CHAPTER 2

LITERATURE REVIEW
2. Oral Drug Delivery

Oral drug delivery is the choicest and most readily accepted form of drug administration because of its non-invasive nature. It is preferred because of the various advantages over other routes of drug delivery. The oral route presents the advantage of avoiding pain and discomfort associated with injections as well as eliminating contaminations. The other advantages include patient convenience and compliance, which increase the therapeutic efficacy of the drug. Oral formulations are also cheaper to produce because they do not need to be manufactured under sterile conditions (Salama et al; 2006).

Despite these potential advantages, oral formulations face several common problems: (i) poor stability in the gastric environment, (ii) poor bioavailability and (iii) the mucus barrier can prevent drug penetration and subsequent absorption. Many drugs are currently used as parenteral formulations because of their poor oral bioavailability. This is due to several unfavourable physicochemical properties, such as large molecular size, susceptibility to enzymatic degradation, poor stability in the gastric low pH environment, poor penetration of the intestinal membrane, short plasma half-life, immunogenicity, and the tendency to undergo aggregation, adsorption, and denaturation, enzymatic degradation prior to absorption and poor penetration of the intestinal membrane (Yun et al; 2012).

For many years, many studies have been focused on the improvement of oral delivery and bioavailability of drugs. One of the approaches to overcome various limitations associated with oral delivery is nanoparticle formulation that encapsulates and protects drugs and releases them in a temporally or spatially controlled manner. The nanoparticle surface can also be modified to enhance or reduce bioadhesion to target specific cells. Polymeric nanoparticles are of especial interest from the pharmaceutical point of view. First, they are more stable in the gastrointestinal tract than other colloidal carriers, such as liposomes, and can protect encapsulated drugs from gastrointestinal environment. Second, the use of various polymeric materials enable the modulation of physicochemical characteristics (e.g. hydrophobicity, zeta potential), drug release properties (e.g. delayed, prolonged, triggered), and biological behaviour (e.g. targeting, bioadhesion, improved cellular uptake) of nanoparticles (Florence; 1997). Third, their submicron size and their large specific surface area favour their absorption compared...
to larger carriers. Also, the particle surface can be modified by adsorption or chemical grafting of certain molecules such as poly (ethylene glycol) (PEG), poloxamers, and bioactive molecules (lectins, invasins).

**Nanoparticles**

Nanoparticles as a carrier or a device have become the focus of attention in this field recently. The nanoparticles possess certain advantages such as greater stability during storage, stability *in vivo* after administration and ease of scale-up without an aseptic process for oral administration (Kreuter; 1995).

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. This system also helps to increase the stability of drugs/proteins and possess useful controlled release properties (Mohanraj and Chen; 2006).

The advantages of using nanoparticles as a drug delivery system includes:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited Poor drug loading, which is usually less than 5% (weight % of the transported drug with respect to the carrier material). As a result, either the quantity of the drug administered is not sufficient to reach a pharmacologically active concentration in the body, or the amount of the carrier material required is too great, leading to toxicity or undesirable side-effects, as well as, too rapid release (so called “burst release”) of the encapsulated drug after administration. As a consequence, a significant fraction of the drug will be released before reaching its pharmacological target in the body, leading to lower activity and more side-effects. These practical problems have to be overcome before nanoparticles can be use clinically or made commercially available (Couvereur; 2013).

Nanoparticles have versatile potential for efficient exploitation of different drug delivery formulations and routes because of the properties provided by their small size. These possible benefits include controlled release, protection of the active pharmaceutical ingredient and drug targeting. Nanoparticles are expected to offer new solutions e.g. for gene therapy and delivery of peptide drugs. Generally, nanoparticles are applied as an injectable or oral solution, but their use as dried material in formulations such as tablets or inhalable powders is equally conceivable (Langer et al; 2000, Lee et al; 2005).

Pharmaceutical nanoparticles are submicron-sized, colloidal vehicles that carry drugs to the target or release drugs in a controlled way in the body. Nanoparticles are usually dispersed in liquid. Such a system can be administered to humans for example by injection, by the oral route, or used in ointments and ocular products. Alternatively, nanoparticles can be dried to a powder, which allows pulmonary delivery or further processing to tablets or capsules (Vila et al; 2002).
2.1. Physiological Considerations of Gastro-intestinal Tract for Oral Delivery of Nanoparticles:

The gastrointestinal tract is a continuous tube-like structure beginning with the mouth (oral cavity) and extending further as pharynx, esophagus, stomach, small intestine, large intestine, rectum and finally culminating into the anal canal.

The human intestinal epithelium is highly absorptive and is composed of villi that increase the total absorptive surface area in the gastrointestinal (GI) tract to 300–400 m² (Ensign et al; 2012). Enterocytes (absorptive) and goblet cells (mucus secreting) cover the villi, which are interspersed with Follicle Associated Epithelium (FAE). These lymphoid regions, Peyer’s patches, are covered with M cells specialized for antigen sampling. M cells are significant for drug delivery, since they are relatively less protected by mucus and have a high transcytotic capacity (Plapied et al; 2011).

Figure 2.1 provides a quick understanding of the GI targets, principles of formulation development that could be utilized and the application opportunities of the nanoparticles-based drug delivery system throughout the GIT (Amiji et al; 2006).

Different types of cells and structures compose the intestinal epithelium. Epithelium of villi is mainly constituted of enterocytes and goblet cells. One of the main functions of enterocytes is to control the passage of macromolecules and pathogens, and, at the same time, to allow the digestive absorption of dietary nutrients. Goblet cells secrete the mucus gel layer, a viscous fluid composed primarily of highly glycosylated proteins (mucins) suspended in a solution of electrolytes. Dispersed through the intestinal mucosa, lymphoid nodules called O-MALT (Organized Associated Lymphoid Mucosa), individually or aggregated into Peyer’s patches, have interested scientists, mainly due to the presence in these structures of particular cells, named M cells (Gebert et al; 1996).

M cells are mainly located within the epithelium of Peyer’s patches, called Follicle Associated Epithelium (FAE) (Fig. 2.2), which is also composed of enterocytes and few goblet cells. M cells deliver samples of foreign material from the lumen to the underlying organized mucosa lymphoid tissues in order to induce immune responses. (Anne et al; 2006)
Fig. 2.1 GIT targets, formulation principles, opportunities and applications

M cells are specialized for antigen sampling, but they are also exploited as a route of host invasion by many pathogens. Furthermore, M cells represent a potential portal for oral delivery of peptides and proteins and for mucosal vaccination, since they possess a high transcytotic capacity and are able to transport a broad range of materials, including nanoparticles. Uptake of particles, microorganisms and macromolecules by M cells, have been described to occur through adsorptive endocytosis by way of clathrin coated pits and vesicles, fluid phase endocytosis and phagocytosis. In addition, M cells, compared with normal epithelial cells have reduced levels of membrane hydrolase activity, which can influence the uptake of nanoparticles. The relatively sparse nature of the glycocalyx facilitates the adherence of both microorganisms and inert particles to their surfaces. Villous-M cells located outside the FAE have been also observed, but the transport of antigens and microorganisms across the intestinal mucosa is carried out mainly by the FAE-M cells. Although less numerous than enterocytes, M cells present
enhanced transcytosis abilities which made them very interesting for oral drug delivery applications (Florence; 2005).

Fig. 2.2 Schematic transverse sections of a Peyer's patch lymphoid follicle and overlying follicle-associated epithelium (FAE), depicting M cell transport of particulate delivery vehicles

The general structure of intestinal organized mucosa-associated lymphoid tissues (O-MALT) is represented by the schematic transverse section of a Peyer's patch lymphoid follicle and associated structures in (A). The FAE is characterized by the presence of specialized antigen sampling M cells (B) (Reprinted from Adv. Drug. Del. Rev., 50, 2001, Clark et al., Exploiting M cells for drug and vaccine delivery, 81–106.)

2.2 Transport of Nanoparticles across the Intestinal Mucosa:

There are four distinct mechanisms for molecules to cross the cell membrane: via paracellular, transcellular, carrier-mediated, and receptor-mediated transport. Absorption through each pathway is dependent on different physical characteristics,
such as molecular weight, hydrophobicity, ionization constants, and pH stability of absorbing molecules as well as biological barriers that restrict protein absorption from the GI tract. To deliver their drug content in the blood, lymph, or target organs, NPs have to cross the gastrointestinal barrier either by passive diffusion via transcellular or paracellular pathways or by active processes mediated by membrane-bound carriers or membrane-derived vesicles.

A schematic diagram of uptake mechanisms of NPs administered orally is shown in Fig. 2.3 (Vivekananda et al; 2007)

![Schematic diagram of uptake mechanisms of NPs administered orally](image)

**Fig. 2.3 Mechanism of uptake of orally administered NPs: (I) M cells of the PP, (II) enterocytes, and (III) GALT**

The direct uptake of NPs through the lymph into the systemic circulation bypassing the liver reduces the first pass metabolism, thus improving bioavailability (Vivekananda et al; 2007).

