Chapter 1

Introduction

Contents

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1.1 Biomaterials

In the last decade the progress in biomaterial science has giant impact in the scientific world, particularly in the field of regenerative medicine. The peculiarity of biomaterial science is its multidisciplinary nature, which develops connections between basic science, medical science and engineering disciplines. The constant flow of information from material science, molecular and cell biology, human physiology and pathology and the continuous demand for new technological solutions have motivated the biomaterial science to be one of the most promising areas of scientific and social interest. Biomaterials of one type or the other have been in clinical use for many years. A reasonable degree of success since 1960 and a rapidly expanding range of materials being made available by advances in basic material science have more recently led to a greater abundance of biomaterials. The basic idea of these 1st generation biomaterials was to "achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host"[1]. The main characteristic of these biomaterials are biological "inertness", so as to cause minimum immune response. The superior approaches concerned the production of 2nd generation bioactive materials that could either show a controlled action and reaction in the physiological environment or the improvement of bioresorbable property of biomaterials that exhibited controlled chemical breakdown and elimination[2], the third generations of biomaterials involved the amalgamation of the former concepts. These materials are thus designed to stimulate precise cellular responses at the molecular level and simultaneously have bioresorbable features.

1.1.1. Definitions and classification of biomaterials

Presently, the one most widely accepted definitions of biomaterials are:

- "A biomaterial is a nonviable material used in a medical device, projected to interact with biological systems[3]".
- "A biomaterial is a material intended to interface with biological systems to estimate, treat, enhance or restore any tissue, organ or function of the body[3]".

In fact, the design and manufacture of biomaterials address numerous scientific aspects and have to assure specific requirements. Especially, in depth research of the chemical, physical and biological attributes of the environment in which the material is anticipated to execute its action is of outmost significance. The acquired information may direct to a better understanding of the issues that determine the final performances of the materials and therefore allowing for the optimization of their design. The bifunctionality of the device under investigation should hence be associated to appropriate mechanical and chemical characteristics. Nevertheless, these products must retain their functionality and safety over the desired time, as ensured by their biocompatibility.

A general classification based on the chemical nature of biomaterials comprises of following types[4]

- **Biologically derived materials** include natural proteins (collagen, elastin, gelatin), polysaccharides (hyaluronic acid, chitosan, dextran, cellulose), and proteins produced biotechnology (genetic engineering), as well as entire cells and natural tissues. A variety of biopolymers find applications as biomaterials. The prominent among them are collagens, cellulose, chitin, chitosan and their derivatives. Collagens, which are major animal structural proteins, are widely used in a variety of forms such as solution, gel, fibers, membranes, sponge and tubing for large number of biomedical applications including drug delivery, sutures, vessels, valves, corneal prosthesis, wound dressing, cartilage substitute and dental applications. Cellulose and its derivatives are mainly employed in fabrications of membranes utilized in hemodialysis machines. Heparin finds applications in improving blood compatibility of other materials. Chitosan and chitin are emerging as biomaterials with wide applications.

- **Synthetic polymers** can be utilized in a broad range of applications, of either intracorporeal or extracorporeal type. The most general uses include short term devices such as sutures, adhesives, contact lenses and long term devices such as cardiac valves, complex systems intended at substituting the function of body organs e.g. artificial kidney, heart, pancreas, catheters, filters for hemodialysis, orthopaedic material and drug delivery devices. The synthetic polymers which find applications in

medical devices, include polyolefins, polyesters, polyamides, polycarbonates, polyurethanes, synthetic rubbers, polyethers and silicone rubber. Various physical forms of polymers which are in use as biomaterials include fibers, textiles, membranes, films, foams, solid rods, powders etc.

- **Metals** are generally used in orthopaedic and dental implants, in addition to in the construction of pacemakers and heart valves. The most commonly used metals are stainless steel, cobalt and titanium based alloys and conducting metals such as platinum and iron. Most metals used for manufacturing implants can be tolerated by the body in minute amounts.

- **Composites** are mixtures (combination of polymers, ceramics, and metals) of two or more phases bonded together so that stress transfer occurs across the phase boundary. Composites presently used in biomaterial applications comprise bone particles or carbon fiber reinforced methyl methacrylate bone cement, porous surface orthopedic implants, and dental filling composites.

- **Ceramics** are extensively engaged in orthopaedic implants, as bone fillers, and in dental, ear, nose and throat implants due to its excellent biocompatibility. The types of ceramic materials used in biomedical applications can be divided into three classes according to their chemical reactivity with the environment:

  [i] Completely resorbable, e.g. hydroxyapatite and β-tricalcium phosphate
  [ii] Surface reactive, e.g. Bioglass and calcium phosphate and
  [iii] Nearly inert, e.g. alumina and carbon.

The most ordinary bioceramics are aluminum oxides, calcium phosphates, titanium oxides, zirconium oxides and glass.

1.1.2. Polymeric biomaterials

The curiosity for polymeric biomaterials and their use in various medical applications such as ophthalmology, dentistry, orthopaedics, implantation of medical devices, bioartificial organs, drug delivery system and many more is increasing rapidly[5]. Compared with other types of biomaterials, such as metals and ceramics, polymers offer the benefit of being engineered and processed in diverse ways to meet

precise end use requirements. In addition the broad variety of commercially available polymers and the research in both advanced organic chemistry and polymer synthesis has led to the development of valuable tools for the design of polymer architecture and the grafting of bioactive molecules[6, 7]. Therefore, according to key requirement of "device", polymers can be selected and specifically tailored for appropriate surface or bulk properties. Nowadays, two important fields of application of polymeric biomaterials i.e. tissue engineering and controlled drug delivery have gained interest both in academia and industry.

Tissue engineering is aimed at restoring, maintaining, or enhancing tissues and organ's functions by assuring synergistic interactions of living cells and synthetic materials. Although transplantation and reconstructive surgery are the most used therapies to address the loss and failure of organs and tissues, they remain imperfect solutions. Tissue engineering could actually afford a valid alternative[8].

In drug delivery main issues are the control of the pharmacokinetics and biodistribution of drugs in order to confine their action to the treated tissue and keep their concentration at therapeutically relevant levels for the preferred time. Polymers are currently used as physical carriers for drugs, components of prodrugs, conjugates or in complexes with proteins or nucleic acids, as well as direct therapeutics in their own right[9]. The role of polymers in drug delivery covers multiple aspects, from the improvement of the physical and chemical stability of the drug to the regulation of drug release and targeting. Hence the goal of drug delivery and the participation of polymeric biomaterials in it are to boost drug efficacy and reduce the toxicity of traditional drug administration, create easier dosage regimes and achieve a better patient compliance[10].

In the recent years, an additional step in the direction of the development of "smart" materials for biomedical uses through synergistically combining the approaches of both conventional tissue engineering and controlled drug delivery has been imagined. The aim of such association is to afford systems that not only constitute a supportive scaffold for cell proliferation, but also guide cell development and organization by progressive and controlled release of cell growth and differentiating factors. This concept, which could promote the formation of highly

organized and complex tissues, is actually under investigation by the most prominent research groups involved in biomaterial science[11].

Generally, polymeric biomaterials are classified as synthetic polymers and polymers of natural origin. The field of applications of synthetic and natural polymeric biomaterials is in fact very wide, including surgical devices, implants and supporting materials (e.g. artificial organs, prostheses and sutures), drug delivery systems with different routes of administration and design, carriers of immobilized enzymes and cells, biosensors components of diagnostic assays, bioadhesives, ocular devices, and materials for orthopedic applications[12]. Biopolymers and their applications are summarised in ***Table 1.1-1.2*** [5, 9, 11-13].

Table 1.1 Natural polymers and their biomedical applications[14].

Polymers	Main biomedical applications and comments
Proteins and Protein base polymers	
Collagen	Sutures, Drug delivery
Albumin	Drug stabilizer and drug delivery
Gelatin	Gels and drug release
Polyaminoacids	Non toxic, non antigenic and biocompatible. Used as carriers for oligomeric drugs.
Polysaccharides and vegetable derived polymers	
Carboxymethylcellulose	Drug release, dialysis membranes, cell immobilization
Starch	Drug delivery
Agarose	Used in clinical analysis and as matrix
Alginate	Biocompatible, Applied in gel preparation and to immobilize cell matrix and enzymes. Injectable microcapsules for neurodegerative diseases and hormones deficiency.
Polysaccharides and human/ animal derived Polymers	
Hyaluronic acid	Moisturizing agent, wound dressing, artificial tears in ophthalmology, various orthopedic applications.
Eparin and Eparin like glycosaminoglycans	Trombolytic anticoagulant properties, Used in surgery and in capsules preparation.
Microbial Polysaccharides	
Dextran and Derivatives	Excellent rheological properties, Plasma Expander and in drug delivery.
Chitosan and derivatives	Natural polycations. Biocompatible, non toxic. Used in gel and film preparations. Apllied in drug delivery.

Table 1.2 Synthetic polymers and their biomedical applications.

