CHAPTER 1

INTRODUCTION
1.1 Introduction

Despite the extensive research and success stories with other routes for drug delivery, the oral route is still the most preferred route because of its basic functionality and the advantages that ensue. Oral delivery is by far the easiest and most convenient way for drug delivery, especially when repeated or routine administration is necessary (Florence and Jani; 1993). But, the challenges associated with oral route include exposure to extreme pH variations, intestinal motility, mucus barrier, P-glycoprotein efflux pump and impermeability of the epithelium (Vincent and Johnny; 2001). Moreover, the gastrointestinal tract provides a variety of barriers to the delivery of drugs, including proteolytic enzymes in gut lumen and on the brush border membrane, mucus layer, gut flora and epithelial cell lining.

One of the most attractive areas of research in drug delivery today is the design of nanosystems that are able to deliver drugs to right place, at appropriate times and at the right dosage. Nanoparticles are solid polymeric colloidal drug carriers ranging from 1 to 1000 nm. Nanoparticulate delivery systems have the potential power to improve drug stability, increase the duration of the therapeutic effect and permits administration through enteral or parenteral administration, which may prevent or minimise the drug degradation and metabolism as well as cellular p-glycoprotein efflux (Ping et al; 2008, Sarmento et al; 2007).

Nanoparticles have been extensively studied for peroral drug delivery, for systemic effect following uptake from enteron, or to act locally in the gastrointestinal tract. Nanoparticles are expected to address the specific issues for drug delivery like low mucosal permeability, absorption windows, low solubility of the drugs, gut metabolism and first pass effect. The potential advantages of nanoparticles as oral drug carriers are enhancement of bioavailability, delivery of vaccine antigens to the gut associated lymphoid tissues (GALT), controlled release, and reduction of the gastrointestinal irritation caused by drugs (Hariharan et al; 2005). The nanocarriers can improve the oral bioavailability of poorly bioavailable drugs due to their specialised uptake mechanism by preventing first pass metabolism of encapsulated drugs (Bhardwaj et al; 2005).
The nanoparticles by virtue their size and colloidal properties can be targeted to GALT (Gut associated lymphoid tissue) to deliver high loads of drug to lymphatic tissue and then to systemic circulation. The nanoparticles are taken up intact by M cells of peyer’s patches in the intestine associated Lymphoid tissue. M cells lack fully developed microvilli in comparison to the neighbouring absorptive cells and deliver the particles taken up to the lymphatics from where they, in a size-dependent manner, are then released into the bloodstream (Ravikumar and Bhardwaj; 2006). This mechanism provides a chance to target cancers of lymphatics as well as targeting antiretroviral drugs to the viral reservoirs. Furthermore, nanoparticles are capable of sustaining drug release in plasma for longer time period, thus reduces frequency of administration (Sonaje et al; 2007).

Nucleoside analogues, together with nucleobases and nucleotide analogues are commonly used in the treatment of viral infections and cancer. In both cases, they act as antimetabolite agents and interfere with the synthesis of cellular or viral nucleic acids. However the need of high doses due to rapid elimination of these compounds, to their poor activation and/or to their non specific distribution, often leads to side effects, toxicities and resistances.

For anticancer agents, higher toxic levels in blood by infusions and lower levels reaching at lymphatic tumour sites lead to resistances and ineffective therapy. Moreover, many of the most potent anticancer therapies can be administered only by injection, which means that cancer patients must travel to receive their medication. Hence, they prefer oral medicaments and home therapy. Oral chemotherapy is convenient, preferred by the patients and can greatly improve the quality of life of old age patients with advanced or metastatic cancer. Oral chemotherapy can eventually promote a new concept of chemotherapy: “chemotherapy at home” (Yun et al; 2012, Feng et al; 2003).

Unfortunately, orally administered anticancer drugs have little chance to get into the blood system and reach the tumor site due to their pure solubility, stability and permeability. As orally administered anticancer drugs would be eliminated by the first metabolic process with cytochrome P450 and by the efflux pump of P-glycoproteins (P-gp). P450/P-gp suppressors such as cyclosporine A can make oral chemotherapy feasible but they fail the immune system of the patients and thus may cause complex medication problems to the patients (Win and Feng; 2005).
Thus, providing oral delivery of anticancer drugs is a challenge and should provide a long-time, continuous exposure of the cancer cells to the anticancer drugs of a relatively lower thus safer concentration and thus give little chance for the tumour blood vessels to grow, resulting in much better efficacy and fewer side effects.