**2.2.1 Paracellular Transport:**

Paracellular transport is the pathway of substances across an epithelium by passing through the intercellular spaces in between epithelial cells. Paracellular transport is passive and results from diffusion. This transport is under the control of tight junctions. A tight junction constitutes the major rate limiting barrier towards the paracellular
transport for permeation of ions and larger substances (Madara; 1998). The dimension of the paracellular space is on the order of 10 Å. The average size of aqueous pores created by epithelial tight junctions is approximately 7–9 Å for the jejunum, 3–4 Å for the ileum, and 8–9 Å for the colon in the human intestine. The solutes with a molecular radius exceeding 15 Å (approximately 3.5 kDa) cannot be transported via this route. Furthermore, tight junctions comprise only about 0.01% of the total absorption surface area of the intestine (Rubas et al; 1996). In physiological conditions, the paracellular route is limited, on one hand, by the very small surface area of the intercellular spaces and, on the other hand, by the tightness of the junctions between the epithelial cells (pore diameter between 3 and 10 Å). Paracellular transport can be enhanced by some polymers in solution or in the form of nanoparticles. Chitosan and poly (acrylic acids) in solution can enhance paracellular transport of drugs through interactions between the negatively-charged cell membrane and the positive charges of the polymer, or by complexing Ca$^{2+}$ involved in the structure of tight junctions (Shakweh et al; 2004, Smith et al; 2004).

### 2.2.2 Transcellular Transport:

Transcellular transport occurs through the intestinal epithelial cells by transcytosis, a particular process by which particles are taken up by cells. A typical example is the movement of glucose from the intestinal lumen to extracellular fluid by epithelial cells. This starts with an endocytic process that takes place at the cell apical membrane. Then, particles are transported through the cells and released at the basolateral pole. The basolateral membrane is thinner and more permeable than the apical membrane because the protein-to-lipid ratio is very low in the basolateral membrane. Transport of particles by the transcellular transport depends on several factors: (i) various physicochemical properties of particles, such as size, lipophilicity, hydrogen bond potential, charge, surface hydrophobicity or the presence of a ligand at the particle surface; (ii) the physiology of the GI tract; Enterocytes and M cells are the primary intestinal cells for transport (Florence; 2004). Furthermore, M cells represent a potential portal for oral delivery of proteins and peptides due to their high endocytosis ability. M cells possess a high transcytotic capacity and transport a wide variety of materials, including nanoparticles. M cells take up macromolecules, particles and
microorganisms by adsorptive endocytosis via clathrin-coated pits and vesicles, fluid phase endocytosis and phagocytosis. Although there has been some controversy in the literature on the extent of particle absorption, there is evidence that particle translocation can occur across enterocytes in the villi part of the intestine (Jani et al; 1992). However, the number of particles translocated through these routes is mostly very low because of the low endocytic activity of the enterocytes. It has been generally observed that the bulk of particle translocation mainly occurs in FAE (Lavelle et al; 1995, Hagan et al; 1990). As a result, many researchers have studied with great interest the Peyer’s patches and M cells which have adapted to absorb a large range of materials. Nevertheless, this route is limited to the transport of relatively low molecular-weight lipophilic drugs. Transcellular transport of nanoparticles occurs by transcytosis, a particular process by which particles are taken up by cells. This begins with an endocytic process that takes place at the cell apical membrane. Then, particles are transported through the cells and released at the basolateral pole (Shakweh et al; 2005) Figure 2.4 shows the different pathways taken up by the drug to cross intestinal barrier.

2.2.3 Carrier Mediated Transport

Drugs are transferred across the cell membrane or entire cell and then released from the basal surface of the enterocyte into circulation. The process is suitable and utilized by small hydrophilic molecules. Active absorption requires energy-dependent uptake of specific molecules by carriers (Russell; 1996). The carriers recognize target molecules through membrane receptors and transport them across the membranes into the GI epithelium, even against the concentration gradient and in trace quantities. For example, small di/tripeptides (including β-lactam antibiotics and angiotensin converting enzyme (ACE) inhibitors), monosaccharides, and amino acids are transported transcellularly by a carrier-mediated transport process. Shah and Shen investigated the carrier-mediated transport of insulin across Caco-2 cell monolayers.

2.2.4 Receptor Mediated Transport

In receptor-mediated transport, protein drugs act either as a receptor specific ligand for surface-attached receptors or as a receptor for surface-attached ligands. Receptor-mediated transport has also been exploited to increase the oral bioavailability of
protein and drugs by modification such as receptor specific ligands with peptide and protein drugs. This transportation entails cell invagination, which leads to formation of a vesicle. This transportation, in general, is known as endocytosis and comprises phagocytosis, pinocytosis, receptor-mediated endocytosis (clathrin-mediated), and potocytosis (non-clathrin-mediated). After protein drugs are transported to the GI tract, they take access to the systemic circulation via two separate and functionally distinct absorption pathways: portal blood and the intestinal lymphatics. The physicochemical and metabolic features of the protein drug and the characteristics of the formulation largely control the relative proportion of protein drug absorbed via these two pathways. Portal blood represents the major pathway for the majority of orally administered protein drugs. During this process, hydrophilic ligands are carried to the liver via the hepatic portal vein, and then by the hepatic artery gain access to the systemic circulation, for subsequent delivery to their sites of action. On the other hand, highly lipophilic ligands (log $P>5$) that cross the same epithelial barrier are transported to the intestinal lymphatics, which directly deliver them to the vena cava, thereby bypassing the hepatic first-pass metabolism.

![Diagram of drug absorption pathways](image.png)

**Fig. 2.4** The pathways that a drug can take to cross the intestinal mucosal barrier
Pathway, A is the transcellular route in which a drug passively permeates the cell
membranes. Pathway B is the paracellular route; the drug passively diffuses via the intercellular junctions. Pathway C is the route of active transport of the drug by transporters. Pathway D is the route of drug permeation that is modified by efflux pumps (Wang et al; 2005).

2.3 Nanoparticles for Anticancer Drug Delivery

To deliver therapeutic agents to tumor cells in vivo, one must overcome the following problems: (i) drug resistance at the tumor level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) distribution, biotransformation and clearance of anticancer drugs in the body.

A strategy could be to associate antitumor drugs with colloidal nanoparticles, with the aim to overcome non-cellular and cellular based mechanisms of resistance and to increase selectivity of drugs towards cancer cells while reducing their toxicity towards normal tissues. If designed appropriately, nanoparticles may act as a drug vehicle able to target tumor tissues or cells, to a certain extent, while protecting the drug from premature inactivation during its transport. Indeed, at the tumor level, the accumulation mechanism of nanoparticles relies on a passive diffusion or convection across the leaky, hyper permeable tumor vasculature. The uptake can also result from a specific recognition in case of ligand decorated nanoparticles (‘active targeting’). Moreover, nanoparticles may also act at the cellular level. They can be endocytosed/phagocytosed by cells, with a resulting cell internalization of the encapsulated drug. Nanoparticles were also found to be able to overcome MDR resistance, which is due to the presence of the P-glycoprotein efflux system localized at the cancerous cell membrane (Brigger et al; 2012).

Today, most of the anticancer drugs are administered through i.v. injection or infusion. Such a way causes high peak above the maximum tolerable concentration (MTC) of the drug in the plasma and then fast excretion of the drug from the circulation system, resulting in a limited area-under the- curve (AUC), which is a quantitative measurement of the therapeutic effects, and a large part of AUC would be associated with high drug concentration above MTC, thus causing serious side effects. Instead, oral
chemotherapy could maintain a sustained moderate concentration of the drug in the circulation to achieve a prolonged exposure of cancerous cells to the drug as well as to avoid high peak above MTC. This will increase the therapeutic efficacy and decrease the side effects. Oral chemotherapy is a key step towards “Chemotherapy at Home”, a dream of cancer patients, which will radically change the clinical practice of chemotherapy and greatly improve the quality of life of the patients. Moreover, oral chemotherapy can provide an easy way for the patients to take the drug by themselves at home. This will greatly reduce their medical expenses and improve their quality of life. Oral chemotherapy is especially important for cancer patients at the latest stage, who are too weak to withstand harsh medical treatment. Oral chemotherapy can provide at least a palliative treatment to give them hope for survival prolongation (Mei et al; 2013, Feng et al; 2011).

Unfortunately, most anticancer drugs especially those with excellent anticancer effects such as Taxanes (paclitaxel and docetaxel) are not orally bioavailable, i.e., not absorbable/interactive in the gastrointestinal (GI) tract. For example, the oral bioavailability of paclitaxel has been found less than 1%. It is well-known that our body is so perfectly structured that all important organs are protected from external toxins by the so-called physiological drug barriers such as the gastrointestinal barrier (GI barrier) and the blood–brain barrier (BBB). The molecular basis of the various physiological drug barriers has been intensively investigated in the past decades and the various solutions including the various medical solutions and pharmaceutical nanotechnology solutions have thus developed (Farokhzad and Langer; 2009).

For oral bioavailability of Taxanes, which are the #1 seller among the various anticancer drugs and had $3.5 billion annual sale in the world market, an intensive investigation showed that orally administrated anticancer drugs such as paclitaxel would be eliminated from the first-pass extraction by the cytochrome P450-dependent metabolic processes and the over expression of plasma membrane transporter P-glycoprotein (P-gp) in the involved physiological systems especially intestine, liver, kidney (Mei et al; 2013).

