechanical properties and most importantly, is a FDA approved polymer and they described that these PLGA microparticles showed biocompatibility and biodegradability due to peptides and proteins present in the polymeric devices [137]. The drug release rate can be accelerated by greater hydrophilicity, increase in chemical interactions among the hydrolytic groups, less crystallinity and larger volume to surface ratio of the device.

Sahoo et. al., [138] focused that 5-Fluorouracil released from calcium-zinc-gellan and calcium-zinc-gellan-ethyl cellulose microbeads. They successfully prepared these micro beads
by simple ionotropic gelation and oil in water ionotropic gelation technique, respectively. Microbeads were characterized by SEM, FTIR, and evaluated for particle size, drug content, and encapsulation efficiency. The release profile of 5-FU from microbeads has followed as dissolution controlled manner. They concluded that the 5-FU loaded calcium-zinc-gellan microbeads were found to be spherical with rough surface and increased proportion of GG and EC has increased encapsulation efficiency, particle size and sustained drug release effect of 5-FU. These beads afford a platform to develop an oral controlled release drug delivery system of 5-FU that not only improves the patient condition but also reduces systematic toxicity and thus reduced side-effects of chemotherapy.

Reddy et. al., [139] prepared novel biodegradable aliphatic poly(ether-urethane)s micro particle devices based on PLF-68 and castor oil. These devices were characterized by FTIR, $^1$HNMR and GPC to confirm the PEU formation and the molecular weight, and to study the controlled released parameters for 5-flurouracil (5-FU). Sizes of the microspheres range measured between 15 and 42 $\mu$m. The microspheres exhibited encapsulation efficiencies up to 98% with spherical innature and have wrinkled surfaces. And also demonstrated that a hydrophilic and lipophilic balance of the matrices can be achieved by varying the ratio of two different diols to obtain the suitable PEU matrices for the release of 5-FU. The formation and dissociation of hydrogen bonds result in swelling and collapse of PEUs and this special property can be used to control the delivery of drugs or other active molecules.

Merve et. al., [140] studied the release of 5-FU from different ionically crosslinked alginate (Alg) beads. They used Fe$^{3+}$, Al$^{3+}$, Zn$^{2+}$ and Ca$^{2+}$ ions as crosslinking agent to prepare the micro beads and these beads were characterized by FTIR, DSC and SEM. The drug release studies were carried out at two pH values 1.2 and 7.4 respectively. They observed that 5-FU release from the beads followed the order of Fe > Zn > Al > Ca-Alg and increased with increasing drug/polymer ratio. Their studies on the release of 5-FU from micro beads crosslinked with the ions of Fe$^{3+}$, Al$^{3+}$, Zn$^{2+}$ and Ca$^{2+}$ indicated that the crosslinking with Fe$^{3+}$ lead to more release of 5-FU from the NaAlg beads. Release of 5-FU from NaAlg beads crosslinked with FeCl$_3$ increased with the decrease in the drug content and also observed that release of 5-FU was much higher at high pH values compared to low pH values.
Maraym et. al., [141] prepared chitosan and alginate films for wound dressing and controlled release of an anti-cancer drug 5-FU. They were used propyleneglycol (PG) and calcium chloride (CaCl₂) as crosslinkers to develop the thickness (p = 0.004), elasticity (p = 0.003), tensile strength (p = 0.324), sorption ability (p = 0.001) of the prepared films. They demonstrated that there is no significant differences in tensile strength (p = 0.324) for alginate and chitosan-based formulations. The size, morphological and porosity of the films were carried out through the SEM analysis and the function of release of 5-FU from the films characterized by the absence of a lag phase, are supported by AFM pore size measurements. In-vitro cytotoxicity testing will be added to the protocol to assess the effect of the glutaraldehyde removal from the chitosan formulations.

Rathod et. al., [142] prepared bovine serum albumin olive oil based microspheres by heat denaturation method and studied an anti-cancer drug 5-FU is an antimetabolite with a broad spectrum activity against solid tumors. The prepared microspheres are biocompatible, nontoxic and non-immunogenic drug carrier. The 5-FU loaded microspheres are more stable at 4°C then at room temperature (25° to 40°C). Release rate of freshly prepared microsphere and freeze dried microspheres are 88.30% and 87.86 % respectively on 7th day, maximum amount of drug was detected in liver and adequately remained in spleen at least up to 24 hours. Studies revealed that after in vivo administration of drug loaded microspheres, maximum amount of drug was detected in liver and adequately remained in spleen at least up to 24 hours. Their study demonstrates that the drug loaded albumin microspheres could be efficiently targeted at the liver by intravenous injection.
References