Researchers have been extensively working for oral delivery of anticancer formulations through nanoparticulate systems. The potential of PLGA nanoparticles of small enough size and appropriate surface coating for oral delivery of anticancer drugs showing enhanced uptake of nanoparticles in Caco-2 cells was proved and studied (Win and Feng; 2005). PLGA/ montmorillonite nanoparticles promoting the oral delivery of paclitaxel were synthesized and evaluated (Dong and Feng; 2005). Bhardwaj et al formulated PLGA nanoparticles for oral delivery of paclitaxel to treat breast cancer (Bhardwaj et al; 2009). Kalaria et al designed PLGA nanoparticles for oral delivery of doxorubicin to improve the bioavailability and found that PLGA nanoparticles have the potential for oral delivery of anticancer drugs (Kalaria et al; 2009). Feng et al formulated docetaxel loaded biodegradable polymeric NPs for oral delivery and improved bioavailability (Feng et al; 2009). Zhang et al formulated nanocarriers to improve oral delivery of anticancer drug and achieved higher drug concentration in tumour (Zhang et al; 2013).

Similarly, for anti-retroviral agents, the reduced bioavailability and short residence time at viral reservoir sites lead to resistance on discontinuation of therapy and inefficient eradication. HIV is able to re-seed the systemic circulation and continue to propagate the infection (Vyas et al; 2008). The combination therapy (HAART) can suppress the HIV replication below the limit of detection in peripheral blood. But it has issues like toxicity, insufficient efficacy, and drug resistance. Moreover, most of antiretroviral drugs suffer from poor solubility, permeability and stability. The major problem with antiviral treatment is to maintain adequate drug levels in the lymphoid tissue which is a major site for storage and replication of virus (Briesen et al; 2000). Main anatomical reservoir sites of HIV include the lymphoid organs (particularly the spleen, lymph nodes, and GALT) and the central nervous system (CNS) [Certain types of lymphoid cells, such as memory CD4+ T lymphocytes are seen in GALT where HIV persists and replicate even after HAART] (Macal et al; 2008, Tincati et al; 2009).
Various nanotechnology based systems are studied extensively by scientists to address the specific issues associated with antiretroviral therapy. Dussere et al (1995) developed a liposomal formulation of foscarnet and found higher drug concentrations with liposomes in lungs and lymph nodes, both the organs are viral reservoir sites. But the main obstacle with liposomal formulations is the physical stability as the systems are digested by physiological enzymes, which would result in immediate release of entrapped drug in the GI tract (Kreuter; 1991).

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 may nevertheless be pointed out as a major drawback in SLN formulations. Polymeric nanoparticles containing antiretroviral drugs can overcome the drawback of physiological stability as well as drug loading. PLGA nanoparticles containing combination of Lopinavir and ritonavir were developed and studied for cytotoxicity (Destache et al; 2009). It has been found that the size of the nanoparticles plays a key role in their adhesion to and interaction with the biological cells. The possible mechanisms for the particles to pass through the gastrointestinal (and other physiological) barriers could be (1) paracellular passage; particles “kneading” between intestinal epithelial cells due to their extremely small size (50 nm); (2) endocytotic uptake—particles absorbed by intestinal enterocytes through endocytosis (particles size 500 nm) (3) lymphatic uptake—particles adsorbed by M cells of the Peyer’s patches (particle size 5 mm) (Florence et al; 1995).

Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers and polysaccharides have been developed and tested over past decades. Biodegradable polymers may be synthetic or natural in origin. Natural biodegradable polymers include human serum albumin, low-density lipoproteins (LDLs), bovine serum albumin, gelatin, collagen, hemoglobin, polysaccharides like chitosan etc. Today, many synthetic biodegradable polymers are being employed successfully for drug delivery applications. Some synthetic biodegradable polymers are widely used in drug delivery technology are polyesters, polylactic acids, polylactones, poly (amino acids), and polyphosphazenes, Alkylcyanoacrylate, PLGA etc.