Research work using wild-type and P-glycoprotein knock-out mice has shown the role of P-gp in multi-drug resistance and enhancing the bioavailability of paclitaxel and other anticancer drugs. Measurements of paclitaxel concentration in the plasma after oral
administration indicated that the area-under-the-curve (AUC) of the drug concentration in the plasma versus time was 6-fold higher for the P-gp knock-out mice than that for the wild-type mice. After intravenous administration of paclitaxel, the AUC was only 2-fold higher in the P-gp knock-out mice compared to the wild-type. Many anticancer drugs are substrates by P-gp. P-gp transporter impedes the permeability of drugs through physiological barriers producing limited pharmacological response (Malingre et al; 2001)

Thus, the inhibition of this efflux pump is also a common strategy to overcome the low oral bioavailability of many anticancer drugs. Inhibition of P-gp may be tackled by (i) co-administration of drugs known as P-gp substrates in order to act as inhibitors of the transporter, (ii) development of novel drugs that are non P-gp substrates, and (iii) design of novel delivery systems that allow the drug to bypass efflux pump transport.

The most popular and prospective strategies used in pharmaceutical cancer nanotechnology include prodrugs, nanoemulsions, dendrimers, micelles, liposomes, solid lipid nanoparticles and nanoparticles of biodegradable polymers for controlled, sustained and targeted drug delivery across the various physiological drug barriers including the gastrointestinal barrier for oral chemotherapy (Feng et al; 2009).

The use of biodegradable polymeric nanoparticles for oral drug delivery has shown significant therapeutic potential for cancer treatment. Polymeric nanoparticles that combine bioadhesive properties with a certain inhibitory activity of the cytochrome P-450 complex and P-gp efflux system and thus promote drug permeability across the mucosal membrane can be an adequate strategy for oral chemotherapy. Another advantage from these polymeric nanoparticles can be their inherent properties to control the release of the incorporated drug, which may be of interest to obtain sustained release of the drug.

Biodegradable polymers and phospholipids are the two most important materials that have been widely used in development of the nanoparticle-based drug delivery systems for oral chemotherapy.

Biodegradable polymers can be seen as a special kind of excipients for drug formulation, which carry the drug, improve its pharmaceutical properties and enhance its ADME process, thus strongly influencing PK or/and PD of the drug. They are biodegradable and thus can be easily eliminated from the body after fulfilling their task.
as a drug carrier. Various FDA approved biodegradable and biocompatible polymers are used most often in the research of polymeric nanoparticle-based drug delivery systems include poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) PLGA), poly(ε-caprolactone) (PCL), etc. However, they are highly hydrophobic and thus not friendly to hydrophilic drugs such as peptides and proteins. They are too strong in mechanical strength and their degradation rates are too slow, thus resulting in too slow drug release to meet the therapeutic needs. Moreover, nanoparticles made from those polymers are hard to be directly conjugated to hydrophilic targeting ligands, for which amphiphilic linker molecules are needed, causing complications in targeting technology. Since it usually takes quite a long time to develop a new biomaterial and have it approved for clinical use, two simple and practical strategies have been adopted to solve this problem. One is to coat the nanoparticles by hydrophilic polymer such as PEG, Chitosan and TPGS, and the other is to synthesize copolymers to incorporate hydrophilic elements in the hydrophobic chains of the polymers. It is well known that drug conjugation to polyethylene glycol (PEG) or formulated in PEG copolymer nanocarriers can enhance its solubility, permeability, stability and thus oral bioavailability (Zhang et al; 2012, Arima et al; 2001).

2.4 Nanoparticles for Antiretroviral Drug Delivery

HIV/AIDS is a global pandemic that has become the leading infectious killer of adults worldwide. By 2006, more than 65 million people had been infected with the HIV virus worldwide and 25 million had died of AIDS. At the end of 2007, around 33 million people were living with the virus, with 2.7 million new infections and 2 million deaths each year. This has caused tremendous social and economic damage worldwide, with developing countries, particularly Sub-Saharan Africa, heavily affected (Merson et al; 2006) After fast development of antiretroviral resistance in individuals treated with single drug regimens, the concept of highly active antiretroviral therapy (HAART) was introduced in the late 1990s, comprising the intense use of combination drug regimens. There are now around 30 individual drugs and fixed-dose combinations available to treat HIV infection. Currently used antiretroviral drug classes include reverse
transcriptase inhibitors (RTIs), protease inhibitors (PIs), entry inhibitors (CCR5 antagonists and fusion inhibitors), and integrase inhibitors (Neves et al; 2010).

Introduction of a combination of three or more different classes of drugs; triple-drug therapy (HAART) revolutionized HIV/AIDS treatment. The use of the HAART regimen, particularly in the developed world, has resulted in tremendous success in improving the expectancy and quality of lives for patients. However, some HAART regimens have serious side effects and, in all cases, HAART has to be taken for a lifetime, with daily dosing of one or more pills. Some patients also develop resistance to certain combinations of drugs, resulting in failure of the treatment. The absence of complete cure under current treatment underscores the great need for continued efforts in seeking innovative approaches for treatment of HIV/AIDS (Walensky et al; 2006, Richman et al; 2001).

But, HAART is not able to provide a cure mainly because of HIV’s ability to persist in latency state in cellular and anatomical reservoir sites. Beside this fact, problems of current antiretroviral therapy also include prolonged treatment periods with drugs possessing important adverse effects, poor drug-regimen compliance, drug resistance, drug-drug interactions, poor drug pharmacokinetics, viral levels rebound after therapy cessation, and costs (Marsden and Jack; 2009).

Even if current antiretroviral therapy is able to reduce the viral load to undetectable levels, HIV is able to persist in the human body, namely in several reservoir sites. Reservoir sites are able to protect the virus from biological elimination pathways, immune response and/or antiretroviral drugs, making it impossible to eradicate the virus and achieve a cure with currently available therapy.

Generally, cellular reservoirs are able to sustain HIV infection by allowing its residence in a physical state capable of surviving for prolonged periods despite otherwise therapeutic levels of antiretroviral drugs. In the case of anatomical reservoir sites, the problem is mainly to achieve and sustain adequate levels of antiretroviral agents within these spaces (Blankson et al; 2002). After initial HIV infection and local amplification at the mucosal site, infected cells migrate to regional lymph nodes, leading to a mild initial viral amplification in naïve T cells. The viral infection is then quickly disseminated by T cells to lymphoid organs, particularly the gut-associated lymphoid tissues (GALT),
spleen, and bone marrow, being accompanied by a burst in the viral load (acute infection) (Shrager et al; 1998).

Main anatomical reservoir sites of HIV include the lymphoid organs (particularly the spleen, lymph nodes, and GALT) and the central nervous system (CNS). Other potential sites have also been reported as possible reservoirs, namely the testicles and the female genital tract. The importance of lymphoid organs is directly related with their role in the circulation and production of lymphocytes and the abundant presence of HIV-susceptible immune cells, namely those able to constitute reservoirs as discussed above. Poor penetration of antiretroviral in the CNS due to insufficient blood-brain barrier (BBB) permeation is a matter of concern, resulting in suboptimal drug levels that allow continuous replication of HIV (Mamo et al; 2010).

**Protease inhibitors (PIs)**, one of the components of HAART are substrate for the efflux cellular membrane transporter P-glycoprotein, which is able to mediate unidirectional transport of these drugs to the cell exterior. The presence of this membrane transporter in macrophages and endothelial cells of the BBB explains the poor concentrations achieved by PIs in these reservoir sites. Conversely, incomplete absorption of some PIs when administered by the oral route can be partially explained, alongside with their poor aqueous solubility, by the presence of this transporter in intestinal epithelial cells. Poor placental penetration of PIs, which may have important clinical implications in mother-to-child transmission, is also justified by the presence of high levels of P-glycoprotein placenta (Neves et al; 2010).

Affordability of antiretroviral drugs is an increasingly huge burden for developed countries and an unattainable goal for developing ones. An interesting approach for reducing overall costs with antiretroviral therapy would be to increase older drugs therapeutic lifespan (i.e. before treatment-compromising adverse effects or drug resistance occurs) by improving their delivery.

Although at an earlier stage, applications of nanotechnology for prevention and treatment of HIV/AIDS have also gained attention in recent years. There are emerging novel approaches in which nanotechnology can enhance current treatment as well as advance new therapeutic strategies, such as gene therapy and immunotherapy. Nanoscale delivery systems enhance and modulate the distribution of hydrophobic and hydrophilic drugs into and within different tissues due to their small size. This
particular feature of nanoscale delivery systems appears to hold the most promise for their use in clinical treatment and prevention of HIV.

General properties of nanoparticles that favour their use in antiretroviral drug delivery are well known and include versatility (virtually all drugs may be encapsulated), good toxicity profile (depending on used excipients), possibility of drug-release modulation, high drug payloads, relative low cost, easiness to produce and possible scale-up to mass production scale. Their ability to incorporate, protect and/or promote the absorption of non-orally administrable anti-HIV drugs, namely mono- or oligonucleotides, is of importance to improve the bioavailability of several molecules. Once bioavailable, protection of incorporated drugs from metabolism is a favorable feature of nanosystems, allowing prolonged drug residence in the human body, thus reducing needed doses and prolonging time between administrations.

Nanoparticles seem to be able to reduce antiretroviral drugs toxicity, namely at the cellular level, providing that rigorous selection of materials and adequate preparation techniques are assured (Vyas et al; 2006). Even if drug uptake is increased when encapsulated in nanocarriers, cell toxicity seems to be diminished, probably due to the slow-release properties of these systems. This possibility is particularly interesting taking in consideration the well-known toxicity associated with anti-HIV therapy (Mamo et al; 2010).