Polymer	Main Applications and comments	Ref
Poly(lactic acid), Poly(glycolic acid) and their copolymers	Biodegradable, copolymerized to regulate degradation time, used in sutures, drug delivery systems, tissue engineering.	15-20
Poly(hydroxybutarayte)s	Biodegradable, used as a matrix for drug	21
Poly (γ-caprolactone) and copolymers	Cell micro encapsulation	22,23
Polyanhydrides	Biodegradable, useful in tissue engineering and for encapsulation of bioactive molecules.	24-26
Polyorthoesters	Surface eroding polymers, ophthalmology.	27-29
Polycyanoacrylates	Biodegradable, depending upon the length of the alkyl chain, used as surgical adhesive, glues and in drug delivery.	30
Polyphosphazenes	Properties can be tailored with side chain functionalization, applications in drug delivery	31-33
Polyamides	Sutures, hemofiltration membranes, inhibitors for DNS transcription	34-37
Polyurethanes	Prosthesis, vascular grafts, catheters tissue adhesives, coatings.	38, 39
Poly(methyl methacrylate)	Dental implants, bone replacement and in intraocular lens	40, 41
Poly (hydroxyethyl methacrylate)	Contact lens, ocular prosthesis, skin coatings, catheters, drug delivery.	42
Poly(ethylene glycol)	FDA approved polymer used in variety of biomedical applications.	43-45
Poly(N-vinylpyrrolidone)	Hydrogels for controlled release of drugs or used in a blood substitutes.	46
Polyamidoimine	Vectors of oligonucleotide transfer and gene delivery	47,48
Poly(N-Isopropyl acrylamide) and Poly (N-Vinylcaprolactam)	Smart polymers used for various medical applications	49, 50

1.2 Drug delivery technology

Controlled drug delivery technology represents one of the most rapidly developing areas of science in which several disciplines, such as chemistry, pharmaceutical technology and medicine are contributing to human health care. For many years, fundamental and applied research has focused on the development of pharmaceutical formulates allowing maximization of the therapeutic efficacy and minimization of the adverse effects of the drugs of interest. The attempts in the identification of upcoming compounds are often followed by weakness in the late clinical trials or in the postmarket follow-up. The main reason of this failure is that the 95% of all new potential therapeutics have poor pharmacokinetics and pharmaceutical properties[51, 52]. Therefore, the growth of appropriate drug delivery system with appropriate pharmacokinetics and pharmaceutical properties of active entrapped bioactive compound is critical. Certainly, drug delivery technology offers the possibility of justifying the costs associated with drug failure, makes possible the administration of labile drugs with good therapeutic potential, as well as provides solutions to many problems related to classical therapeutics currently available on the market.

Conventional drug administration usually leads to a "pulsed pattern" of the drug concentration in the blood, characterized by a typical peak and valley trend. During the therapy, the plasmatic concentration of the bioactive agent may fall outside the therapeutic range and the administration of the drug must be repeated after precise time intervals in order to maintain the therapeutic action. Moreover, the active agent, sensitive to changes in pH as well as been attacked by enzymatic degradation, may loose some activity and have a reduced half-life. To avoid the rapid breakdown or clearance in vivo, the drug is commonly administered in high concentration, resulting sometimes in strong toxic effects or induced inflammatory processes. On the other hand, when the plasmatic concentration drops below the therapeutic level there is a significant decrease of the therapeutic benefits ***(Figure 1.1).*** In the past 30 years pharmaceutical research has become increasingly focused on the development of novel systems for drug delivery that overcome the disadvantages of conventional methods of drug treatment. A controlled release device is designed to release enough drug, initially to reach therapeutic levels and then continue releasing at a constant rate

to replace used or deactivated drug, to reduce or eliminate the overdose-underdose cycle in the drug concentration and to maximize the efficiency of the drug. Targeted or site specific release in the body would also improve the efficiency of treatment and limit the drug exposure to undesired areas that may result in side effects. Also, controlled release devices provide a protective barrier to the metabolic processes in the body, thus enabling the delivery of drugs with short half-life which were previously unusable with conventional methods. In addition, new delivery methods may circumvent the oral administration route for drugs and provide constant drug release from implantable devices.

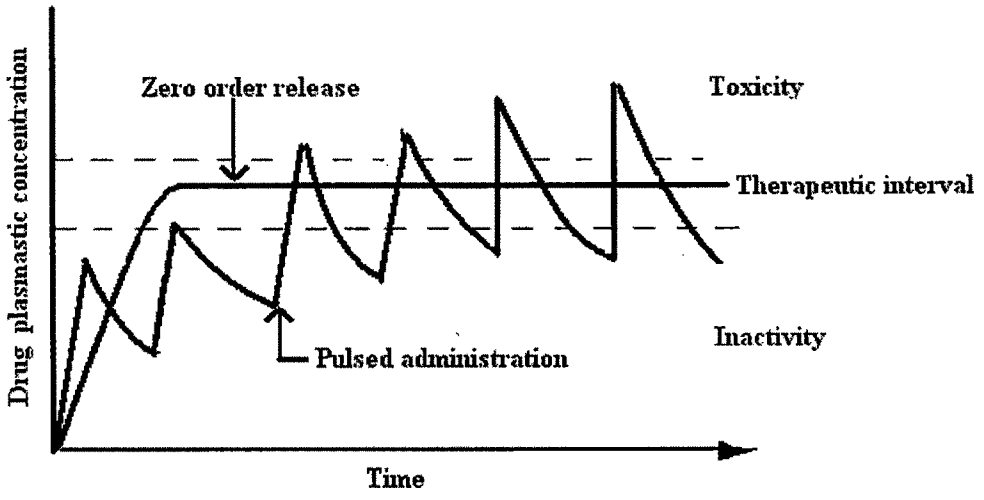

Figure 1.1 Variation in drug plasmatic concentration with time following conventional administration and controlled release devices[53].

The drug delivery systems offer several advantages as compared to conventional dosage forms. The system is usually a reservoir of the therapeutic agent, with a specific time release of the drug, thus leading to a partial control of the pharmacokinetics that becomes complete if the system is also designed for the targeted release of the drug and influences its biodistribution. In addition, the delivery system safeguards the drug from the attack of enzymatic degradation, it can enhance the penetration of the active agent in the diseased tissues improving its efficiency and reducing the toxicity. A better compliance and convenience of the patient are also

achieved [54, 55]. Compared to traditional drug formulation and administration, the advantages of a controlled delivery system can be summarized as follows:

- Continuous maintenance of drug levels within the desired therapeutic range;
- Reduction of harmful side effects;
- Potentially decreased amount of drug;
- Lower number of administrations and possibly less invasive, leading to improved patient compliance;
- Administration of pharmaceuticals with short in vivo half–life (for example peptides and proteins).

In the development of drug delivery systems, the rational should be modified according to the specific biological substance and/or the particular therapeutic situation. Although clinical introduction of the first controlled release systems occurred 30 years ago, nowadays these systems have an incredible impact on nearly every branch of modern medicine, including cardiology, ophthalmology, endocrinology, oncology, pneumology, immunology and pain management. In the United States alone, the market size for drug delivery systems in 1997 was over $ 13 billions, in 2002 it raised to about $ 47 billions and was projected to grow to about $ 87 billion in 2007 with a rate at 8% p.a.[56] As a matter of fact, every year a considerable number of improved devices are presented and adopted for health care. In addition, many of highly profitable blockbuster drugs have reached patent expiry by 2006 and lost about $37 billion in market value to generic competition. Hence optimizing products through drug delivery might be a successful strategy to re-launch competitive pharmaceutics[56, 57].

Common types of controlled drug release systems are:

- Oral systems
- Formulates for parenteral administration
- Therapeutic transdermal delivery systems
- Occular systems
- Intrauterine and intravaginal systems

Recently, the interest of pharmaceutical scientists is mainly focused on the formulation of nanosized drug delivery devices for the administration of protein drugs, oligonucleotides, DNA and RNA for gene regulation[58-61]. Next generation of drug delivery systems will be influenced by the "intelligent material design" in terms of developing systems sensitive to drug concentration itself and to certain physiological stimuli. Recognitive molecular systems with biosensing properties will be integrated in the delivery device so that the release of the therapeutic will occur only under specific conditions[62]. Among the general characteristics that drug delivery systems should present are the ability to incorporate the drug without damaging it, tuneable release kinetics, long in-vivo stability, biocompatibility, lack of toxicity and immunogenicity and potential of targeting specific organs and tissues. All of these characteristics are related to the nature of the materials that constitute the delivery system. In particular, the detection of an ample compromise of both bulk and surface property represents an important issue to be addressed. Organic macromolecules have highly tuneable physicochemical characteristics and in some cases, polymeric materials can be further processed or functionalized. Accordingly, they especially expected stand for the best suited class of materials for drug delivery technology[62].

1.2.1. Polymer based drug delivery systems

Polymeric systems had an enormous impact on pharmacological therapy. In the past, many efforts were focused on using polymers that have a history of medical use and then adapting their microstructures to provide the desired delivery rate. Tailor-made design of materials is a more modern approach to solve specific issues of drug delivery[62]. First generation drug delivery devices were based on drug encapsulation in non–degradable macromolecular matrices from which the drug could diffuse. Therefore, the release kinetics was completely dependent on the diffusive behavior of the drug through the polymer matrix[57]. A search for a second generation drug delivery devices is going on, where the polymer matrix is engineered in order to play an active role and permit the selective release of the incorporated drug to a specific site or against a selected tissue (drug targeting). Besides, due to the in-vivo degradability, the release kinetic profile can be precisely modulated[63]. The first category of release systems consisted of simpler devices that could not be easily applied to particular cases, for example to protein delivery. On the other hand, the

second generation systems are characterized by high selectivity and efficiency, although they need careful control of the macromolecular structure of the polymer matrix. The particular requirements for controlled release applications dictate on each circumstance the choice of polymer. Normally, the demand is less severe if the device is intended to design for external use or for oral administration; it is much more rigorous if the system is given intravenously, subcutaneously or for application into internal body compartments. For the external use, the polymer may be non biodegradable, such as having carbon–carbon or silicon– oxygen backbones, whereas biodegradable materials are in great demand for internal applications. Typical examples of biodegradable and non–biodegradable polymers used in controlled release technology are listed in ***Table 1.3.***

Table 1.3 Non-biodegradable and biodegradable polymers commonly used in controlled release applications.

Non biodegradable Polymers	Biodegradable Polymers	
Polysiloxanes	Polylactides	Polyglycolides
Polyacrylates	Polycyanoacrylates	Polysaccharides
Polyurethanes	Polyorthoesters	Polyanhydrides
Polyethylene	Polyphosphazenes	Poly (ε-caprolactone)

Drug delivery systems can be roughly classified in two core categories, namely systems in which the biological substance release is controlled respectively by the drug diffusion and by the degradation of the device.

