CHAPTER 1

INTRODUCTION
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1.1 Introduction to Viral Infection and Disease

AIDS was first characterized in 1980 and attributed to human immuno deficiency virus (HIV), which is a single stranded RNA retrovirus.

In Retrovirus, DNA is transcripted from RNA (reverse transcription) by an enzyme called reverse transcriptase (Viral RNA dependent - DNA polymerase).

Two viruses associated with AIDS are HIV-1 and HIV-2.

HIV-1 - World wide infections are caused by this.

HIV-2 - Infection occurs in Western Africa and India.

Viruses are obligate intracellular parasites composed of either DNA or RNA wrapped in a protein nucleocapsid. Some viruses produce a glycoprotein envelop that surrounds the nucleocapsid. Viruses shed their capsid after invading the host cell. The host cell then synthesizes new viruses using the message encoded by the viral DNA or RNA.¹

Viruses present a more difficult problem of chemotherapy than do higher organisms, e.g. bacteria, for they are intracellular parasites that use the metabolism of host cells. Highly selective toxicity is, therefore, harder to achieve. Identification of differences between viral and human metabolism has led to the development of effective antiviral agents, whose roles are increasingly well defined.²

Viruses are obligate intracellular parasites that use many of the host cell’s biochemical mechanisms and products to sustain their viability. A mature virus (virion) can exist outside a host cell and still retain its infective properties. However, to reproduce, the virus must enter the host cell, take over the host cell’s mechanisms for nucleic acid
and protein synthesis, and direct the host cell to make new viral particles.  

1.2 Classification of Viruses\(^3\)

Viruses are composed of one or more strands of a nucleic acid (core) enclosed by a protein coat (capsid). Many viruses possess an outer envelope of protein or lipoprotein. Viral cores can contain either DNA or RNA; thus, viruses may be classified as DNA viruses or RNA viruses. Further classification is usually based on morphology, cellular site of viral multiplication, or other characteristics.

Examples of DNA viruses and the diseases that they produce include:
1. Adenoviruses (colds, conjunctivitis)
2. Hepadnaviruses (hepatitis B)
3. Herpesviruses (cytomegalovirus, chickenpox, shingles)
4. Papillomaviruses (warts)
5. Poxviruses (smallpox).

Pathogenic RNA viruses include:
1. Arboviruses (tick-borne encephalitis, yellow fever)
2. Arenaviruses (Lassa fever, meningitis)
3. Orthomyxoviruses (influenza)
4. Paramyxoviruses (measles, mumps)
5. Picornaviruses (polio, meningitis, colds)
6. Rhabdoviruses (rabies); rubella virus (German measles)
7. Retroviruses (AIDS).

1.3 Pathogenesis of HIV-Related Disease

Human immunodeficiency viruses (HIV) are lentiviruses, a family of mammalian retroviruses evolved to establish chronic persistent infection
with gradual onset of clinical symptoms. Unlike herpesviruses, replication is constant following infection, and although some infected cells may harbor nonreplicating but infectious virus for years, there generally is no true period of viral latency following infection. Humans and chimpanzees are the only known hosts for these viruses.

There are two major families of HIV. Most of the epidemic involves HIV-1; HIV-2 is a close relative whose distribution is concentrated in western Africa. HIV-1 is genetically diverse, with at least five distinct subfamilies or clades. HIV-1 and HIV-2 have similar in vitro sensitivity to most antiretroviral drugs, although the nonnucleoside reverse transcriptase inhibitors (NNRTIs) are HIV-1-specific and have no activity against HIV-2. Within HIV-1 isolates, clade per se does not seem to have a major effect on drug sensitivity.

1.4 Virus Structure

HIV is a typical retrovirus with a small RNA genome of 9300 base pairs. Two copies of the genome are contained in a nucleocapsid core surrounded by a lipid bilayer, or envelope, that is derived from the host cell plasma membrane.

The viral genome encodes three major open reading frames: gag encodes a polyprotein that is processed to release the major structural proteins of the virus; pol overlaps gag and encodes three important enzyme activities—an RNA-dependent DNA polymerase or reverse transcriptase with RNAase activity, protease, and the viral integrase; and env encodes the large transmembrane envelope protein responsible for cell binding and entry. Several small genes encode regulatory proteins that enhance virion production or combat host defenses. These include tat, rev, nef, and vpr.
1.5 Viral Replication

Although the specific details of replication vary among types of viruses, the overall process can be described as consisting of five phases: (1) attachment and penetration, (2) uncoating, (3) synthesis of viral components, (4) assembly of virus particles, and (5) release of the virus. An overview of the viral replication cycle is shown in Figure 1.1.