Specifically, targeted delivery of antiretroviral drugs to CD4+ T cells and macrophages as well as delivery to the brain and other organ systems could ensure that drugs reach latent reservoirs. Moreover, by controlling the release profiles of the delivery systems, drugs could be released over a longer time and at higher effective doses to the specific targets (Nowacek et al; 2009, Amiji et al; 2006).

Passive targeting is based in the inherent properties of different nanosystems, namely size, particle shape, and surface charge, which can modulate its bioavailability, biodistribution and/or targeting.

Active targeting strategies have also been employed for antiretroviral drug delivery. In the case of active targeting, nanotechnology-based systems are conveniently modified, most commonly by surface attachment of specific ligands that are able to recognize target cells or sites, and/or escape bio elimination processes. Once opsonization and endocytosis occur, nanoparticles are incorporated in an endolysosome, being degraded;
however, the ability of various nanoparticles to escape the endolysosomal compartment allows incorporated drugs to be delivered to the cytoplasm and, eventually, to the nucleus. Nanoparticles have the ability to enhance uptake of drug by macrophages, particularly when these cells were infected by HIV (up to 60% more than for uninfected macrophages (Dou et al; 2007).

The presence of wide amounts of HIV-susceptible immune cells in the lymphoid organs makes its interesting to target antiretroviral drug to these sites in HIV therapy. This strategy comprises targeting nanosystems to immune cell populations, particularly macrophages. The normal uptake of nanoparticles by macrophages present in the RES is indeed an important passive method for targeting this anatomical reservoir site, as early demonstrated in vivo by Lobenberg et al (1997).

In one study, [14C]-zidovudine-loaded PHCA nanoparticles were administered intravenously in a rat model; soon after administration, the drug was detected in the organs of the RES in concentrations above 18-fold of those for the drug aqueous solution. Identical effects were also observed by these investigators after oral intake, providing evidence that cell/organ drug targeting may also be achieved by administering drug-loaded nanoparticles through more patient-friendly routes (Lobenberg et al; 1997).

Dembri and coworkers studied the applicability of oral administration for zidovudine-loaded poly (iso-hexylcyanoacrylate) nanoparticles in rats and observed drug accumulation in the intestinal mucosa after direct gastric administration, being the concentration of zidovudine in Peyer’s patches around 4-times higher for nanoparticles than for drug solution; also, tissue concentrations (30–45 μM) were much higher than those reported for HIV IC50 (0.06–1.36 μM). This approach showed to be efficient in concentrating zidovudine in the gastrointestinal tract and GALT, which are important sites for HIV replication and perpetuation, as highlighted previously (Dembri et al; 2000).

Kinman and coworkers optimized indinavir loaded liposomes in order to improve lymphoid tissue localization and pharmacokinetic profile. PEGylation of liposomes demonstrated to provide 6-fold higher indinavir levels in lymph nodes and enhance drug exposure in blood and also indicate that the concomitant use of the proposed nanocarrier and conventional oral indinavir regimens could be a valuable strategy in
order to prolong the utility (i.e. drug lifespan before resistance occurs) of antiretroviral drugs (Kinman et al; 2006).

2.5 Methods of Preparation of Nanoparticles

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity. (Kreuter et al; 1994)

2.5.1 Nanoparticles Prepared by Polymerization Process of Monomers:

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The Nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used. Two types of polymerization processes have been adopted to prepare polymeric nanoparticles (Jain et al; 2000, Mohanraj and Chen; 2006).

a) Dispersion Polymerization: Dispersion polymerization starts with monomer, an initiator, solvent in which the formed polymer is insoluble, and a polymeric stabilizer. Polymer forms in the continuous phase and precipitates into a new particle phase which is stabilized by the polymeric stabilizer. Small particles are formed by aggregation of growing polymer chains precipitating from the continuous phase as these chains exceed a critical chain length. Coalescence of these precursor particles with themselves and with their aggregates results in the formation of stable colloidal particles, which occurs when sufficient stabilizer covers the particles.

b) Emulsion Polymerization: In this technique the monomer is emulsified in non-solvent containing surfactant, which leads to the formation of monomer swollen micelles and stabilized monomer droplets. The polymerization is performed in the
presence of initiator. Emulsion polymerization may be performed using either organic or aqueous media as continuous phase. Poly (methyl methacrylate), poly (alkyl cyanoacrylate), acrylic copolymer, polystyrene, poly (vinyl pyridine) and polyacrolen nanoparticles are prepared by emulsion polymerization technique.

2.5.2 Nanoparticles Prepared from Dispersion of Preformed Polymers: Several techniques have been suggested to prepare the biodegradable polymeric nanoparticles from preformed polymers such as poly (D,L-lactide) (PLA), poly (D,L-glycolide) (PLG) and poly (D,L-lactide-co-glycolide) (PLGA) (Kompella et al, 2001, Ravikumar et al; 2004). The basic methodologies of the commonly used preparation methods are as follows:

a) Emulsion/evaporation

This is one of the most frequently used methods. The preformed polymer and drug are first dissolved in a water-immiscible organic solvent, which is then emulsified in an aqueous solution containing stabilizer. The emulsification is brought about by subsequent exposure to a high-energy source such as an ultrasonic device, homogenizer, or colloid mill. The organic phase is evaporated under reduced pressure or vacuum, resulting in the formation of the aqueous dispersion of nanoparticles. The nanoparticles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residues or any free drug and lyophilized for storage (Guarrero et al 1998; Song 1997). Modification of this method, known as high-pressure emulsification solvent evaporation (HPESE), has been reported by (Jaiswal et al; 2004) This method involves preparation of a coarse emulsion, which is then subjected to homogenization under high-pressure followed by overnight stirring to remove organic solvent. This method has the advantage of obtaining small, monodispersed nanoparticles with high encapsulation efficiency and reproducibility. The emulsion evaporation method can be used for preparation of particles with sizes varying from a few nanometers to micrometers by controlling the stirring rates and conditions (Ubrich; 2004). A diagrammatic representation of this method is shown in Fig. 2.5
The emulsion evaporation method suffers from the limitation of poor entrapment of hydrophilic drugs because of their diffusion and partitioning from the dispersed oil phase into the aqueous continuous phase. Therefore, to encapsulate hydrophilic drugs and proteins, the double-emulsion technique is employed, which involves the addition of aqueous drug solution to organic polymer solution under vigorous stirring to form a w/o emulsion. This w/o emulsion is added into second aqueous phase containing more stabilizers with stirring to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by evaporation (Vandervoort et al; 2002). A number of hydrophilic drugs like the peptide leuprolide acetate, a lutenizing hormone-releasing agonist, vaccines, proteins/peptides and conventional molecules have been successfully encapsulated by this method. After evaporation of organic solvent under reduced pressure, the polymer precipitates and nanoparticles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilization (Jain; 2000). A diagrammatic representation of this method is shown in Fig. 2.6.
Fig. 2.6 Schematic diagram of w/o/w in-liquid drying process for preparation of Nanoparticles

c) Salting-out

This technique involves the addition of polymer and drug solution in a slightly water miscible solvent such as acetone to an aqueous solution containing the salting out agent and a colloidal stabilizer under vigorous mechanical stirring. When this o/w emulsion is diluted with a sufficient volume of water, it induces the formation of Nanoparticles by enhancing the diffusion of acetone into the aqueous phase. The remaining solvent and salting-out agent are eliminated by cross-flow filtration (Allemann et al; 1998). Several manufacturing parameters can be varied including stirring rate, internal/external phase ratio, concentration of polymer in the organic phase, type of electrolyte, concentration, and type of stabilizer in the aqueous phase. By considering the entrapment efficiency of nanoparticles, this method is most suitable for water insoluble drugs. Salt permeate biological systems and are crucial for life. However salts also affect the stability of proteins. It has been reported since many years that neutral salts perturb various protein structures in ways that go well beyond simple, non-specific charge effects (Doming et al; 2002).
d) Emulsification-diffusion

This is another widely used method involving polymer solution in partially water miscible solvent (such as ethyl acetate, benzyl alcohol, propylene carbonate) presaturated with water, added to an aqueous solution containing stabilizer under vigorous stirring. The subsequent addition of water to the system destabilizes the equilibrium between the two phases and causes the solvent to diffuse into the external phase, resulting in reduction of the interfacial tension and in nanoparticle formation, which gradually becomes poorer in solvent.

Although this method is a modification of the salting out procedure, it provides the advantage of avoiding the use of salts and thus eliminates the need for intensive purification steps. While this method also suffers from low entrapment efficiency of hydrophilic drugs in nanoparticles, incorporation of medium chain glyceride into aqueous solution has been found to improve the encapsulation efficiency of watersoluble drugs into nanospheres offering the advantage of simplicity, narrow particle size distribution, and ready dispersibility of the resultant particles (Jain; 2000).

e) Nanoprecipitation

In nanoprecipitation, introduced by Fessi and co-workers (Fessi et al; 1995), the particle formation is based on precipitation and subsequent solidification of the polymer at the interface of a solvent and a non-solvent. Thus, the process is often called solvent displacement or interfacial deposition. This method is usually employed to incorporate lipophilic drugs into the carriers based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution (Molpeceres et al; 1996, Barichello et al; 1999).