1.2.1. A. Non biodegradable delivery systems

Non biodegradable delivery systems are mainly diffusion controlled release systems where non biodegradable polymeric materials are used [57, 63], e.g.

- Ethylene - vinyl acetate copolymers (EVAc)
- Ethylene - vinyl alcohol copolymers (EVA)

- Polymers and copolymers of 2–hydroxyl ethylmethacrylate (HEMA) and more in general poly (methacrylate)s.
- Polysiloxanes (silicones)

In these systems, the drug diffusion through the polymer matrix or membrane is because of the thermodynamic driving force arising from the difference in concentration of the drug inside and outside the device. Two types of release devices, namely membrane and matrix are used for the purpose.

Membrane systems

In membrane systems, the drug is surrounded by a polymeric film ***(Figure 1.2)*** whose porosity is not uniform, and which determines the drug release rate[64]**.** These types of systems are more complex than matrix systems, but they offer a higher control of release profiles.

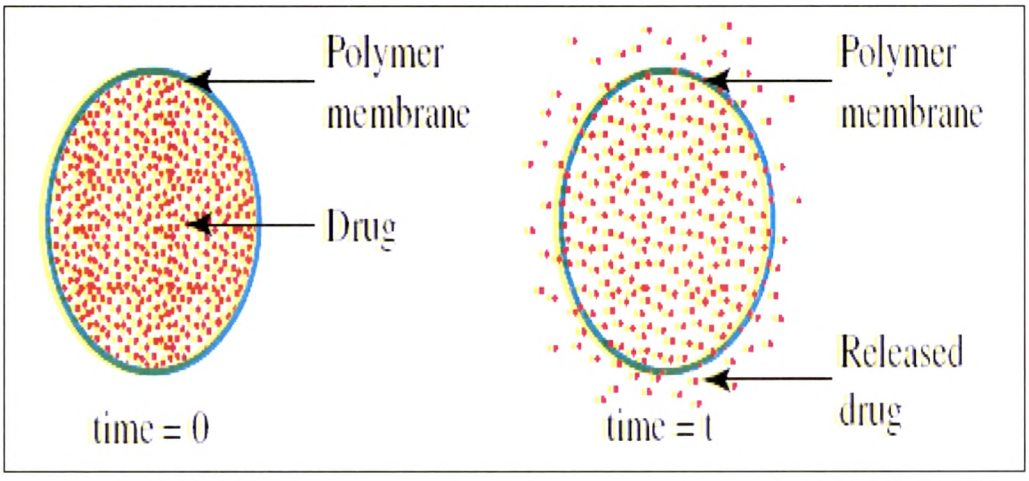

Figure 1.2 Schematic representation of membrane release systems[53].

The drug can be a dry powder dispersed in a liquid or entrapped in a solid polymer matrix. The membrane can be a microporous or macroporous polymer film. Its composition changes from a single component to a mixture of polymers, or to a heterogeneous matrix in which hydrophilic polymer particles are dispersed in a hydrophobic polymer matrix. The membrane and the active core can be assembled using different technologies:

- Drug and solid membrane lamination into films.
- Drug coating with a volatile solution of the polymer.
- Drug micro-encapsulation
- Tubular membrane loading with dissolved or dispersed drug and
- Drug loading in membrane capsules.

This latter approach led to the development of Norplant, small silicone capsules containing contraceptives that are slowly released by diffusion through the polymer for 5 years. The main limitation of these systems is the sustained delivery of ionic species and of molecules with molecular mass over 400 can not be realized.

Matrix systems

To address the issues of matrix systems, drug can be physically embedded in polymers at large enough concentration to create a series of interconnecting pores through which the drug can slowly diffuse. In these systems, the matrix may consist of hydrophobic or viscous hydrophilic polymers in which the solid drug is dispersed ***(Figure 1.3)***. Generally, the drug is sparingly soluble in the polymer matrix. These release systems are inexpensive and readily available, since they are prepared simply by mixing the polymer matrix and the drug. The desired device shape is obtained later by extrusion. The release mechanism is based on the diffusion of the drug molecules to the surface from where they are delivered. This process takes place as long as the higher concentration of the drug in the system core affords a constant flow of drug molecules through the matrix.

In this dissolution–diffusion process, the interface between the drug reservoir and the release moiety progressively moves towards the core of the device.

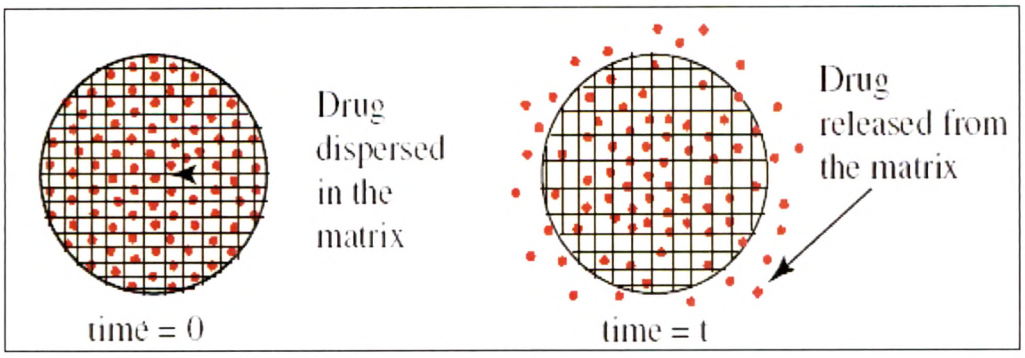

Figure 1.3 Schematic representation of matrix release systems[53].

1. 2. 1. B. Biodegradable delivery systems

In degradable delivery systems, the drug is loaded in a bioerodible and/or biodegradable polymer matrix. The release takes place because of a combination of processes, such as matrix degradation and drug diffusion. The use of these materials was considered in order to avoid the problems related to the physiological secretion or mechanical removal of the non degradable drug delivery devices after their function is completed. Certainly, these devices are the preferred ones for internal application. It is important to note that the combination of diffusion through pores and polymer degradation process provides an additional control of the drug release rate. In ideal systems, the degradation occurs only at the surface of the device, affording a progressive delivery of the drug ***(Figure 1.4).***

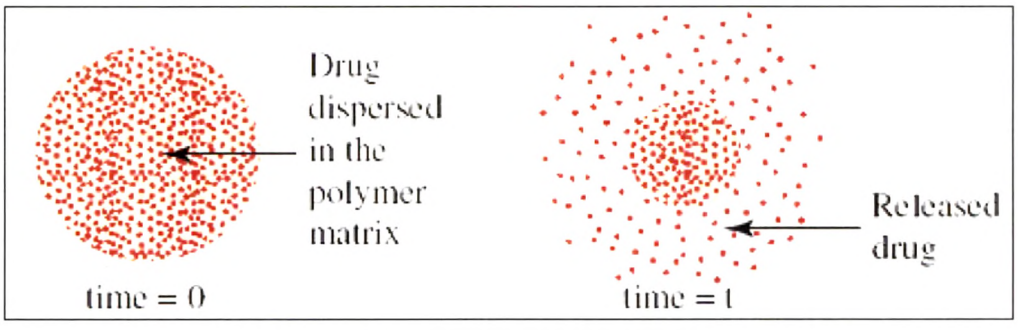

Figure 1.4 Schematic representation of degradable matrix systems[53].

However, swelling phenomena and/or internal polymer degradation usually take place simultaneously making the overall kinetics more complex. Especially, bulk erosion makes constant release rates complicated to be achieved and creates the possibility of dose dumping, as the system ultimately hydrolyzes. To address these issues, it would be desirable to design polymers that display only surface erosion. One strategy to achieve this goal is based on the use of polymers constituted by hydrophobic units connected by water labile bonds. This approach has the advantage of keeping water away from the polymer bulk while allowing for controlled erosion when water reacts with the matrix surface[64]. In general, an almost constant release profile over long periods can be achieved by fine modulation of geometry, chemical degradation and porosity characteristics of the device. In this respect, degradable drug delivery systems can be formulated in different shapes, i.e. cylinders, sticks, microcapsules, microspheres, nanoparticles, films and fibers. It is convenient to classify biodegradable release systems according to the mechanism responsible for matrix degradation and hence of drug delivery. Accordingly three main types of polymer matrixes can be identified[65].

- ***Bioerodible cross linked matrix:*** This kind of matrix is constituted of water soluble polymers that are crosslinked to give non–soluble networks. However, by hydrolytic or enzymatic cleavage they are converted into water soluble macromolecules, and the release of the incorporated drug is achieved ***(Figure 1.5).***

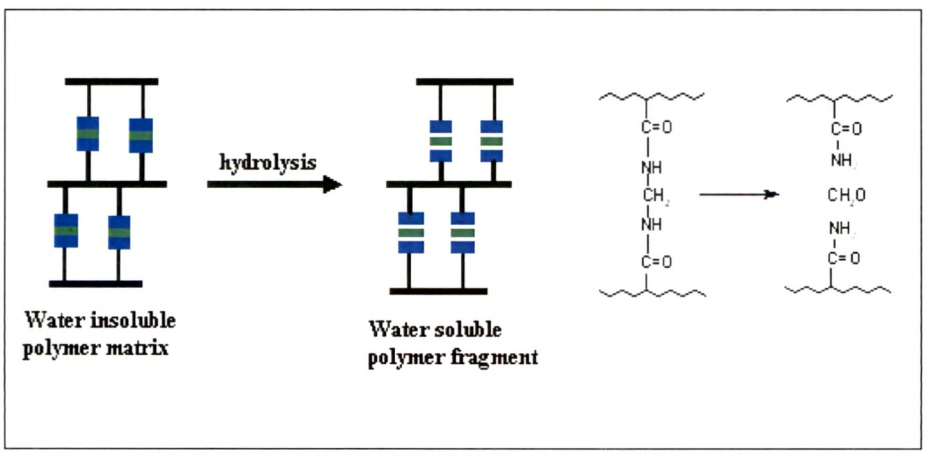

Figure 1.5 Schematic representation of bioerodible crosslinked matrix[53].