Fig: 1.1. Replicative cycle of HIV-1 Showing the sites of Action of Antiretroviral Agents.
(Source: Goodman & Gilman's The Pharmacologic Basis of Therapeutics - 11th Ed. (2006))

1.5.1 Replicative Cycle

A. Replicative cycle of a herpesvirus, an example of a DNA virus.
B. Replicative cycle of an influenza virus, an example of an RNA virus.


Infection begins when specific receptor sites on the virus recognize corresponding surface proteins on the host cell. The virus penetrates the host membrane by a mechanism resembling endocytosis and is
encapsulated by the host cell’s cytoplasm, forming a vacuole. Next, the protein coat dissociates and releases the viral genome, usually into the host cell’s nucleus. Following the release of its genome, the virus synthesizes nucleic acids and proteins in sequence. In DNA viruses, the first genes to be transcribed are the immediate– early genes. These genes code for regulatory proteins that in turn initiate the transcription of the early genes responsible for viral genome replication.

After the viral DNA is replicated, the late genes are transcribed and translated, producing proteins required for the assembly of the new virions. RNA viruses have several major strategies for genome replication and protein expression. Certain RNA viruses contain enzymes that synthesize messenger RNA (mRNA) using their RNA as a template; others use their own RNA as mRNA. The retroviruses use viral reverse transcriptase enzymes to produce DNA using viral RNA as a template. The newly synthesized DNA integrates into the host genome and is transcribed into mRNA and genomic RNA for progeny virions. Following their production, the viral components are assembled to form a mature virus particle. The viral genome is encapsulated by viral protein; in some cases (e.g. adenovirus, poliovirus), it is not encapsulated. In certain viruses, such as the poxviruses, multiple membranes surround the capsid. Release of the virus from the host cell may be rapid and produce cell lysis and death. A slower process resembling budding may allow the host cell to survive.

1.6 Overview of Antiviral Therapy

Three basic approaches are used to control viral diseases: vaccination, antiviral chemotherapy, and stimulation of host resistance mechanisms. Vaccination has been used successfully to prevent measles, rubella, mumps, poliomyelitis, yellow fever, smallpox, chickenpox, and hepatitis B. Unfortunately, the usefulness of vaccines appears to be limited when many stereotypes are involved (e.g., rhinoviruses, HIV).
Furthermore, vaccines have little or no use once the infection has been established because they cannot prevent the spread of active infections within the host. Passive immunization with human immune globulin, equine antiserum, or antiserum from vaccinated humans can be used to assist the body’s own defense mechanisms. Intramuscular preparations of immune globulin may be used to prevent infection following viral exposure and as replacement therapy in individuals with antibody deficiencies. Peak plasma concentrations of intramuscular immune globulins occur in about 2 days. In contrast, intravenously administered immune globulin provides immediate passive immunity. The chemotherapy of viral infections may involve interference with any or all of the steps in the viral replication cycle. Because viral replication and host cell processes are so intimately linked, the main problem in the chemotherapy of viruses is finding a drug that is selectively toxic to the virus. Stimulation of host resistance is the least used of the antiviral intervention strategies.

1.7 FDA Regulation of Oral Controlled Release Drugs

In the 1980s, FDA introduced rigorous regulations governing bioequivalence and in vitro–in vivo correlations for controlled-release products. Required pharmacokinetic evaluations involve.

- Relative bioavailability following single dose.
- Relative bioavailability following multiple dose effect of food.
- Dose proportionality.
- Unit dosage strength proportionality.
- Single-dose bioequivalence study (experimental versus marketed formulations at various strengths).
- \textit{Invivo–invitro} correlation.
- Pharmacokinetic/pharmacodynamic(PK/PD) relationship.
In general, for drugs in which the exposure–response relationship has not been established or is unknown, applications for changing the formulation from immediate release to controlled release requires demonstration of the safety and efficacy of the product in the target patient population. When an NME is developed as a controlled-release dosage form, additional studies to characterize its absorption, distribution, metabolism, and excretion (ADME) characteristics are recommended.