The polymer is dissolved in a water miscible organic solvent (or solvent mixture) and added to an aqueous solution, in which the organic solvent diffuses (Fig. 2.7). Particle formation is spontaneous, because the polymer precipitates in the aqueous environment. According to the current opinion, the Marangoni effect is considered to explain the process: solvent flow, diffusion and surface tensions at the interface of the organic solvent and the aqueous phase cause turbulences, which form small droplets containing the polymer. Subsequently, as the solvent diffuses out from the droplets, the
polymer precipitates. Finally, the organic solvent is typically evaporated with the help of a vacuum.

![Fig. 2.7 Schematic illustration of the nanoprecipitation process](image)

The injection rate of the organic phase into the aqueous phase affects the particle size. It was observed that a decrease occurs in both particle size and drug entrapment as the rate of mixing of the two phases increase. This method gave relatively narrow particle size distribution for different formulations evaluated.

The drug loading efficiency was found to be lower for the hydrophilic drugs than lipophilic drugs because of their poor interaction with the polymer leading to diffusion of the drug, from the polymer in the organic phase, to the external aqueous environment, although exceptions were found, as seen in case of proteins and peptides. Govender et al (1999) showed improved incorporation of the water-soluble drug, procaine hydrochloride, into PLGA nanoparticles by increasing the aqueous phase pH and replacing procaine hydrochloride by procaine dehydrate base. The difficulty faced in this preparation technique is the choice of drug/polymer/solvent/nonsolvent system in which the nanoparticles would be formed and the drug efficiently entrapped.
f) Emulsion-diffusion-evaporation

Employing the emulsion-diffusion-evaporation method, Nanoparticles are prepared by dissolving PLGA in ethyl acetate at room temperature. The organic phase is then added to an aqueous stabilizer mixture containing PVA and chitosan in water under stirring. The emulsion is stirred at room temperature for 3 hours before homogenizing for 10 minutes.

To this emulsion, water is added under stirring, resulting in Nanoprecipitation (Ravikumar et al; 2004). Stirring is continued in a water bath maintained at 40 °C to remove organic solvent. Stirring causes the dispersion of the solvent as irregularly sized globules in equilibrium with the continuous phase, and the stabilizer is then adsorbed on the larger interface created. Homogenization further results in smaller globules. Addition of the water and heating step destabilizes the equilibrium and causes the diffusion of organic solvents to the external surface.

2.6 Characterization of Nanoparticles

The unique qualities and performance of nanoparticulate systems as device for drug delivery arises directly from their physicochemical properties. Hence, determining such characteristics is essential in achieving the mechanistic understanding of their behaviour. A good understanding allows prediction of in vivo performance as well as allowing particle designing, formulation development and process troubleshooting to be carried out in a rational fashion. After preparation, nanoparticles are characterized at two levels. The physicochemical characterization consists of the evaluation of the particle size, size distribution, and surface properties (composition, charge, hydrophobicity) of the nanoparticles. The biopharmaceutical characterization includes measurements of drug encapsulation, in vitro drug release rates, and in vivo studies revealing biodistribution, bioavailability, and efficacy of the drug. Nanoparticles are generally characterized for the following parameters

- Particle size
- Surface charge (Zeta potential)
- Crystalline state
- Surface morphology
Drug release studies

Stability

**Particle size**

The most basic and important property of any nanoparticulate system is its size. The saturation solubility, dissolution velocity, physical stability and even biological performance of these systems depend on their particle size. Saturation solubility and dissolution velocity showed considerable variation with change in particle size of the drug (Muller and Peters; 1998). The most frequently used techniques for particle size measurement of nanosized systems are dynamic light scattering techniques, static light scattering techniques and microscopy. Each method has its own advantages as well as disadvantages. The mean size and width of distribution (polydispersity index) is typically determined by photon correlation spectroscopy (PCS). This technique can be used for rapid and accurate determination of the mean particle diameter of nanoparticles (Muller; 1984). It records the variation in the intensity of scattered light on the microsecond time scale (Pecora; 2000). The measuring range of PCS is limited to approximately 3 nm–3mm. Therefore, Laser Diffractometry (LD) is also used to detect any particles in the micrometer range or aggregates of drug nanoparticles. For nanoparticles intended for intravenous use, particle size determination by coulter counter is also essential as few particles with particle size more than 5 µm may cause problem of blockage of blood vessels. Depending on the type of equipment employed, the measuring size range is approximately 0.01–80 µm. The instrument and the material to be analyzed are important parameters which will affect the accurate particle size measurement. The stability of the sample during analysis is the most important requisite for correct and reproducible results (Keck; 2010). Thus, all above things must be considered during selection of appropriate technique for particle size determination for a particular sample.

**Surface charge (Zeta potential)**

Particle charge is a stability determining parameter in nanoparticles. It is measured by electrophoresis and typically expressed as phoretic mobility \([(\text{mm/}S) / (\text{V/cm})]\) or zeta
potential (mV). Zeta potential is used as surrogate for surface charge, and is often measured by observing the oscillations in signal that result from light scattered by particles located in an electric field. There are a number of instrumental configurations with different approaches implemented in different equipments, with mostly used Doppler shift. The zeta potential of a nanosuspension is governed by both the surfactant and the drug itself. For a physically stable nanoparticulate suspension solely stabilized by electrostatic repulsion, a zeta potential of ±30 mV is required as minimum. In case of a combined electrostatic and steric stabilization, ±20mV is sufficient as a rough guideline (Muller and Jacobs; 2002).

**Crystalline state**

Drug particles in amorphous form are likely to be generated when nanoparticles are prepared. Hence, it is essential to investigate the extent of amorphous drug particles generated during production of nanoparticles. The crystalline status of the nanosuspension can be assessed by differential scanning calorimetry (DSC) (Muller et al; 2001). This is particularly very important when the drug exhibits polymorphic forms. The changes in the physical state of the drug particles as well as extent of amorphous fraction can be determined by X-ray diffraction analysis (Muller and Grau; 1998) and can be supplemented by DSC studies. The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic and morphological changes that a drug undergoes when subjected to nanosizing.

**Surface morphology**

Nanoparticles can be directly observed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) with the former method being better for morphological examinations (Molpeceres et al; 2000). TEM has a smaller size limit of detection and provides structural information via electron diffraction, but staining is usually required. Researchers must be cognizant of the statistically small sample size and the effect of applied vacuum on the particles during analysis. Very detailed images can be obtained from freeze fracture approach in which a cast is made of the original sample (Mosqueira et al; 2001). Sample corruption resulting from the extensive sample preparation is always a possibility, though lower vacuum instrumentation reduces this manipulation, albeit at the loss of some resolution (Nizri et al; 2004).
microscopy (AFM) microscopy can also be used to confirm the size and shape of nanosized particles. AFM is capable of scanning the surfaces in controlled environmental conditions and is a complementary to SEM imaging.

**Drug release studies**

*In vitro* release studies are generally performed to accomplish one or more of the following aims:

1. As an indirect measurement of drug availability, especially in preliminary stages of product development
2. Quality control to support batch release and to comply with specifications of batches proven to be clinically and biologically effective
3. Assess formulation factors and manufacturing methods that are likely to influence bioavailability
4. Substantiation of label claim of the product
5. As a compendial requirement

Currently, research is focused on shortening the time span of *in vitro* release experiments with the aim of providing a quick and reliable method for assessing and predicting drug release. For commercial dosage forms that release drug for 30 to 90 days or even longer, accelerated or short-term release provides the potential for conducting an *in vitro* release test in a matter of days rather than months. Release testing of these dosage forms at 37-C would require the addition of preservatives and impose certain limitations on the *in vitro* method, such as stability and compatibility of the components of the release device, like tubings and membranes. Therefore, a short-term release test might even be more reliable for quality-control purposes. In addition, short term studies can provide a rapid assessment of formulation and processing variables that affect drug release from the delivery system, especially in the developmental stage. These short-term studies can be performed by accelerating one or more conditions employed in a real-time *in vitro* release study. Such accelerating conditions include elevated temperature, altering pH, and use of surfactants. As with the real-time *in vitro* release study, the method should be simple, reproducible under the conditions of study, inexpensive, and applicable to biodegradable nanoparticulate formulations that have varying duration of action *in vivo*.
Generally aqueous media such as simulated gastric fluid without enzymes, simulated intestinal fluid without enzymes, water and buffers have been employed to study release of water soluble drugs. For water insoluble drugs, surfactants, bile acids, bile salts and lecithins have been shown to increase the rate of drug release. The level of interest in the in vitro dissolution of poorly water soluble drugs has increased in recent years due to the need of finding a suitable dissolution media for pharmaceutical formulations that may reflect their in vivo performance.

In vivo poorly water-soluble drugs are solubilised through complex endogenous surfactants such as bile acids, bile salts and lecithin. However, in vitro dissolution models in less complex micelle systems have been used. The use of surfactants in the dissolution system for poorly water-soluble drugs may be physiologically more meaningful due to the presence of natural surfactants in the gastrointestinal tract. Additionally, the following should be considered prior to studying drug release:

1. Sink conditions: Although sink conditions may not exist at the in vivo site of action, it is wise to employ sink conditions during in vitro testing. In the event that a small volume of media can be used (based on the method employed and assay sensitivity), total media replacement may be used to ensure drug solubility, maintain sink conditions, and prevent accumulation of polymer degradation products.