- ***Water insoluble/soluble matrix:*** In this case, water insoluble polymers may become soluble because of ionization, hydrolysis, or protonation of the side chains, allowing the drug to be released ***(Figure 1.6).***

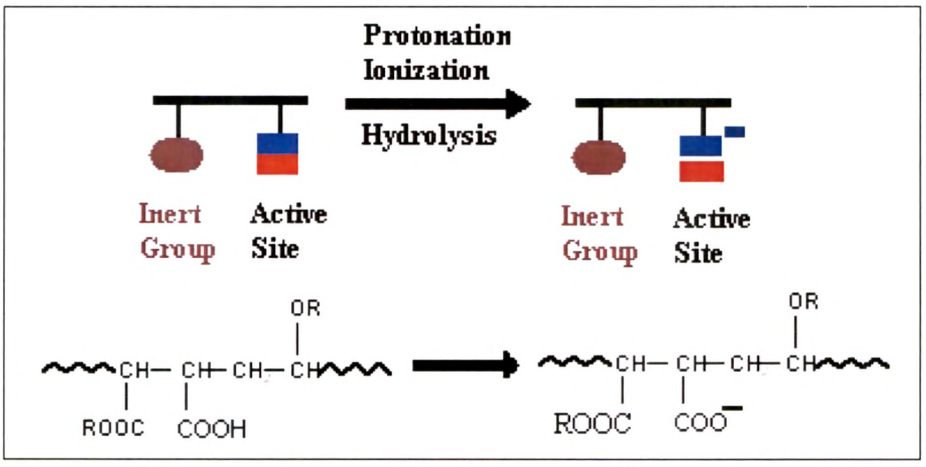

Figure 1.6 Schematic representation of water insoluble/soluble matrix[53].

- ***Bioerodible/biodegradable matrix:*** Hydrolytic cleavage of main chain bonds of water insoluble polymers leads to medium to low molecular weight fragments that are soluble in water ***(Figure 1.7).***

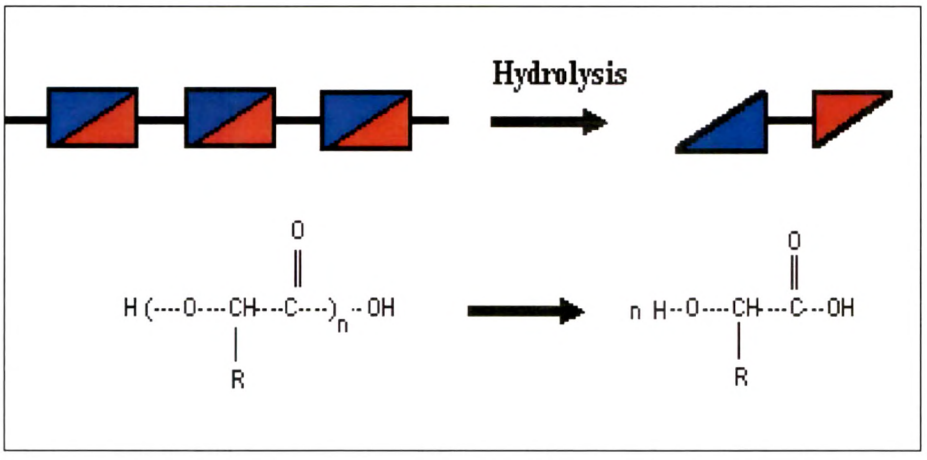

Figure 1.7 Schematic representation of bioerodible/biodegradable matrix[53].

In recent years, the deeper understanding of pathophysiology of several diseases, have given rise to the concept of novel pulsatile drug delivery systems. In these systems, the release of the drug is modulated in order to match the effective need of drug action. This improvised release profiles would increase drug effectiveness, reduce the toxicity as well as avoid the development of tolerance. Polymers able to receive and

respond to specific stimuli have been prepared and applied for the development of such drug delivery systems[51, 66]. The mentioned stimuli can be physiological (pH, temperature, presence of specific agents and chemicals, e.g. enzymes, antigens, sugars etc.) or external (ultrasonic radiations, magnetic fields, light, mechanical, electrical, etc.) responsive.[67, 68].

1.2.2. Colloidal drug delivery systems

Colloidal drug delivery systems represent one of the most studied and developed systems during the last decades, due to

- Increase in high development costs for new therapeutic molecules.
- Increase in the proportion of drug approvals comprising new formulations of approved drugs and
- Less time required to bring such products in market than for new chemical entities.

Colloid science is often defined as the science of materials with size below a micron. While particle size is often the primary property of concern in a colloidal system for its final application, the huge surface area that results from dividing a mass of materials down to colloidal dimensions means that colloidal materials offer other important advantages in terms of tailoring surface properties and subsequent modification of particle behaviors. In terms of drug delivery, the flexibility in tailoring the internal structure and surface has lead to adoption of colloidal drug carriers as the platform for a wide range of existing products as a means to achieve new delivery modes for existing drugs and improve their therapeutic profiles. Because of their small particle size, suitably modified, colloidal drug carriers are the most promising systems to attain the goal of targeted release. The outcome of a drug after administration in-vivo is determined by the combination of drug absorption, distribution, metabolism, and elimination [ADME]. Regardless of the administration path, the bioavailability of the drug depends mainly on its physicochemical properties and therefore on its chemical structure. In particular for intravenous injection, the limit of drug solubility can be solved in different ways. General approaches consist in specific or non-specific complexation (e.g. with PEG or cyclodextrins), use of solvent mixtures, micro-ionization, micro-precipitation or disintegration techniques (pearl milling, high pressure homogenization).[69] Release control from these products is

hardly tuneable, whereas the loading of the therapeutic agent into drug carriers allows for a better control of drug biodistribution, bioavailability and pharmacokinetics[70]. Colloidal drug delivery systems include liposomes, niosomes, dendrimers, micelles, nanoparticles and microemulsions. Ointments, which also can be conceived as colloidal drug delivery systems, are mainly used topically and therefore are very different in their way of action from the above mentioned systems. Colloidal carriers are very similar in their size, shape and mode of administration and for this reason they may be used alternatively. Nevertheless, they present a number of different advantages and disadvantages, so that the most suitable system will have to be chosen depending on the drug and the therapeutic goal to be reached [71,72]. Examples of different colloidal drug delivery system and their applications are summarised in ***Table 1.4***.

Table 1.4 Colloidal drug delivery systems and their applications.

Nanoparticles	Description	Applications	Ref.
Dendrimers	Macromolecular compound that comprises a series of branches around an inner core	Rapid and complete release of Paclitaxel	73
Liposomes	Artificial spherical vesicles produced from natural phospholipids and cholesterol	Liposomal doxorubicine has been employed successfully in the treatment of recurrent uterine adenosarcoma in mice	74,75
Ceramic nanoparticles	Nanoparticles are made from inorganic compounds such as silica and titania	Ultra fine silica based nanoparticles that release water insoluble anticancer drug.	76
Nanocapsules	Vesicular systems in which drug is surrounded by polymeric membranes	Artificial red blood cell nanocapsules with PEG-PLA membranes; circulation half life of blood cells is doubled	77
Micelles	Amphiphilic block copolymer that can self-associate in aqueous solution	PCL-PEO block copolymers that can selectively deliver drug to specific sub cellular targets.	78
Hybrid nanodevices	Hybrid nanoparticles covalently bound to oligonucleotide	DNA-TiO_2 nanoparticles with the ability to target, bind and cleve DNA	79, 80
Nanospheres	Matrix systems in which the drug is physically and uniformly dispersed	Uptake of PLGA nanospheres by human dendritic cells in vivo; has implications for the selective activations of the T-cell mediated immune response	81,82

1.2.2. A. Liposomes and noisomes

Liposomes were first recommended as carriers for bioactive agents in 1971[83] and nowadays they are the most developed and accessible carrier systems[84]. Liposomes are small artificial spherical vesicles constituted of one or more concentric lipid (usually phospholipids and cholesterol) layers delimiting an inner water phase. When mixed in water under low shear conditions, the phospholipids arrange themselves in sheets, the molecules aligning side by side in "heads" up and "tails" down orientation. Once the critical concentration of lipids is overcome, these sheets join tails– to–tails to form a bilayer membrane, which encloses some of the water in a phospholipids sphere. Typically, several of these vesicles will form one inside the other in diminishing size, creating a multilamellar structure of concentric phospholipid spheres separated by layers of water. The diameter of these vesicles ranges from 200 nm to 1 μm. By sonication or ultra high–shear processing, unilamellar vesicles and bilayer spheres having 30–200 nm diameter can be obtained ***(Figure 1.8).***

Based on their size and structure, liposomes are generally classified in three categories: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV) and multilamellar vesicles (MLV). The introduction of positively or negatively charged lipids provide liposome surface charge, whereas liposome bilayer can be chemically grafted to functional moieties or polymers, such as PEG in order to avoid detection by the body's immune system[85]. Liposomes are used as biocompatible carriers of conventional and unconventional drugs, for pharmaceutical, cosmetic, and biochemical uses.

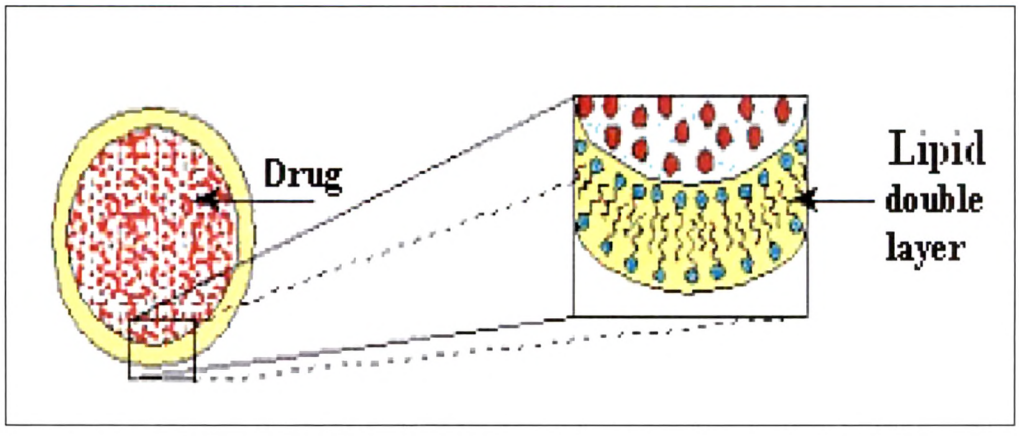

Figure 1.8 Schematic representation of unilamellar liposomes.