**Gemcitabine HCl**, an anticancer agent, is currently in clinical use for the treatment of several types of cancer. Gemcitabine is a difluoro analog of deoxycytidine.
Unfortunately, the drug is rapidly metabolised with a short plasma half-life and its cytostatic action is strongly exposure-time dependent. It is rapidly and extensively deaminated by cytidine deaminase in blood, liver, kidney and other tissues (Derakhshandeh and Fathi; 2012). In order to achieve the required concentration over sufficient periods of time, repeated application of relatively high doses is required (Vandana and Sahoo; 2010). This, in turn, leads to dose-limiting systemic toxicity. The plasma half life after intravenous infusion is 8-17 min in human plasma. Therefore, it is required in high doses. Furthermore, Gemcitabine is highly hydrophilic molecule with log P value 1.4 (Trickler et al; 2010). Till now, there is no oral formulation of Gemcitabine HCl in the market. It is available in the market in the freeze-dried form of an aqueous solution of the HCl salt known as Gemzar. After reconstitution Gemzar is used for intravenous administration as an infusion only (EliLilly; 1997).

**Lopinavir** is a potent protease inhibitor used as a leading component in combined chemotherapy commonly referred as Highly Active Anti-Retroviral Therapy (HAART). Lopinavir has poor oral bioavailability due to poor drug solubility characteristics as well as extensive first pass metabolism, primarily mediated by cytochrome P450 and P-glycoprotein efflux which limits intestinal uptake (Chattopadhyay et al; 2008, Griffin and O Driscoll; 2008 and Jain et al; 2009). In marketed preparations, Lopinavir is always co-administered with ritonavir, as ritonavir inhibits the cytochrome P450 enzyme, responsible for extensive first pass metabolism (Prot et al; 2006).

### 1.2 Aims and Objectives

The present investigation was aimed at development and characterization of PLGA nanoparticles of Gemcitabine HCl and Lopinavir for oral delivery with the following objectives:

- To formulate and optimize orally delivered nanoparticles of Gemcitabine HCl and Lopinavir, capable of absorption through M cell of Peyer’s patches in intestine, therefore, bypassing presystemic hepatic metabolism and enhancing the bioavailability of drugs.
- To prove the utility of PLGA nanoparticles in improving oral bioavailability of anticancer drug and antiretroviral drug.
• To enhance the absorption of drugs by entrapping in nanocarrier, so that Gemcitabine HCl could be administered orally and Lopinavir could be administered alone without need of Ritonavir.

• To compare the prepared nanoparticulate systems with respect to ease of formulation, characterization, in vitro and ex vivo drug release, in vitro cell uptake and transport studies in Caco 2 cells, cytotoxicity studies, stability and in vivo performance viz. pharmacokinetic studies.

**Hypothesis:** Nanoparticles of biodegradable polymers may provide an alternative solution for oral delivery of anticancer drugs and targeting anti-retroviral drugs to the intestinal lymphatic tissue with tumour sites and high viral load respectively across the gastrointestinal barrier due to their extremely small size and their appropriate surface coating to escape from the recognition by P450/P-gp. Further, nanoparticles have the ability to circumvent the p-glycoprotein efflux which is present on the membranes of HIV reservoir cells as well as intestinal epithelial cells. This in turn, increases the absorption as well as target the antiretroviral drug to HIV reservoir sites and anticancer drugs to lymphatic tumours as well as systemic circulation.

It was hypothesized that PLGA nanoparticles would be absorbed through the M-cells of the Peyer’s patches and then undergo lymphatic uptake, thereby bypassing liver metabolism and increasing bioavailability of the drug. Moreover, reach the lymphatic sites directly before reaching the systemic circulation.

**1.3 Plan of Work**

1. Literature survey, procurement of APIs and excipients.
2. Preformulation studies – Screening of excipients and characterization of API.
3. Analytical methods.
4. Formulation of Nanoparticles by multiple emulsification and nanoprecipitation method.
5. Optimization of process and formulation variables by factorial design.
6. Characterization (Particle size, Zeta potential, TEM, DSC and FTIR) and in vitro and ex vivo drug release studies of formulation in comparison with plain drug solutions.
7. *In vitro* cell line studies: quantitative uptake studies by FACS and qualitative uptake studies by confocal laser microscope, transport/permeability studies in Caco 2 cells and cytotoxicity studies by MTT assay in K562 cell lines.

8. Stability studies – Short term stability studies as per ICH guidelines.

9. *In vivo* absorption and pharmacokinetic studies of Gemcitabine HCl loaded PLGA nanoparticles.

10. *In vivo* absorption and pharmacokinetic studies of Lopinavir loaded PLGA nanoparticles.
1.4 References

- Dong Y and Feng SS. Poly(dl-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials, 26(30), 2005, 6068-6076.
Florence AT. Nanoparticles uptake by the oral route; fulfilling its potential. Drug Disc Today Tech, 2, 2005, 75-81.