2. Burst release: The release method employed should be able to identify a high initial release or burst from the formulation. Additionally, the method should provide information about the onset and duration of burst to assess its influence on the in vivo efficacy and safety window of the drug being studied.

3. Robustness of technique: The in vitro release method employed should be able to assess the influence of changes in the manufacturing procedure on the formulation. This would be useful from a quality-control standpoint and could also aid in the design and development of drug delivery systems. Ideally, an in vitro test method should mimic in vivo conditions and release mechanism as much as possible (D'Souza and DeLuca 2006).

Methods to study the in vitro release are: (i) side-by-side diffusion cells with artificial or biological membranes; (ii) dialysis bag diffusion technique; (iii) reverse dialysis sac technique; (Meneau and Ollivon) ultra centrifugation; (v) Ultra filtration; or (vi) centrifugal ultra filtration technique. Despite the continuous efforts in this direction, there are still some technical difficulties to study in vitro drug release from NPs. These
are attributed to the separation of NPs from the release media. In order to separate NPs and to avoid the tedious and time-consuming separation dialysis has been used; here, the suspension of NPs is added to the dialysis bags/ tubes of different molecular mass cut-off. These bags are then incubated in the dissolution medium. Another technique involves the use of a diffusion cell consisting of donor and acceptor compartments; this technique was used to separate through the artificial / biological membranes. In this method kinetic study was not performed under the perfect sink conditions, because the NPs were not diluted in the release media, but were separated from the release media through the membrane. Thus, the amount of drug in the release media did not reflect the amount of drug released. In order to avoid the enclosure of NPs in the dialysis bag, Leavy and Benita used a reverse dialysis technique for o/w emulsion. In this method, NPs were added directly into the dissolution medium. The same technique was adopted by Calvo et al. for the release from the NPs, nanocapsules and nanoemulsions (Soppimath, Aminabhavi et al. 2001).

The release rates of NPs depend upon (i) desorption of the surface-bound /adsorbed drug; (ii) diffusion through the NP matrix; (iii) diffusion (in case of nanocapsules) through the polymer wall; (Meneau and Ollivon) NP matrix erosion; and (v) a combined erosion / and diffusion process. Thus, diffusion and biodegradation govern the process of drug release. Release profiles of the drugs from NPs depend upon the nature of the delivery system. In the case of a matrix device, drug is uniformly distributed / dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix degradation, then the mechanism of drug release occurs mainly by diffusion, otherwise it depends upon degradation. Rapid initial release is attributed to the fraction of the drug which is adsorbed or weakly bound to large surface area of the NPs, than to the drug incorporated in NPs.

**Stability**

Physical stability is crucial in formulation of drug nanosuspension. As nanoparticles have mean particle diameter in nanometer range, they are prone to aggregation of the particles. The aggregation may be due to Ostwald ripening which occurs due to different saturation solubilities in the vicinity of very small and larger particles. Stabilizers like surfactants or polymeric macromolecules are required to stabilize the nanoparticles against inter-particulate forces and prevent them from aggregation. Surfactants are
used to minimize the free energy and stabilize the system. The stabilization provided by the stabilizers is by steric, electrostatic or combination of these two processes. Steric stabilization is achieved by adsorbing surfactants/polymers onto the particle surface while electrostatic stabilization is obtained by adsorbing charged molecules, which can be ionic surfactants or charged polymers, onto the particle surface. Generally, steric stabilization alone is sufficient to provide stability to the nanosized particles but electrostatic stabilization is often combined with it as an additional measure.

Formation of impurities due to process and formulation parameters must be studied. The impurities could be identified by various techniques such as infrared spectroscopy (IR), high performance liquid chromatography (HPLC) and mass spectroscopy (MS).

Beside characterization of above properties, additional characterization of the nanoparticles is required if surface modification is done for particles. The parameters for which surface modified nanoparticles are evaluated include adhesion properties, surface hydrophilicity/hydrophobicity and interaction with body proteins. The adhesiveness of the drug nanoparticles is considered to be a major factor contributing towards increasing the bioavailability and reducing variability of absorption. Surface hydrophobicity determines the interaction with the cells prior to phagocytosis and is relevant parameter for adsorption of plasma proteins. It is considered as important parameter affecting in vivo organ distribution after i.v. injection. Separation by Hydrophobic Interaction Chromatography (HIC) depends on the reversible adsorption of biomolecules according to their hydrophobicity. HIC is widely used for the separation and purification of proteins in their native state. HIC technique is used for determination of surface hydrophilicity/hydrophobicity. Hydrophobicity of nanoparticles is characterized by HIC in which hydrophilic particles pass the column faster while elution of hydrophobic particles is retarded.

2.7 Biodegradable Polymers

Biodegradable polymers have been extensively used in controlled drug delivery because they have the advantage of not requiring surgical removal after they serve their intended purpose. It offers various advantages like versatile degradation kinetics, non-
toxicity, and biocompatibility. In recent years, additional polymers designed primarily for medical applications have entered the arena of controlled release.

Many of these materials are designed to degrade within the body, among them are following:

- Polylactides (PLA).
- Polyglycolides (PGA).
- Poly (lactide-co-glycolides) (PLGA).
- Polyanhydrides & Polyorthoesters.

Originally, polylactides and polyglycolides were used as absorbable suture material, and it was a natural step to work with these polymers in controlled drug delivery systems. The greatest advantage of these degradable polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. However, biodegradable materials do produce degradation by-products that must be tolerated with little or no adverse reactions within the biological environment.

**POLY (D, L-LACTIDE-CO-GLYCOLIDE) PLGA**

**PLGA** or **poly(lactic-co-glycolic acid)** is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. PLGA is synthesized by means of random ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this polymer include tin (II) 2-ethylhexanoate, tin (II) alkoxides, or aluminum isopropoxide. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding linear, aliphatic polyester as a product. Fig. 2.8 depicts the structure of PLGA and its monomers.
Fig. 2.8 Structure of Poly glycolic acid (PGA), Poly lactic acid (PLA) and Poly (lactic-co-glycolic) acid (PLGA)

The understanding of physical, chemical and biological properties of the polymer is helpful before formulating a controlled drug delivery device. Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water and subsequently degrade more slowly. The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to the molecular weight.

The mechanical strength, swelling behaviour, capacity to undergo hydrolysis, and subsequently the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The crystallinity of the PLGA copolymer is directly dependent on the type and molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGA polymers containing 50:50 ratios of lactic and glycolic acids are hydrolyzed much faster than those containing higher proportion of either of the two monomers. Gilding and Reed have pointed out that PLGA containing less than 70% glycolide are amorphous in nature. The degree of crystallinity and the melting point of the polymers are directly related to the molecular weight of the polymer.
The Tg (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 37° C and hence they are glassy in nature. Thus they have a fairly rigid chain structure which gives them significant mechanical strength to be formulated as drug delivery devices.

**Degradation and metabolic pathway of PLGA**

The degradation rate of PLGA in water is a function of the molecular weight and the lactide: glycolide ratio. Higher the glycolide content and lower molecular weight increase the degradation rate. For e.g. the degradation time of PLGA 50:50 is 1-2 months while that of PLGA 70:30 and PLGA 85:15 are higher (approx. 12-24 months).

PLGA show a glass transition temperature in the range of 40-60 °C. The inherent viscosity of PLGA is dependent on their molecular weight as shown in Table 2.1. For e.g. for PLGA 50:50 the molecular weight increases with the increase in its inherent viscosity (Purac biomaterials).

**Table 2.1: Inherent viscosity and molecular weight for PLGA 50:50 (Purac Biomaterials)**

<table>
<thead>
<tr>
<th>IV [dl/g]</th>
<th>Mw[g/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>17,000</td>
</tr>
<tr>
<td>0.3</td>
<td>30,000</td>
</tr>
<tr>
<td>0.4</td>
<td>44,000</td>
</tr>
<tr>
<td>0.5</td>
<td>59,000</td>
</tr>
<tr>
<td>0.6</td>
<td>76,000</td>
</tr>
<tr>
<td>0.7</td>
<td>94,000</td>
</tr>
<tr>
<td>0.8</td>
<td>113,000</td>
</tr>
<tr>
<td>0.9</td>
<td>133,000</td>
</tr>
<tr>
<td>1.0</td>
<td>153,000</td>
</tr>
<tr>
<td>1.1</td>
<td>174,000</td>
</tr>
<tr>
<td>1.2</td>
<td>196,000</td>
</tr>
</tbody>
</table>

Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.
Degradation of PLA or PLGA occurs by autocatalytic cleavage of the ester bonds through spontaneous hydrolysis into oligomers and d, l-lactic and glycolic acid monomers. Lactate converted into pyruvate and glycolate enter the Krebs' cycle to be degraded into CO$_2$ and H$_2$O. The polymer erosion in delivery devices is the degradation of polymers to water-soluble fragments, accompanied by a progressive weight loss of the matrix. Generally, the polymer erosion could be classified into two mechanisms, namely surface or bulk erosion (Gopferich; 1996).