1.8 Beneficial Characteristics of Controlled Drug Delivery System

<table>
<thead>
<tr>
<th>Benefit</th>
<th>Reason</th>
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<tbody>
<tr>
<td>Therapeutic advantage</td>
<td>Reduction in drug plasma level fluctuations, maintenance of study plasma level of the drug over a prolonged time period, ideally simulating an intravenous infusion of a drug.</td>
</tr>
<tr>
<td>Reduction in adverse effects and improved tolerability</td>
<td>Drug plasma level are maintained within a narrow window with no sharp peaks and with AUC of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release dosage forms. This greatly reduces the possibility of side effects, as the scale of side effects increases as we approaches to the MSC.</td>
</tr>
<tr>
<td>Patient comfort and compliance</td>
<td>Oral drug delivery is the most common and convenient for patient, and a reduction in dosing frequency entrances compliance.</td>
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<tr>
<td>Reduction in healthcare cost</td>
<td>The total cost of therapy of the controlled release product could be comparable or lower than the immediate release product. The overall expenses in disease management also be reduces.</td>
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1.9 Drawbacks of Conventional Antiretroviral Drugs

Majority of the currently marketed antiHIV agents are formulated as solid dosage forms, viz., tablets and capsules for oral use; or liquid dosage forms, viz., solutions, suspensions for oral and parenteral use. While the oral dosage forms offer convenience, delivery of drugs via this route suffers from significant first pass effect, variation of absorption and degradation in the gastrointestinal tract due to enzymes and extreme pH conditions\textsuperscript{15}. For example, zidovudine the first antiretroviral developed, although rapidly absorbed from the intestine, loses considerable potency by the hepatic first pass metabolism (40\%) and then rapid elimination from the body with a biological half life of only 1 h\textsuperscript{16}.

As a shortfall for all the conventional oral dosage forms, the duration of action is limited since the absorption of the drug depends on the resident time of the drug in the gastrointestinal tract \textsuperscript{17}. Also, many of these compounds exhibit poor or low bioavailability due to various other factors, namely, physicochemical properties such as dissolution rate and solubility, or biological properties such as permeability (didanosine exhibits low intestinal permeability) and metabolism\textsuperscript{18}.

The performance of a drug is a function of its physicochemical properties, such as aqueous solubility and drug stability. Studies have shown that solution stability and acid liability have become a significant concern in the dosage form development of dideoxynucleosides. Thus the causes for poor and variable absorption are vast, but they can primarily be related to physicochemical properties such as dissolution rate and solubility. For example, the oral bioavailability of NNRTIs is limited due to their low aqueous solubility. Thus the bioavailability variability of antiretrovirals may be a significant factor in the failure of some of the drug regimens. Also, after an antiHIV drug is absorbed and enters the blood circulation, metabolism/elimination and transport barriers will substantially decrease the effective amount of drug reaching the target action site. In order to succeed in an effective therapy for AIDS, it is
crucial to maintain the systemic drug concentration consistently above their target antiretroviral concentration throughout the course of the treatment and to enhance localization and intracellular delivery of the drug.

However, because of the short biological half life of number of these drugs, conventional routes are inherently limited in that they can not maintain a constant plasma level with the target therapeutic range for a prolonged duration\textsuperscript{19}. Due to virustatic nature of the drugs, they must be administered for the life of the patient. All these therapeutic moieties exhibit dose-dependent toxic side effects such as hepatotoxicity, hyperglycaemia, hyperlipidemia, lactic acidosis, lipodystropy, osteonecrosis, osteoporosis, osteopenia, skin rashes, resulting from excessive systemic concentration, and they often require dosage reduction or even cessation of treatment, since conditions like lactic acidosis may even be fatal. Thus despite their undisputed effectiveness, several complicated clinical issues are associated with the use of these agents. Adherence of the patient to the treatment is critical, as loss of antiviral efficacy has been correlated with poor pill taking resulting in loss of viral suppression. The high pill burden [examples: fosamprenavir-1400 mg twice daily, nelfinavir-1250 mg twice daily (10 tablets daily), amprenavir 1200 mg three times daily (16 tablets daily)] and coordination with the meals make these agents laborious to take\textsuperscript{20}.