The enormous flexibility in particle size and in the physical characteristics of the lipids makes them an attractive potential for constructing tailor–made vehicles for a wide range of applications. Liposomes can be custom designed for almost any need by varying the lipid content, size, surface charge and method of preparation. A special quality of liposomes is that, they enable water soluble and water insoluble materials to be used together in a formulation without use of surfactants or other emulsifiers. Water soluble materials are dissolved in the water in which the phospholipids are hydrated, and when the liposomes are formed, these materials are trapped in the aqueous centre. Conversely, the liposome wall has hydrophobic features. Even though liposomes are characterized by positive aspects, such as high drug payloads and good bio–acceptability, they present some drawbacks related to their in-vitro and in-vivo instability (sensitivity of the phospholipid membranes to environmental degradation, rapid drug leakage across the phospholipid bilayer), unreliable reproducibility and difficult surface functionalization. Liposome based formulation of hydrophobic drugs like amphotericine B (Ambisome B™) and verteporfin (Visudyne™) and hydrophilic drugs, including doxorubicin hydrochloride (Doxil™) have already got FDA approval[86].

Niosomes are vesicles made of non–ionic surfactants[87] characterized by an increased chemical stability that gives them a significant advantage over liposomes. Examples of substances entrapped in this kind of carrier include anticancer[88] and anti–leishmanial drugs. Moreover, niosomes are intended as vaccine adjuvant, since the formulation of antigens as niosomes in water–in–oil emulsion further increases the activity of antigens[89].

1.2.2. B. Nanoparticles

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. Over the past few decades, pharmaceutical principles and nanotechnology have been applied for the preparation of nanoparticles for either therapeutical or diagnosis purposes[90-94]. Polymeric nanoparticles have been developed since 1976 by using non–biodegradable and biodegradable polymeric systems[95]. The biologically active molecules can be adsorbed or attached onto nanoparticle surface, or entrapped, dissolved, or encapsulated inside the carrier. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. A

nanoparticle is a collective name for nanosphere and nanocapsule. The first one has a matrix type structure in which the drug is dispersed physically and uniformly, whereas the second one has a polymeric outer shell and an inner liquid core[96] ***(Figure 1.9)***.

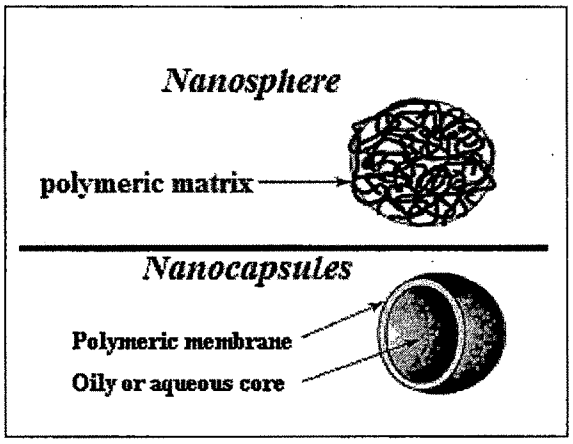

Figure 1.9 Comparison of the structure of nanospheres and nanocapsules.

The major goals in designing nanoparticles as a delivery systems are to control particle size, surface properties and release of pharmacologically active agents in order to achieve site-specific action of the drug at the therapeutically optimum rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drug from degradation, targeting to site of action and reduction in toxicity and side effects; their applications are limited due to inherent limitations such as low encapsulation efficiency, rapid leakage of water soluble drug in the presence of blood components and poor storage stability[97]. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins.

The advantages of use of nanoparticles as drug carrier mainly result from their two basic properties. First, because of their size, they can penetrate through small capillaries and be taken up by cells, thus inducing efficient drug accumulation at the target sites[98]. Secondly, the availability of a wide range of polymeric materials and further possibility of biofunctionalization of particle surface with targeting and stealth moieties[99]. Nanoparticles can be administered in fluidized form with a liquid carrier. This permits their use in the preparation of intravascular infusions and of injectable

suspensions for both parenteral and enteric administration. Nanoparticles for sustained release can be formulated based upon the composition and material characteristics of the specific polymers, drugs, and excipients used[100]. Nanoparticles can be designed for different kind of administration routes: ***intravenous, intramuscular, subcutaneous, oral, nasal, ocular, transdermal.*** Depending on the desired mode of administration, the size of the carriers should be optimized. By taking into account that the diameter of the smallest blood capillaries is about 4 μm, a reduction of the particle size enables intravenous injection; this is desirable also for intramuscular and subcutaneous administration, because it minimizes possible irritant reactions[95, 98]. Among the most used synthetic nanoparticle matrices, poly (alkyl cyanoacrylate)s, poly (ethylene glycol) (PEG) and polyesters, such as poly (lactic acid) (PLA), poly (lactic-co–glycolic acid) (PLGA), poly (ε–caprolactone) (PCL) and their copolymers can be mentioned. With regard to available toxicological and clinical data, the aliphatic polyesters based on lactic and glycolic acids are unquestionably the most widely investigated polymers. Their high biodegradability and biocompatibility are well assessed. In fact, it is known that lactide/glycolide polymer chains are cleaved by hydrolysis to form natural metabolites (lactic and glycolic acids), which are eliminated from the body through the Krebs cycle. The use of biodegradable materials avoids the problems related to the physiological excretion or mechanical removal of the delivery device after drug depletion. Moreover, the presence of a biodegradable matrix can provide a further control of the release rate, by joining the typical diffusive mechanism with tunable polymer degradation[94]. As mentioned above, a fundamental characteristic of nanoparticles is their ability to deliver drugs across several biological barriers. With the development in nanotechnology, it is now possible to produce drug Nanoparticles which can be utilized in a variety of innovative ways. For example, in 2005, the U.S.FDA approved intravenously administered 130-nm albumin nanoparticles loaded with Paclitaxel (Abraxane™) for cancer therapy. Recently, it has been demonstrated that poly (butyl cyanoacrylate) nanoparticles coated with polysorbate 80 and synthesized through emulsion polymerization technique, are effective in crossing blood–brain barrier (BBB) and transporting the hexapeptide dalargin and other agents into the brain effectively[98]. Since then, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) are known as long-circulating particles, which have been used as potential drug delivery devices because of their ability to circulate for a prolonged

time period, target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.[101, 102]. Nanoparticles have been produced also for the sustained intracellular drug delivery. As an example, nanoparticles were effective in sustaining intracellular dexamethasone levels in the treatment of vascular smooth muscle cells[98, 103]. The delivery of antigens for vaccination has also been considered as a novel application of nanoparticles[103].

In spite of these advantages, nanoparticles do have some limitations. For example, their small size and large surface area can lead to particle-particle agglomeration, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, burst release of drug from nanoparticles and limited drug loading problems have to be overcome before nanoparticles can be used extensively for clinically or made commercially available.

1.2.3 Effect of characteristics of nanoparticles on drug delivery

Nanoparticles are of great interest in drug delivery because of the comparable size of the components in the human cells. It appears that nature, in making the biological systems, has extensively used nanometer scale. If one has to go hand in hand with nature in treating the diseases one needs to use the same scale, whether it is correcting a faulty gene, killing leprosy bacteria sitting inside the body cells, blocking the multiplications of viral genome, killing a cancer cell, repairing the cellular metabolism, or preventing wrinkles or other signs of aging[104].

- ***Particle size***

Particle size and particle size distribution are the most important characteristics of nanoparticle systems. They determine the in-vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition they can also influence the drug loading, drug release capacity and stability. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as drug delivery systems. Generally, intracellular uptakes of nanoparticles are higher as compared to microparticles and hence able to deliver the therapeutical agents to wider range of biological targets. Desai *et al*[105] studied the effect of particle size (100 nm, 500 nm, 1μm and 10 μm) of poly (d, l-lactide-co-glycolide) (PLGA) on uptake in rat gastrointestinal tissue. They found that

100 nm nanoparticles had a 2.5 fold greater uptake than 1 μm microparticles and 6 fold greater uptakes than 10 μm microparticles in a Caco-2 cell line. The nanoparticles penetrated throughout the sub mucosal layers in a rat in situ intestinal loop model, while microparticles were mostly localized in the epithelial lining. Drug release is also affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which slowly allow more drug to be encapsulated and slowly diffuse out. Dynamic light scattering technique is most routine and fast method for determination of particle size and the results obtained by scattering technique can be verified by use of microscopic techniques such as SEM/TEM/AFM.

- ***Surface properties of nanoparticles***

When nanoparticles are administered intravenously, they are easily recognized by the body's immune system, and are then cleared by phagocytes from the circulation[106]. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins. This in turn influences in-vivo fate of nanoparticles.[107, 108]. The process of binding of these opsonins (mainly, proteins) onto the nanoparticles surface are called opsonization, which acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes systems (MPS) such as liver, spleen, lungs and bone marrow. Certainly, once in the blood stream, conventional surface unmodified nanoparticles are rapidly opsonized and massively cleared by the macrophages of MPS rich organs[109]. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to reduce the opsonization and to prolong the circulation of nanoparticles in vivo. This can be achieved by surface modification of nanoparticles, and it can be done either with the help of hydrophilic biodegradable polymers/surfactants or by preparing the nanoparticles by copolymerization at specific composition of hydrophobic/ hydrophilic monomers, so as hydrophilic polymer remains at the surface. The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the

particles and medium in which it is dispersed. Generally, it was observed that, nanoparticles with a zeta potential near or above ± 30 mV have been shown to be stable in suspension, as the surface charge prevents agglomeration of the particles.