In the case of surface erosion, the degradation is faster than the water diffusion. Thus the degradation and erosion take place on the surface of the matrix; in contrast, with bulk erosion, the water penetration is faster and the degradation and erosion affect all the polymer bulk (Fig. 2.9). PLGA are bulk erosion polymers. The weight loss of the polymer devices doesn't take place at the beginning of the degradation of the PLGA. Accompanying with the produced water soluble oligomers, significant weight loss occurs when the molecular weight of the PLGA reaches certain threshold.

Fig. 2.9 Schematic illustration of the changes of polymer matrix during (a) surface erosion and (b) bulk erosion (Burkersrodaa et al; 1997)

Bulk erosion is the main degradation pathway for PLGA copolymer. This occurs by random scission of ester bonds in the polymer backbone proceeding homogenously throughout the device (Vandervoort et al; 2002). A three-phase mechanism for PLGA biodegradation has been proposed (Jain; 2000). Initially, a significant decrease in
molecular weight of polymer is observed, with no appreciable weight loss and no
soluble monomer products formed after random chain scission. This phase is followed
by a decrease in molecular weight with rapid loss of mass and formation of soluble
mono and oligomeric products. Finally, soluble monomer products are formed from
soluble oligomeric fragments, resulting in complete polymer degradation. It has been
reported that the drug(s) having amino functional groups such as amines, basic drugs,
protein, and peptides have the potential to interact with polymer pendant groups,
accelerating the polymer degradation rates and the release of the drugs incorporated in
the polyester matrix (Guarrero et al; 1998).

Degradation rate depends on four basic parameters: hydrolysis rate constant
(depending on the molecular weight, the lactic/glycolic ratio, and the morphology),
amount of water absorbed, diffusion coefficient of the polymer fragments through the
polymer matrix, and solubility of the degradation products in the surrounding aqueous
medium. All of these parameters are influenced by temperature, additives (including
drug molecules), pH, ionic strength, buffering capacity, size and processing history,
steric hindrance etc. Polymer properties such as molecular weight, crystallinity and
glass transition temperature also control the degradation rate of polymers.

The biodegradation rate of the PLGA copolymers are dependent on the molar ratio of
the lactic and glycolic acids in the polymer chain, molecular weight of the polymer, the
degree of crystallinity and Tg of the polymer. The role of enzymes in any PLGA
biodegradation has not been well established. Most of the literature indicates that the
biodegradation of PLGA does not involve any enzymatic activity and is purely through
hydrolysis.

Literature data (Huh et al 2003) indicate that in vivo degradation times for copolymers
of lactides and glycolides vary from a few weeks to more than 1 year. The most widely
used PLGA copolymer composition of 50:50 has the fastest degradation rate of the d,l-
lactide/glycolide materials, with that polymer degrading in about 50-60 days. The
65:35, 77:25, and 88:15 d,l-lactide/glycolides have progressively longer in vivo
lifetimes, with the 88:15 lasting about 150 days in vivo. Poly (d,l-lactide) requires about
12-16 months to biodegrade completely, and poly (1-lactide), being more crystalline
and less hydrophilic, can be found in vivo even after 1.5-2 years.

### 2.8 Stabilizers

A stabilizer is required to avoid coalescence and formation of agglomerates during and after the emulsification process of nanoparticles. The large interfacial tension of small droplets drives the system to coalescence. Adsorption of stabilizers at the interface prevents this coalescence by lowering the interfacial tension and the energy of the system. The type and concentration of stabilizer used may influence the particle size and particle properties such as ζ potential and mucoadhesion. Both particle size and ζ potential are important physicochemical properties because they determine the physical stability and biopharmaceutical properties of nanoparticles, influencing drug release rate, biodistribution, mucoadhesion, and cellular uptake (Vandervoort et al 2002). Pluronics (Poloxamers) are nonionic block copolymer of poly (oxyethylene) and poly (oxypropylene). Various grades of poloxamers available are Poloxamer 124, Poloxamer 188, Poloxamer 237, Poloxamer 338 and Poloxamer 407 based on ratio of ethylene oxide and propylene oxide units and having molecular weight range from 2090-17400. Poloxamers are widely used as stabilizers for nanoparticles formulated by various polymers like PLGA, Chitosan etc. These are coated on the surface of PLGA and chitosan NPs and can affect the zeta potential, particle size and particle surface properties. Poloxamer coated nanoparticles has long circulating properties and
capabilities of bypassing reticuloendothelial system uptake (Jain D et al; 2013). Polyvinyl alcohol (PVA) used in combination with other polymer resulted in nanoparticle formation, but exclusion of PVA from the formulation increased the size of particles to above 1 μm (Vandervoort et al; 2002). The type of PVA used influences the physical properties such as the particle size and redispersibility of PLGA nanoparticles. The use of less hydrolyzed PVA provided higher percentage yield and uniform sized nanoparticles, whereas the highly hydrolyzed grade resulted in poor productivity and poor redispersibility. It has been shown that a fraction of PVA resides on PLGA nanoparticles surface, even on repeated washing, because PVA forms an interconnected network with the polymer at the interface.
2.9 Drug Profiles

2.9.1 Drug Profile of Gemcitabine HCl

Gemcitabine HCl (USP30 NF27, Drug Bank, Goodman Gilman’s the pharmacological basis of therapeutics; 2006, Rx drug List, Martindale; 2009)

Category: Antineoplastic, Antimetabolite (Pyrimidine analogue)

CAS No: 122111-03-9

Proprietary Names: Gemcitera, Gemsar Gemzar, Zefei

Molecular Formula: C$_9$H$_{11}$F$_2$N$_3$O$_4$•HCl

Molecular Weight: 299.66

Structural formula and Chemical name:

(2’-deoxy-2’,2’-difluorocytidine monohydrochloride (β-isomer)).

Physicochemical properties: Gemcitabine HCl is a white to off-white solid powder.

Solubility: It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

pKa: 3.6

Mechanism of Action: Gemcitabine HCl enters cells via active nucleoside transporters. Kills malignant cells undergoing DNA synthesis; arrests progression of cells at G1/S border. Intracellularly, deoxycytidine kinase phosphorylates Gemcitabine to produce difluorodeoxycytidine monophosphate (dFdCMP), from which point it is converted to
difluorodeoxycytidine di- and triphosphate (dFdCDP and dFdCTP). Unlike cytarabine, the cytotoxicity of Gemcitabine HCl is not confined to the S phase of the cell cycle, and the drug is equally effective against confluent cells and cells in logarithmic growth phase. The cytotoxic activity may be a result of several actions on DNA synthesis: dFdCTP competes with dCTP as a weak inhibitor of DNA polymerase; dFdCDP is a potent inhibitor of ribonucleotide reductase, resulting in depletion of deoxyribonucleotide pools necessary for DNA synthesis; and dFdCTP is incorporated into DNA and after the incorporation of one more additional nucleotide leads to DNA strand termination. This "extra" nucleotide may be important in hiding the dFdCTP from DNA repair enzymes, as the incorporated dFdCMP appears to be resistant to repair. The ability of cells to incorporate dFdCTP into DNA is critical for Gemcitabine HCl-induced apoptosis.

**Pharmacokinetics**

**Absorption, Fate, and Elimination:** Gemcitabine HCl is administered as an intravenous infusion. Patients receiving Gemcitabine HCl 1000 mg/m2 once weekly generally demonstrate Cmax values of 10 to 40 μg/ml and achieve steady state after 15 to 30 minutes, during 30-minute infusion protocol.

The pharmacokinetics of the parent compound are largely determined by deamination, and the predominant urinary elimination product is the inactive metabolite difluorodeoxyuridine (dFdU). Gemcitabine has a short plasma half-life of approximately 15 minutes, with women and elderly subjects having slower clearance. Gemcitabine HCl half-life for short infusions ranged from 42 to 94 minutes, Clearance is dose-independent but can vary widely among individuals. Conversion of Gemcitabine HCl to dFdCMP by deoxycytidine kinase is saturated at infusion rates of approximately 10 mg/m2 per minute, which produce plasma drug concentrations in the range of 15 to 20 uM. In an attempt to increase dFdCTP formation, the duration of infusion at this maximum concentration has been extended to 150 minutes. In contrast to fixed infusion duration of 30 minutes, the 150-minute infusion produces a higher level of dFdCTP within peripheral blood mononuclear cells, increases the degree of myelosuppression, but has uncertain effects on antitumor activity.
**Therapeutic Uses and administration:** The standard dosing schedule for Gemcitabine HCl (GEMZAR) is a 30-minute intravenous infusion of 1 to 1.2 g/m² on days 1, 8, and 15 of each 28-day cycle.

Gemcitabine HCl is a highly hydrophilic drug, first approved by FDA in 1996 for the treatment of breast cancer in combination with paclitaxel. In 2006 it was further approved for ovarian cancer in combination with paclitaxel and has been recently approved as the first line of treatment for pancreatic cancer that is advanced or has metastasized.

**Non-small cell lung cancer:** First line treatment of locally advanced (Stage IIIA or IIIB), or metastatic (Stage IV) non-small cell lung cancer.

**Pancreatic cancer:** Treatment of locally advanced (non resectable Stage II or Stage III) or metastatic (Stage IV) adenocarcinoma of the pancreas.

**Bladder cancer:** Treatment of bladder carcinoma at the invasive stage.

**Breast cancer:** Indicated in combination with cisplatin for the treatment of relapsed metastatic breast cancer after adjuvant/neoadjuvant chemotherapy. Prior chemotherapy should have included an anthracycline unless clinically contra-indicated.