1.10 Rationale for Controlled Drug Delivery of Anti Retroviral Drugs

To fulfill the need of a long-term treatment with anti HIV agents, where most of the patients suffer from the drawbacks of frequent administration, plasma concentration fluctuation, significant adjustment in the lifestyle; it is desirable to have controlled- or sustained-release drug delivery systems to improve the overall therapeutic benefit and to achieve an ideal therapy. By sustained or controlled delivery, it is possible to achieve effective plasma concentration without significant fluctuation, to avoid sub-therapeutic and toxic plasma concentrations, to
facilitate release of the medication in a controlled manner to obtain a continuous delivery, to achieve an effective therapy with low dosage of the drug, to reduce the frequency of medication and thus to improve patient adherence, by preventing the interference of therapy with the day-to-day lifestyle.

Percutaneous absorption of a number of antiretrovirals has been studied indicating a promising future for this route for the delivery of antiretrovirals\textsuperscript{21}. Novel drug delivery carriers such as liposomes, microparticles and drug-encapsulated erythrocytes are also under investigation\textsuperscript{22}. Liposomes as drug delivery systems are very versatile in that they can be tailored to suit the delivery of various drug molecules. The ability of liposomes to alleviate the toxicity of various drugs has been established. Liposomal formulations of toxic drugs like doxorubicin and amphotericin B were significantly less toxic compared to the free drug\textsuperscript{23}. The effect of liposomal encapsulation, on the haematopoietic toxicity and antiviral efficacy of AZT has been studied in mice. Liposomal encapsulated AZT was more effective than the AZT in preventing the development of plasma reverse transcriptase activity after infection of mice with the retrovirus\textsuperscript{24}. Thus the liposomal AZT resulted in a significant reduction in the toxicity, a substantial enhancement of antiviral activity coupled with enhanced localization of the drug in the liver and spleen.

Furthermore, it is observed that encapsulation of Dideoxycytidine-5'-triphosphate (DDCTP) in liposomes exhibited good chemical stability of the drug molecule\textsuperscript{25}. Results obtained with this encapsulated DDCTP in the murine acquired immunodeficiency syndrome model indicate that DDCTP encapsulated in liposomes can reduce proviral DNA in cells of the mononuclear phagocyte system (MPS) in both spleen and bone marrow\textsuperscript{26}. Also the liposomal form increases the efficacy, reduces the toxicity and increases the half life of the drug.
1.11 Gastroretentive Drug Delivery Systems (GRDDS)

Dosage forms that can be retained in the stomach are called gastroretentive drug delivery systems (GRDDS). GRDDS can improve controlled delivery of drugs with an absorption window by continuously releasing the drug for a prolonged period before it reaches its absorption site, thus ensuring optimal bioavailability. Drugs with a narrow absorption window are mostly associated with improved absorption at the jejunum and ileum due to the enhanced absorption properties of these sites (e.g. large surface area), or because of enhanced solubility in the stomach as opposed to the more distal parts of the GIT.

1.12 Suitable Drug Candidates for Gastroretention

In general, appropriate candidates for CRGRDF are molecules that have poor colonic absorption but are characterized by better absorption properties at the upper parts of the GIT:

1. Narrow absorption window in GI tract, e.g., riboflavin and levodopa
2. Primarily absorbed from stomach and upper part of GI tract, e.g., calcium supplements, chlordiazepoxide and cinnarazine
3. Drugs that act locally in the stomach, e.g., antacids and misoprostol
4. Drugs that degrade in the colon, e.g., ranitidine Hcl and metronidazole
5. Drugs that disturb normal colonic bacteria, e.g., amoxicillin trihydrate

1.13 Factors Controlling Gastric Retention of Dosage Forms

1. Density of dosage form
2. Size of dosage form
3. Shape of dosage form
4. Single or multiple unit formulation
5. Fed or unfed state: under fasting conditions
6. Nature of meal
7. Caloric content
8. Frequency of feed
9. Gender
10. Age
11. Posture
12. Concomitant drug administration
13. Biological factors

1.14 LITERATURE REVIEW ON DRUGS

The literature review of various anti infective/retroviral drugs had revealed that only limited work was carried out in the subjected area.

1.14.1 Literature Review of Stavudine

F Feleke et al 28 had formulated Stavudine matrix controlled tablets using HPMC.

Robert et al 29 formulated Stavudine controlled release bead-lets.

Sahoo S K et al 30 had formulated Stavudine microcapsules employing ethyl cellulose and PVP in combination.

Suresh et al 31 had carried out studies on Stavudine PLGA microcapsules.

US FDA recently had approved the extended release Stavudine in capsule form under the brand name of ZERIT XR 32.