- ***Drug loading***

Ideally, a successful nanoparticulate system should have high drug loading capacity in order to reduce the quantity of matrix used and ultimately matrix directed side effects. Drug loading can be done by two methods:

- Encapsulation of bioactive compounds at the time of nanoparticle preparation (Incorporation method).
- Absorbing and/or attaching the drug after formation of nanoparticles. (Adsorptions/Absorption technique).

Couvreur *et al*[109] reported the adsorption of two antineoplastic drugs viz, dactinomycin and methotrexate onto poly (methyl cyanoacrylate) and poly (ethyl cyanoacrylate) nanoparticles. The capacity of adsorption is thus related to the hydrophobicity of the polymer and the specific area of the NPs. In the case of entrapment methods, an increase in concentration of the monomer increases the loading efficiency of drug, but a reverse trend is reported with an increase in the initial drug concentration in solution by Radwan[110]. In addition to adsorption and incorporation, an alternative method of drug loading was proposed by Yoo *et al*[111]. In this method, the drug was chemically conjugated to the NPs. Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix materials, which is related to the polymer composition, molecular weight, drug-polymer interaction, and the presence of functional groups in matrix[112-114]. The macromolecules or proteins show greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption[115]. While for small bioactive molecules, studies show the use of ionic interaction between the drug and matrix materials can be very effective way to increase the drug loading[116, 117].

- ***Drug Release***

The following methods for the determination of the in-vitro release are being used:

- Side by side diffusion cells with artificial or biological membranes
- Dialysis bag diffusion technique
- Reverse dialysis sac technique
- Ultracentrifugation
- Ultra-filtration (Centrifugal) technique

The dialysis technique is a very popular method to study the release of drug from colloidal suspensions. To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. Besides polymer biodegradation, a number of interrelated factors govern rate of release from particles e.g. the physicochemical properties associated with particles such as size, shape, porosity, morphology etc. Which, in turn are influenced by a variety of factors, such as, method of preparation, formulation parameters to name a few. In general, drug release rate depends on:

- Desorption of the surface bound/adsorbed drug,
- Drug diffusion through the nanoparticle matrix,
- Nanoparticle matrix erosion/diffusion process,
- Combination of erosion/diffusion process,
- Water permeability and solubility (hydrophilicity/hydrophobicity)
- Chemical composition
- Mechanism of hydrolysis (non catalytic, autocatalytic, enzymatic)
- Additives (acidic, basic, monomers, solvents, drugs)
- Morphology (crystalline, amorphous)
- Device dimensions (size, shape, surface to volume ratio)
- Porosity
- Glass transition temperature (glassy, rubbery)
- Molecular weight and molecular weight distribution
- Physio-chemical factors (ion exchange, ionic strength, pH)
- Sterilization

- Site of implantation

Washington[118] has reported that, particle size has direct correlation with rate of drug release. He found that, increased size and zeta potential of particles, enhanced uptake of particles by the Reticulo Endothelial System (RES) while decrease in size and increased zeta potential lead to increased stability of the particles and also affected fate of the particles after administration. Smaller particles were found accumulated in tumor sites due to facilitated extravasation and greater endocytosis.

Besides particle size, many other parameters have been shown to affect the drug release profiles. The nature of the drug or other additives are obviously very important and also degree of drug loading, various polymer parameters like molecular weight, crystallinity, composition, porosity of matrix and physical state of drug inside the nanoparticles govern the release profile of therapeutic agent from nanoparticles matrix.

Maulding *et al*[119] have reported on the acceleration of microsphere degradation rates by incorporation of a tertiary amino compound, thioridazine. They suggested that the nucleophilic nitrogen of the thioridazine base participated in the degradation of ester bonds and as little effect was seen when the amino group of thioridazine was protonated and the salt (pamoate) was incorporated in the microspheres. Basic compounds can catalyze ester linkage scission and thus accelerate polymer degradation. On the other hand, appropriate amounts of basic compounds can neutralize carboxyl end groups and thus decrease the rate of degradation[120].

Some studies have investigated inclusion of various additives that enhanced drug incorporation and eventually controlled drug release. Encapsulation efficiency of Propafenone hydrochloride in PEG-g-PLA polymer prepared without triethyleneamine (TEA) was 10 % [121]. It was increased to 43 % with the use of TEA probably due to the scavenging action of TEA on Propafenone hydrochloride. Fresta *et al* [122] reported 60 to 70 % initial burst release for particles incorporating drug by adsorption. This method of incorporating drug into the matrix provided more sustained release profile. For chemically conjugated drug, release occurred over a period of 25 days compared to unconjugated drug where all drug release took place

within 5 days[111]. Also, release of conjugated drug was reported to be influenced by the chemistry of attachment to the polymer.

The degree of drug loading is often correlated with the release profile, especially since the drug may be crystallized in the carrier matrix at high loadings. For example, as reported by Leroux *et al*[123], PLA nanoparticles containing 16.7 % savoxepine released 90 % of their drug load in 24 h as opposed to particles containing 7.1 % savoxepine which released their content over 3 weeks.

By appropriate selection of polymer parameters like molecular weight, crystallinity and composition, release profile can be modulated. Duan *et al*[124] synthesized monomethoxy polyethylene glycol-poly (lactic acid-co-glycolic acid) - monomethoxy polyethylene glycol (mPEG-PLGA-mPEG) nanoparticles using mitoxantrone as a model drug. PLGA copolymers with varying molar ratios of lactic acid to glycolic acid and different molecular weights and contents of mPEGs were synthesized and evaluated. They found that rate of mitoxantrone release can be modulated by either increase of mPEG content or decrease in molar ratio of lactic acid in Nanoparticles. Increase in lactic acid led to an increase in hydrohobicity that enhanced drug loading and decreased rate of drug release while at lower molecular weight of the polymer faster drug release rate was observed [125].

Several researchers have tried to explore the mechanisms of burst and control it by several methods such as surface coating of the polymers[126-128], changing the surfactant[129], changing the molecular weight of the polymer[130], modifying the backbone[131] etc.

Altering the porosity of the particles also affects the drug diffusion and polymer degradation rate. Lemaire *et al*[132] suggested that if a drug molecule is present within a network of micropores it will have to diffuse towards the closest pore to be released outside. Movement of the drug within the porous media is highly restricted due to limited space available and is influenced by diffusivity of drug, porosity and tortuosity of matrix. Rapid release of savoxepine, estradiol and dexamethasone from polymeric nanoparticles has been explained by presence of pores in the particles[133-135].

Physical state of the drug inside the nanoparticles plays a key role in the drug diffusion from nanoparticles. An amorphous form of the drug may lead to an increase in the rate of dissolution of the drug. Amorphous materials do not have any long range order such as crystal lattice as crystalline materials do. They have a higher internal energy, a larger free volume, and greater molecular mobility in comparison to the crystalline state. These properties are responsible for greater solubility and hence fast release. Small drug molecules can also easily crystallize and consequently both crystalline and amorphous states coexist. The DSC can provide qualitative and quantitative information about the physicochemical status of the drug in the particles[136]. The related thermal transitions include melting, recrystallization, decomposition or a change in heat capacity. DSC is useful to monitor different samples of the same material to assess their similarities or differences or the effects of additives on the thermal properties of a material. Using DSC analysis of drug, polymeric materials and the resulting drug loaded nanoparticles, the nature of the drug inside the polymer matrix can be assessed, which may be present in a solid solution, metastable molecular dispersion or in a crystalline form and may display relevant properties during in-vitro release. There is no detectable melting endotherm if the drug is present in a molecular dispersion or a solid solution state in the polymeric microspheres loaded with sufficient drug. The physical state of polymer is also instrumental in influencing drug release rate. Amorphous or crystalline drug may be present in either amorphous or crystalline polymer. When the glass transition temperature (Tg) of polymer is higher than ambient temperature, the polymer is in a glassy state and the material is brittle and hard. Below Tg polymer is in a rubbery state and hence promotes mobility and release of drugs. Drugs or additives if well dissolved in the particles may act as a plasticizer and reduce the Tg of nanoparticles. On the other hand, a crystalline form may act as reinforcing filler and cause an increase in Tg[137-138].

1.3. Techniques of preparation of nanoparticles and microparticles

The setting up of polymeric nanoparticles preparation process requires an accurate investigation of physical and chemical properties of polymer and drug. The optimized preparation process should assure for the chemical stability and biological activity of the incorporated therapeutic agents. Particularly, when the loaded active

agent is a protein, its penetration upon contact with hydrophobic organic solvents or acidic/basic aqueous solutions should be avoided by using appropriate preparation conditions. The encapsulation efficiency and the yield of the process should be high enough for mass production and the produced nanoparticles should display a homogeneous diameter distribution, in agreement with their end-use requirements. However, the preparation and purification processes must lead to high reproducible results and the release profile of the drug should meet the specific final application requisites. Finally, in case of final pharmaceutical dosage forms that involve the recovery of a suspension of nanoparticles in appropriate mediums, free-flowing nanoparticles should be prepared and appropriate storage conditions should be investigated.

There are number of techniques available for the preparation of drug loaded nanoparticulate systems such as the emulsion solvent evaporation/extraction method, spray drying, phase separation-coacervation, interfacial deposition and in-situ polymerization. Each method has its own advantages and disadvantages. The choice of a particular technique depends on polymer and drug features, site of action and therapy regimes[139]. An extensive description of several preparation methods has recently been reviewed[140].