**Ovarian cancer:** Indicated alone or in combination with other chemotherapeutic agents in the management of patients with advanced or relapsed epithelial ovarian carcinoma.

**Adverse effects and clinical Toxicities:** Paresthesia, nausea, vomiting, diarrhoea, stomatitis, hematuria, proteinuria, hemolytic uremic syndrome, renal failure, anemia, leukopenia, thrombocytopenia, dyspnea, bronchospasm, alopecia, rash, cellulitis, flulike symptoms, fever, edema, injection site reactions, anaphylactoid reactions are common adverse effects. The principal toxicity of Gemcitabine HCl is myelosuppression. In general, the longer-duration infusions lead to greater myelosuppression. Nonhematologic toxicities including a flu-like syndrome, asthenia, and mild elevation in liver transaminases may occur in 40% or more of patients. Rarely, patients on Gemcitabine HCl treatment for many months may develop a slowly progressive hemolytic uremic syndrome, necessitating drug discontinuation.
Contraindications:

The drug is contraindicated in patients with known hypersensitivity to the drug or any component in this formulation. Concomitant administration with radiation therapy is contraindicated due to risk of radio sensitization and of the onset of severe pulmonary and oesophageal fibrosis. Concomitant administration with cisplatin in patients with severe renal failure is contraindicated.

Analytical methods:

UV-spectrophotometric method

A simple accurate and sensitive UV-spectrophotometric method for estimation of Gemcitabine HCl in distilled water at 269 nm was used by Celano et al; 2004, Arias et al; 2009.

HPLC method

USP method is a gradient HPLC method and uses variable mixture of mobile phase solution A and solution B as mobile phase. [Solution A is filtered and degassed solution of 13.8 gm of monobasic sodium phosphate and 2.5 ml of phosphoric acid in 1000 ml of distilled water] while solution B is filtered and degassed methanol. The liquid chromatography is equipped with a 275 nm detector and a 4.6mm X 25cm column that contain packing 5 µm. The flow rate is 1.2 ml/min. The retention time for Gemcitabine HCl was 6.2 min.

Formulations available: The clinical formulation is supplied in a sterile form for intravenous use only. Vials of Gemzar (Gemcitabine HCl) contain either 200 mg or 1 g of Gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment. Available as Powder for injection: 200 mg in 10-ml vial, 1 g in 50-ml vial.
Research work done on Gemcitabine HCl:

Gang et al (2007) formulated Gemcitabine HCl loaded magnetic polycaprolactone nanoparticles and the anti-tumor effects were examined using nude mice bearing subcutaneous human pancreatic adenocarcinoma cells (HPAC) in vivo. The antitumor effect was shown with 15-fold lower dose when compared to free Gemcitabine HCl. Proved therapeutic benefit of magnetic PCL nanoparticles by delivering drugs efficiently to magnetically targeted tumor tissues, thus achieving safe and successful anti-tumor effects with low toxicity.


Trickler et al (2010) formulated Gemcitabine HCl loaded Chitosan and Glyceryl monooleate for treatment of pancreatic cancer and showed a significant decrease in IC50 value for cell survival as compared to Gemcitabine solution (Trickler et al; 2010).

Derakshandeh et al (2012) formulated Chitosan nanoparticles for oral absorption of Gemcitabine HCl and showed that intestinal transport of Gemcitabine increased 3–5 folds by loading in chitosan nanocarrier.

Lim et al (2012) formulated Gemcitabine HCl loaded microspheres using chitosan as mucoadhesive polymer and Eudragit L100-55 as enteric copolymer for improving oral absorption and found 3.8 fold increased uptake in Caco 2 cells.

Vandana and Sahoo (2010) developed pegylated Gemcitabine HCl for intravenous delivery and achieved long circulation time for Pegylated Gemcitabine HCl in comparison to plain drug.

Hosneiyah et al (2013) formulated Gemcitabine HCl loaded chitosan -pluronic nanoparticles for oral delivery were studied for treatment of colon cancer. Cytotoxicity assay in HT 29 cells showed increased cytotoxicity of Gemcitabine loaded nanoparticles.

Hao et al (2013) developed self micro emulsifying drug delivery system for oral delivery of Gemcitabine HCl and found that the formulation was effective against several cancer types, was metabolized more slowly than Gemcitabine hydrochloride, and exhibited enhanced oral bioavailability.

2.9.2 Drug Profile of Lopinavir

Lopinavir (IP 2007, Rx Drug list, Drug Bank, Goodman Gilman's the pharmacological basis of therapeutics; 2006)

Category: Antiviral, Protease Inhibitor

CAS No: 192725-17-0

Molecular Weight: 628.8

Melting Point: 124 to 127 °C

Physicochemical properties: White to light tan powder.

Solubility: Freely soluble in methanol and ethanol; soluble in isopropanol; practically insoluble in water.

Proprietary Names: Kaletra, Aluvia


Structural Formula:

\[
\text{(alphaS)-Tetrahydro-} \quad \text{N-[(alphaS)-alpha-[(2S,3S)-2-hydroxy-} \\
\text{4-phenyl-3-[2-(2,6-} \quad \text{butyl]phenethyl]-alpha-isopropyl-2-oxo-} \\
\text{pyrimidineacetamide}
\]
Mechanism of Action: Lopinavir is an inhibitor of the HIV-1 protease, preventing cleavage of the Gag-Pol polyprotein and reducing the probability of viral particles reaching a mature, infectious state. Lopinavir is an antiretroviral agent which inhibits HIV protease, causing the enzyme incapable of processing the polyprotein precursor. This leads to the production of non-infectious and immature HIV particles. Lopinavir is used in combination with ritonavir, which increases the bioavailability to therapeutic levels.

Absorption: It is absorbed from the GI tract. Tablet formulation unaffected by food but liquid capsules and oral liquid have improved bioavailability when taken with food.

Distribution: Approx 98-99% drug is plasma bound.

Metabolism: It is metabolised hepatically by isoenzyme CYP3A.

Excretion: Eliminated via urine (10%) and faeces (83%).

Therapeutic uses and indications: The drug is indicated for treatment of AIDS.

Adverse Drug Reactions: Diarrhoea, abdominal pain, asthenia, headache, dyspepsia, vomiting, myalgia, bronchitis, hypertension, palpitation, thrombophlebitis, vasculitis, agitation, anxiety, ataxia, hypertonic, confusion, depression, dyskinesia, peripheral neuritis; Cushing’s syndrome; hypothyroidism, sexual dysfunction, lactic acidosis, arthralgia, abnormal vision, otitis media, tinnitus, acne, alopecia, dry skin, skin discoloration, nail disorders, sweating, Pancreatitis

Contraindications: Hypersensitivity; renal or hepatic failure; lactation; Concomitant use of drugs highly dependent on CYP3A for clearance and associated with serious toxicity.

Analytical methods:

Faux et al, 2001 developed a rapid and simple HPLC method for detection of Lopinavir in plasma samples. In this method, the separation was performed on reverse phase C8 column (150 X 3.9 mm i.d, 5 µm). The mobile phase consisted of mixture of Acetonitrile and water (41:59, v/v). Flow rate was maintained 1.0 ml/min and compound eluted were recorded by UV detector at 210nm. The method was validated for linearity, accuracy and precision.
Another method was developed by Alex et al, 2011. The method employed a C18 column. The mobile phase consisted of a mixture of 55 volumes of acetonitrile and methanol in the volume ratio 80:20 and 45 volumes of 0.02M potassium dihydrogen phosphate solution with pH adjusted to 3 using orthophosphoric acid. The mobile phase flow rate was adjusted to be 1.5 ml/min. The injection volume was 20 microlitre and the maximum wavelength for detection was set as 210 nm.

**Dosage forms available:**

Film-coated tablets containing Lopinavir 200 mg and ritonavir 50 mg. Oral solution containing Lopinavir 80 mg/ml and ritonavir 20 mg/ml. Soft gelatin capsules containing Lopinavir 133.3 mg and Ritonavir 33.3 mg. Film-coated tablets containing Lopinavir 100 mg and Ritonavir 25 mg.

**Research work done on Lopinavir:**

PLGA nanoparticles containing combination of Lopinavir and ritonavir were developed by Destache et al (2009) and pharmacokinetic studies were performed in rats. They showed the sustained delivery of antiretroviral drugs from the PLGA NPs for 28 days. Alex et al (2011) developed solid lipid nanoparticles of Lopinavir for lymphatic targeting and results showed that the percentage bioavailability was enhanced but the poor drug loading was nevertheless pointed out as a major drawback in SLN formulations.

Alex et al (2011) proved the enhanced delivery of Lopinavir to CNS by compritol based solid lipid nanoparticles and demonstrated that SLNs with a poloxamer coating can be effectively absorbed through the lymphatic system, and can effectively target the drug to the CNS due to the combined effect of lipophilicity and surface charge.

Jain et al (2012) formulated surface stabilized nanoparticles of Lopinavir to enhance oral bioavailability without administration of ritonavir and demonstrated 3.11 fold increase in bioavailability in comparison to plain Lopinavir with ritonavir.

Negi et al (2012) developed solid lipid nanoparticles of Lopinavir by hot self nanoemulsifying technique using stearic acid, poloxamer and polyethylene glycol mixture and reported higher oral bioavailability in comparison to plain Lopinavir.
2.10 References

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