1.14.2 Literature Review of Lamivudine

Punnaa R et al 33 had formulated the matrix tablets for controlled release of Lamivudine employing different viscosity grades of hydroxypropyl methylcellulose (HPMC) polymer as the rate retardant .
Sen H et al. formulated anti retroviral drugs viz., Zidovudine and Lamivudine in combination and achieved the desired controlled release.

A Ghosch et al. had developed modified release ethyl cellulose microspheres incorporated with Lamivudine.

V Pratiba had carried studies on Lamivudine microspheres employing various Eudragits in different proportions.

1.14.3 Literature Review of Lopinavir and Ritonavir

I Tho et al. had carried out “Formation of nano/micro-dispersions with improved dissolution properties upon dispersion of Ritonavir melt extrudate in aqueous media”

Ehrhardt Manuela et al. “Monitoring of lopinavir and ritonavir in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC/MS/MS assay” For the determination of the HIV protease inhibitors lopinavir and ritonavir in human plasma, plasma ultrafiltrate, and peripheral blood mononuclear cells (PBMCs) a highly sensitive and selective method has been developed, validated, and applied to samples of a healthy volunteer.

Ray John et al. “Simultaneous determination of indinavir, ritonavir and lopinavir (ABT 378) in human plasma by high-performance liquid chromatography” and developed an isocratic reversed-phase high-performance liquid chromatographic method with ultraviolet detection at 205 nm has been validated for the determination of indinavir, ritonavir and lopinavir (ABT 378) in human plasma.

Maria Eliane Donato et al. “Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on in vivo data” developed and validate a dissolution test for lopinavir soft gel capsules (Kaletra®), using a simulated absorption profile based on in vivo data.
1.14.4 Literature Review of Valganciclovir

Shen Y and Tu Jiasheng et al.\(^{41}\) had prepared and evaluated the Ocular Pharmacokinetics of Ganciclovir Liposomes. Ophthalmic liposomes of ganciclovir (GCV) were prepared by the reverse phase evaporation method, and their ocular pharmacokinetics in albino rabbits were determined.

1.15 LITERATURE REVIEW ON POLYMERS USED IN THE PRESENT STUDY

Rahman et al.\(^{42}\) had formulated a bilayer-floating tablet (BFT) of Captopril employing direct compression technology. HPMC K-grade and effervescent mixture of citric acid and sodium bicarbonate formed the floating layer. The release layer contained Captopril and various polymers such as HPMC-K15M, PVP K30 and Carbopol 934, alone or in combination with the drug.

Xiaoqiang et al.\(^{43}\) had developed a sustained release tablet for phenylpropanolamine hydrochloride because of its short biological half life. Three floating matrix tablets based on a gas-forming agent were prepared. HPMC K4M and Carbopol 971P were used in formulating the hydrogel system.

Srivastava et al.\(^{44}\) had formulated microspheres employing HPMC and ethyl cellulose by solvent evaporation method.

Jain et al.\(^{45}\) designed a controlled release system to increase GRT without contact with gastric mucosa, which was achieved through the floating microspheres by emulsion solvent diffusion technique consisting of calcium silicate (FLR) as a porous carrier, Repaglinide and a Eudragit polymer.
Kamel El et al\textsuperscript{46} had prepared floating microparticles of Ketoprofen by emulsion solvent diffusion technique by using different proportions of Eudragit S 100 (ES) with Eudragit RL (ERL).

Mine et al\textsuperscript{47} investigated the swelling and relaxation properties of lipid matrix on diffusional exponent (n) and determine the desired release profile of Metronidazole lipid matrix tablets, in which the granules were prepared by using carnauba wax, beeswax, stearic acid, cutina Hr, precirol\textsubscript{ATO} 5, and compritol\textsubscript{ATO} 888 by hot fusion method and pressed the tablets of these granules.

Feng qian et al\textsuperscript{48} designed a controlled release matrix formulation for freely water-soluble drug of sodium ferulate (SF) to achieve a 24 h release profile using compritol 888 ATO as an inert matrix-forming agent to control the release of SF.

Tetsuo et al\textsuperscript{49} had formulated two matrix theophylline tablets with different release mechanisms and were compared. Tablet exhibited swelling/disintegration-type release, of which matrix was made of hydrophobic wax granules consisting of Stearic acid, hydrogenated oil and glycerol esters of fatty acids, and hydrophilic polymer granules composed primarily of hydroxypropyl methyl cellulose (HPMC).