1.3.1. Emulsion-solvent evaporation/extraction methods

1.3.1. A. Single emulsion method

This method has been primarily used to encapsulate hydrophobic drugs through oil-in-water (o/w) emulsification process[141]. The hydrophobic drug is dissolved or dispersed in an organic solvent into the polymer solution, and the resulting mixture, after emulsification by high-speed homogenisation or sonication, is added into an aqueous solution to make an oil-in-water emulsion with the aid of amphiphilic macromolecules, which are termed emulsifier/stabilizer/additive[142]. The solvent in the emulsion is removed by either evaporation at elevated temperatures or extraction in a large amount of water, resulting in formation of compact particles. The solvent evaporation method has been used extensively to prepare PLA and PLGA micro and nano-particles containing many different drugs[143]. Several variables have been identified which can influence the properties of the nanoparticles, including drug

solubility, internal morphology, solvent type, diffusion rate, temperature, polymer composition, viscosity, and drug loading[144]. This method, however, is only available for the hydrophobic drugs because the hydrophilic drugs may diffuse out or partition from the dispersed oil phase into the aqueous phase, leading to poor encapsulation efficiencies[145]. Many types of drugs with different physical and chemical properties have been formulated into polymeric systems, including anti-cancer drugs, narcotic agents, local anesthetics, steroids and fertility control agents.

1.3.1. B. Double emulsion method

Several water soluble drugs have been encapsulated by water-in-oil-in-water (w/o/w) methods. The aqueous solution of the water soluble drug is emulsified with polymer dissolved organic solution to form the water-in-oil (w/o) emulsion. The emulsification is carried out using either high speed homogenizers or sonicators. This primary emulsion is then transferred into an excess amount of water containing an emulsifier under vigorous stirring, thus forming a w/o/w emulsion. In the subsequent procedure, the solvent is removed by either evaporation or extraction process. One advantage of this method is encapsulation of hydrophilic drugs in an aqueous phase with the high encapsulation efficiency. For this reason, the w/o/w emulsion system has been used widely for the development of protein delivery systems. The characteristics of the particles prepared by the double emulsion method are dependent upon the properties of the polymer (such as composition and molecular weight), the ratio of polymer to drug, the concentration and nature of the emulsifier, temperature, and the stirring/agitation speed during the emulsification process[145].

1.3.2. Phase separation method

This method involves phase separation of a polymer solution by adding an organic nonsolvent. Drugs are first dispersed or dissolved in a polymer solution. An organic nonsolvent is added to this solution (e.g. silicon oil) under continuous stirring, by which the polymer is gradually extracted and soft coacervate of droplets containing the drug are generated. The rate of adding nonsolvent affects the extraction rate of the solvent, the size of the particles and encapsulation efficiency of the drug. The commonly used nonsolvents include silicone oil, vegetable oil, light liquid paraffin, and low molecular weight polybutadiene. The coacervate phase is then hardened by

exposing it into an excess amount of another nonsolvent such as hexane, heptane and diethyl ether.

The final characteristics of the colloidal product are affected by the molecular weight of the polymer, viscosity of the nonsolvent and polymer concentration. The main disadvantage of this method is a high possibility of forming large aggregates. Extremely sticky coacervate droplets frequently adhere to each other before complete phase separation. This technique is promising for preparation of protein loaded microcapsules. For example, conventional methods of preparing microparticles involve extensive exposure of proteins to the interface between aqueous and organic phases, to hydrophobic polymer matrix, and to acidic/basic microenvironments resulting from degradation of the polymer. These unfavorable interactions are reported to induce conformational changes of proteins. On the contrary, the interfacial phase separation technique is shown to minimize these sources of protein inactivation[146].

1.3.3. Nanoprecipitation method

The nanoprecipitation technique for nanoparticle preparation was first developed and patented by Fessi and co-workers in 1989[147] and it is a straight forward technique, rapid and easy to perform. The nanoparticle formation is instantaneous and the entire procedure is carried out in only one step. The polymer and the drug are dissolved together and precipitated in a nonsolvent, miscible with the former one. Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent. Indeed, as soon as the polymer containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment. The rapid nanoparticle formation is due to interfacial turbulences that take place at the interface of the solvent and the nonsolvent and results from complex and cumulated phenomena such as flow, diffusion and surface tension variations. Nanoprecipitation often enables the production of small nanoparticles (100–300 nm) with narrow unimodal distribution and a wide range of preformed polymers can be used, such as poly (d,l-lactic-co-glycolic acids), cellulose derivatives or poly (caprolactones).

This method does not require extended shearing/stirring rates, sonication or very high temperatures, and is characterized by the absence of oily-aqueous interfaces, all conditions that might damage a protein structure. Moreover, surfactants are not always needed and unacceptable toxic organic solvents are generally excluded from this procedure. However, the original nanoprecipitation method suffers from some drawbacks. This technique is mostly suitable for compounds having a hydrophobic nature such as indomethacin, which is soluble in ethanol or acetone, but displays very limited solubility in water. Recently, formulation and process modifications were investigated to improve the versatility of the nanoprecipitation technique, particularly with respect to the encapsulation of hydrophilic drugs (e.g. proteins)[148]. The nanoprecipitation method has been used extensively to prepare nanoparticles containing many drugs[149-153].

1.3.4. Dialysis method

Some disadvantages of the conventional methods include the difficulties and necessities of removal of solvent and surfactant residues, low particle yield, excessive steps for preparation, and the necessity to use a high concentration of surfactant for the preparation of small spherical particles and polymeric micelles. The dialysis method is a simple and effective preparation method for small and narrow size distributed nanoparticles mostly using block graft copolymers and other amphiphilic materials[154]. Polymer, drug and surfactants are dissolved in the same organic solvent and placed inside a dialysis tube with proper molecular weight cut-off. Dialysis is performed against a nonsolvent, miscible with the former one. The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer, drug and surfactants due to a loss of solubility and by the formation of homogeneous suspensions of micro-nanoparticles ***(Figure 1.10)***. Trials of preparations without surfactants were performed. The dialysis method was applied to PLGA starting from different solvent solutions and avoiding the use of surfactants. The solubility of the particles components in the initial solvents may affect the physicochemical properties such as particle size and drug contents, whereas the absence of surfactant led to some drawbacks such as instability of the re-dispersed freeze dried PLGA nanoparticles and low drug loading efficiency[154]. Poly (Lactide) / Tween 80 copolymers were synthesized, aiming at incorporating a widely used

emulsifier directly in polymer backbone for preparation of biomedical nanoparticles. Nanoparticles loaded with anticancer drug were obtained by using the dialysis method and without use of additional surfactants/emulsifiers by Gupta *et al*[155].

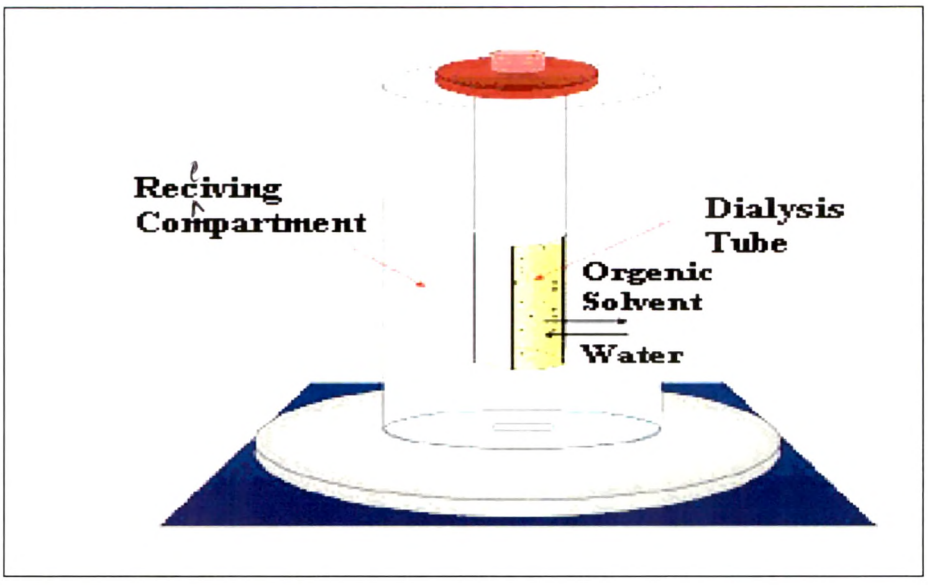

Figure 1.10 Schematic representation of dialysis procedure[53].

1.3.5. Self assembling method

Recently it has been demonstrated that nanoparticles can be obtained by interaction between charged polymers and oppositely charged molecules. Such association depends on many factors including coulombic interactions, hydrophobicity of the polymer–molecule pair, and the conformational features of the polymer ***(Figure 1.11)***. One special class of such systems is the complexes formed by polyions of opposite charges. The solution behavior of these complexes strongly depends on their composition. Electroneutral complexes that contain equivalent amounts of polyion units and monomers are water-insoluble. Non stoichiometric complexes containing an excess of one of the components are generally soluble in water. Since these complexes are capable of forming aggregates of nanometer size, they have been termed as polyion complex (PIC) micelles or block ionomer complexes (BICs) [156]. Micellar drug delivery systems display several advantages over other particulate configurations. For example, they can significantly enhance the water solubility of hydrophobic drugs for improved bioavailability. They usually exhibit low critical micelle concentration (CMC), rendering the drug loaded micelles

stable in the bloodstream to achieve fairly long circulation time[157]. The traditional micelles are unable to sense a signal and respond by changing their structures. Incorporating external control over structure and physical properties offers the possibility of constructing systems capable of both tunable transformation and controlled transmission of energy or information. For this purpose thermosensitive[157], pH sensitive[158], and pH and temperature sensitive micelles[159] had been studied.