Yu et al\textsuperscript{50} had formulated and evaluated sustained drug release after melt granulation and heat treatment. Theophylline (anhydrous) and phenylpropanolamine hydrochloride (ppa) were used as model drugs. Compritol -888 ATO (Glyceryl Behebnate NF) was incorporated as the wax matrix material.

Aiman et al\textsuperscript{51} had formulated an inert matrix to control the release of Tramadol HCL using glyceryl Behenate as a matrix – forming agent.

Alan et al\textsuperscript{52} had used melt granulation process for both immediate and sustained release TAVIST\textregistered (clamastine fumarate USP) tablet formulations. In these formulations, glycerylpalmitostereate (water in
soluble) and poly ethylene glycol 8000 (water soluble0 were used as the melt granulating material in various combinations.

Jaimini et al\textsuperscript{53} formulated Famotidine floating tablets employing two different grades of Methocel K100 (HPMC K100) and Methocel K15 (HPMC K15) by including an effervescent agent.

Dave et al\textsuperscript{54} developed gastroretentive delivery system of Ranitidine HCL. Guar gum, Xanthan gum and HPMC were used as gel forming agents and Sodium carbonate as a gas-generating agent.

Yang et al\textsuperscript{55} had developed a swellable asymmetric triple-layer floating tablet to prolong the gastric residence time of triple drug regimen (tetracycline, metronidazole, and clarithromycin) in Helicobacter pylori-associated peptic ulcers using hydroxy propyl methyl cellulose (HPMC) and polyethylene oxide) (PEO) as the rate-controlling polymers.

Wu et al\textsuperscript{56} formulated floating sustained release tablets of Nimodipine by using HPMC and PEG 6000.

Xiaoqiang Xu et al\textsuperscript{57} developed three floating matrix formulations of phenoporlamine hydrochloride based on gas forming agent. HPMC K4M and Carbopol 971P NF were used in formulating the hydrogel drug delivery system.

Shoufeng Li et al\textsuperscript{58} have reported the effect of HPMC and carbopol on the release and floating properties of gastric floating drug delivery system using factorial design. In the study HPMC of K4M and K100 LV viscosity grades and carbopol 934 were used in formulating the gastric floating drug delivery.

Deshpande A.A, et al\textsuperscript{59} developed a novel controlled release gastric retention tablets containing hydrophilic drug Chlorpheniramine maleate and a poorly soluble drug Riboflavin 5’phosphate using Cabopol 934P as a gelling agent, these tablets were coated with a permeable and elastin
polymer Eudragit RL 30D and NE 30D was used to provide an initial alkaline micro-environment and confer buoyancy to tablet.

R. Margretchandira et al\textsuperscript{60} had formulated and evaluated the floating tablets of Itopride hydrochloride employing direct compression method. Polymers like HPMC K100M, HPMC K15M and Carbopol 934 P, were used along with gas generating agent.

El Kamel, AH et al\textsuperscript{61} had formulated and evaluated sustained release ketoprofen floating micro particles through emulsion-solvent diffusion technique with Euragit S100 with Euragit RL in varied proportions.

Chinam niranjan patra et al\textsuperscript{62} had formulated and evaluated sustained release bilayer tablets of propranolol hydrochloride using superdisintegrant sodium starch glycolate for the fast release layer and ethylcellulose, Eudragit RLPO and Eudragit RSPO for the sustaining layer.

1.16 LITERATURE REVIEW ON ANALYTICAL METHODS

Kano \textit{et al} had developed a novel HPLC method for the determination of Lamivudine in human plasma\textsuperscript{63}.

Namitha \textit{et al} had carried out simultaneously determination of Lamivudine and Stavudine combination as fixed dose \textsuperscript{64}.

Bin \textit{et al} had analyzed the Zidovudine, Lamivudine and Nevirapine form human plasma \textsuperscript{65}.

Emilla \textit{et al} had carried quantitative analysis of Zidovudine and Lamivudine by RP-HPLC method \textsuperscript{66}.

Ashanefi \textit{et al} had performed and reported the analysis of Zidovudine using RP-HPLC method \textsuperscript{67}.
C. M. Phechkrajang et al \(^6\) performed “Quantitative Determination of Lopinavir and Ritonavir in Syrup Preparation by Liquid Chromatography” and reported a simple high-performance liquid chromatographic method (HPLC) for its determination.

Usami Y et al\(^6\)\(^9\) had carried simultaneous determination of Lopinavir (LPV), Ritonavir (RTV) and Efavirenz (EFZ)” by simple HPLC method.