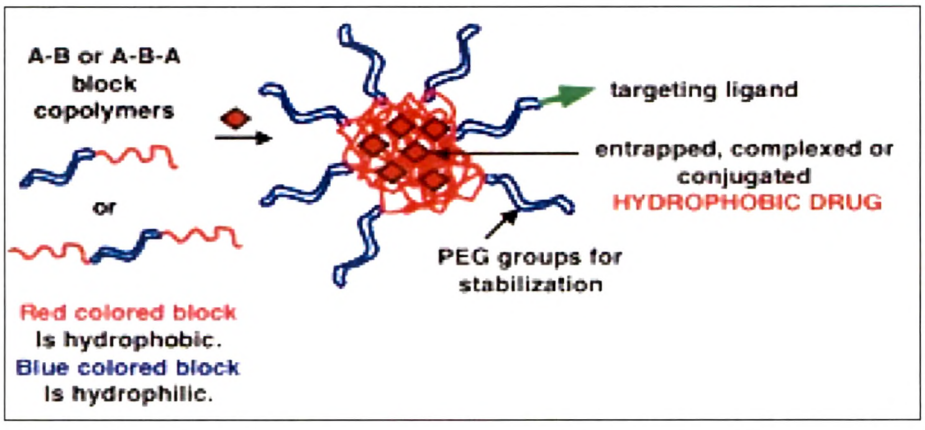

Figure 1.11 Schematic representation of diblock copolymer self assembly[156].

1.3.6. Rapid expansion of supercritical fluid solution method

Supercritical fluid technology has played a significant role in the particle formation. A supercritical fluid is loosely defined as a solvent at a temperature above the critical temperature, at which the fluid remains a single phase regardless of pressure. However, for practical purposes such as high density for solubility considerations, fluids of interest to materials processing are typically at near critical temperatures. Among the most important properties of a supercritical fluid are the low and tunable densities, which can be easily varied from gaslike to liquidlike through a simple change in pressure at constant temperature and the unusual solvation effects at densities near the critical density (often discussed in terms of solute–solvent and solute–solute clustering). The production of polymeric nanoparticles through "Rapid Expansion of a Supercritical Solution" into either air (RESS) or Liquid Solvent (RESOLV) may conceptually be divided into two somewhat related processes. One is the initial formation of nanoparticles in the rapid expansion, and the other is the stabilization of the suspended nanoparticles. Evidently, the protection of initially formed polymeric nanoparticles represents a different set of technical challenges,

which are largely independent on the rapid expansion process itself, especially if the protection agent is added immediately after the expansion. The good news is that many methods for stabilizing nanoparticle suspensions are already available in the literature, some of which have shown promise in use with RESOLV. Supercritical fluid processing techniques can play a significant role in the particle formation and production. In particular, the RESOLV technique offers a unique way to prepare clean and narrowly distributed polymeric nanoparticles. Since the nanoparticles obtained in RESOLV are suspended, they may be protected from agglomeration by using existing stabilization methods and agents. These stable nanoparticle suspensions may find many interesting and important applications, as already discussed in the literature[160]. Furthermore, a new method called Supercritical Fluid Extraction of Emulsions (SFEE) has been investigated. The method combines the advantages of traditional emulsion based techniques, namely control of particle size and surface properties, with the advantages of continuous supercritical fluid extraction process, such as efficient scale-up, higher product purity, and shorter processing times. Fine particles of model compounds cholesterol acetate (CA), griseofulvin (GF), and megestrol acetate (MA) were produced by extraction of the internal phase of oil-in-water emulsions using supercritical carbon dioxide[161].

1.3.7. Spray drying method

Compared to other conventional methods, spray drying offers several advantages. It shows good reproducibility, involves relatively mild conditions, allows for control of the particle size, and is less dependent on the solubility of the drug and the polymer. Generally, the polymer is dissolved in volatile solvents and the drug is dispersed or dissolved in the polymer solution. Solutions or dispersions are sprayed against a stream of cold air (-60 °C; top-spraying) using a two-fluid pneumatic nozzle with heating facilities. The frozen droplets formed by this spray-freezing step are dried during the following atmospheric freeze-drying in the cold desiccated air stream by sublimation. A filter holds the fine product back in the drying chamber, while the water vapor is removed by the circulating air in the cooling systems, where the humidity condenses on the refrigerated surfaces[161]. Recently this method has been used to prepare dry powder aerosol particles[162], powder formulations for controlled delivery of Paciltaxel[163], and powders for aerosol delivery to the lung. In an attempt to

minimize aggregation of the microparticles, a double-nozzle spray-drying technique was developed. While the polymer/drug solution is sprayed from one nozzle, aqueous mannitol solution is simultaneously sprayed in order to coat the particulate with an anti-adherent agent. The results indicate that the coating of the microspheres with mannitol reduces the extent of aggregation and augments the yield of the product[164]. When a protein drug is encapsulated by means of spray drying, a loss of its biological activity may occur especially when aqueous phase systems are involved[165]. The atomization of a w/o emulsion or cryogenic, non-aqueous processes can be used instead[166]. In the latter technique, the liquid droplets of the polymer/drug solution are produced through the spraying nozzle, collected in liquid nitrogen containing frozen ethanol, and hardened by placing them at -80°C where the solvent extraction occurs[167].

1.3.8 In-situ polymerization method

Production of nanoparticles with the desired physicochemical properties to facilitate the targeted drug delivery has been a topic of renewed interest in pharmaceutical industries. But solvent impurities and some stabilizing agent for the stability of colloid remain in the drug-loaded nanoparticles, and then these become toxic and may degrade the pharmaceuticals within the polymer matrix. The majority of the microparticle preparation techniques have been tailored for the fabrication of nano-sized particles but by such efforts, researcher was not able to produce uniform size and relatively monodispersed polymeric nanoparticles with specific geometry for intended applications. Nanoparticles can also be prepared by emulsion and micro emulsion polymerization of monomers. The term emulsion refers to a dispersion of one phase into another continuous phase (immiscible to the dispersed) with the help of an emulsifier (usually an amphipathic molecule). Emulsions are classified according to the nature of dispersed and continuous phase (oil-in-water or direct, water-in-oil or inverse, water-in-oil-in-water or double) and their size/stability (miniemulsions, microemulsions).

These techniques have generated considerable interest over the years as potential drug delivery systems[168-187]. Advantages associated with such systems include their stability, optical clarity and ease of preparation. The existence of microdomains of different polarity within the same single-phase solution enables both

water-soluble and oil-soluble materials to be solubilised, and at the same time if this is so desired. Furthermore it is also possible to incorporate amphiphilic drugs. It should be noted that solubilisate partitions between the droplet and continuous phase and that while there may be a preferred site for solubilisation of drug. For example the likely preferred sites of incorporation of a water-insoluble drug into an o/w microemulsion are the disperse oil phase and/or hydrophobic tail region of the surfactant molecule, while a water-soluble material would be most likely to be incorporated into the disperse aqueous phase of a water-in-oil droplet. The majority of pharmaceutically active agents are hydrophobic in nature and hence due to the ability of o/w emulsion systems to incorporate hydrophobic drugs into the polar oil phase thereby enhancing their solubility. These methods are known for imparting high specific surface area, spherical morphology and lower particle size polymeric nanoparticles enabling a large variety of drugs to be successfully associated, including cytostatics, antibodies, antiparasitic compounds, hormones and antiviral drugs.

Since pioneering works of Peehmann and Rohm in the late 1930s, the industrial use of acrylic polymers has gained popularity[188]. Acrylic polymers such as Eudragit® and Carbapol® are currently being used worldwide in the pharmaceutical industry for control drug release and for the development of specific drug delivery system. Kim *et al*[189] have suggested that, by use of hydrophilic and hydrophobic monomers with appropriate composition, controlled release of bioactive molecules can be successfully achieved. Polyalkylacrylate nanoparticles have been reported to show slow biodegradability[190]. Biodegradability and stimuli responsiveness of the polymer can be improved by copolymerizing it with more biodegradable stimuli-responsive monomers, such as hydroxyl ethyl methacrylate, acrylamide, N-isopropyl acrylamide and N-vinyl caprolactam[191, 192]. Recently, Babu *et al.*[193] carried out polymerization of acrylamide and methyl methacrylate to produce novel core-shell microspheres and investigated the release of anti-cancer agent 5-fluorouracil with respect to absorption - adsorption technique and cross-linking agent. Improved release profile with higher entrapment of drug was reported for 3:1 ratio of acrylamide to methyl methacrylate core shell microparticle prepared by in-situ polymerization method. This has prompted intense research activity to study polymerization reactions.

1.4 Objectives

Polymeric nanoparticles for pharmaceutical applications are in use since 1960's. Since then, various polymerization methods as well as methods such as emulsion crosslinking, ionotropic gelation, emulsification/solvent evaporation, spray drying and coacervation/precipitation involving preformed polymers have been developed to prepare polymeric nanoparticles. They have been used as carriers for cytostatics, antibodies, antiparasitic compounds, anticancer drugs, hormones, antiviral drugs and many more. The primary objective of the present work is the development and optimization of procedures for the preparation of polymeric nanoparticles. The work has been conducted with the practical end in mind of preparing nano delivery structures for the delivery of bioactive agents. In the undertaken research activity, polymers with different origin (natural and synthetic) and characteristics (hydrophobic, hydrophilic, amphiphilic) are used for encapsulation of hydrophobic and hydrophilic model drugs in order to get a better understanding of the suitability and versatility of the methods applied for the preparation of nano drug delivery systems. Effects of nanoparticles morphology, size and surface charge on encapsulation efficiency as well as on drug release profiles will be examined.

Hence we have undertaken

- Synthesis of copolymeric nanoparticles using acrylate monomers e.g. ethyl methacrylate (EMA) & methyl methacrylate (MMA) and hydrophilic 2-hydroxyethylmethacrylate (HEMA) and thermo responsive N-vinylcaprolactam (VCL) monomers through emulsion polymerization.
- Synthesis of natural polymeric nanoparticles of chitosan by ionic gelation method.
- Characterization of copolymeric nanoparticles through FTIR, ^{1}H NMR, DSC, XRD, UV-VIS spectrometry, GPC, DLS, TEM and XPS.
- Use of synthesized synthetic and natural copolymeric nanoparticles for encapsulation of bioactive molecules such as Lamotrigine (anti-epileptic drug), Venlafaxine Hydrochloride (anti-psychotic drug), Etoposide (anticancer drug) and their in-vitro release profiles.

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