CHAPTER 1: TECHNIQUES FOR ENHANCED ANTI-HIV THERAPY

1. 1. Introduction

The acquired immune deficiency syndrome (AIDS) and associated infections caused by human immuno-deficiency virus (HIV), has been identified as one among the dreadful ailments which pose alarming challenges to the community health throughout the world, especially more frightening in certain areas like sub-Saharan Africa.\(^1\) The global level statistics have shown more than 33.2 million patients alive with this infection. Based on Indian health organization estimates India have around 6-7 million HIV infected patients. 2.1 million People are newly infected (2014), 1.5 million as dead with AIDS in 2013 (UNAIDS: http:// cmg.the body.com/unaids/2014) and 2.1 million in India (2-3 million/2013). Intervention through AIDS counseling educational systems and highly active antiviral therapy have promoted HIV infections from serious lethal incurable stage to controllable disease state\(^2\) Presently, there are two known species of human immunodeficiency virus generally mentioned as HIV-1 and HIV-2 along with their sub-species, out of which HIV-1 infections are widespread around the world, whereas HIV-2 is highly prevailing in West Africa that takes longer duration to extend as immunodeficiency syndrome.\(^3,4\) This virus can infect only humans where progressive failure of immune system occurs, as the virus weakens the immune system by killing the important cells that combat against disease allowing life threatening opportunistic infections. HIV, it is unlike other viruses which causes acute coryza or common cold. Over a period, the body defence system can slough off almost all such viruses out of the body, but cannot get rid of HIV and yet, researches are still trying to find out the reason.\(^2\)
1.1.1. Pathway of HIV infection

The foremost step for the cause of HIV infection inside human body is the incorporation of viral genome into host cell, followed by replication of cells, which leads to the advanced stage condition as acquired immune deficiency syndrome. The Gp-120 protein of the virus binds with the two transmembrane receptors of the host cell, one is CD4+ receptor and the other is either of the chemokine receptors namely CCR5 or CXCR4, or HIV macrophages or T-helper lymphocytes. T-Tropic viruses prefer the macrophage of the HIV-1 viruses in tropic types that is predominate in the brain.\(^7,\,8\) The viral genome contains three structural genes - gag, trol, and env and six regulatory genes - tat, rev, nef, vif, vpr and vpo. With the help of these genes and other host cell resources, the viruses maximize its production. J.Cheinen \textit{et al.} has documented well about the immuno pathogenesis of HIV / AIDS from the early stage of disease till the ending of the complete infection.\(^5\) The final phase of this syndrome is usually characterized by a spectrum of diseases including the chances of infection caused by pneumocytosis, carinii and mycobacterium tuberculosis, cancer and dementia.\(^6,\,9\) The susceptible sites of the virus infection are central nervous system, lymph nodes, bone marrow, spleen, lungs, etc. Symptoms in the CNS are more prominent which leads to remarkable damage or loss of neurons ultimately resulting in HIV related dementia, if untreated.\(^10\) The uncontrollable HIV-1 infection often ends with fatal results within 5 to10 years.\(^11\)

The primary mechanism by which HIV transmission occurs is the direct contact of genital mucosal surface to the virus during sexual intercourse. Pettfior \textit{et.al} performed a
reproductive health research study and found that majority of the subjects (more than 93 %) use condom which is the most successful preventive measures.\textsuperscript{12}

1.2. Components of HIV virus

HIV is spherical in appearance having 1/10,000 of a millimeter diameter. It is composed of a viral envelope and the viral core. The viral envelope is the outer cover of the virus, composed of lipid bilayer embedded with virion which protrudes throughout the surface. This virion comprises of a cap containing glycoprotein 120 (gp120) molecules and a stem comprising of glycoprotein 41 (gp41). These glycoproteins enable virus to recognize the surface proteins of the specialized immune cells and enter the cell. The capsid contains 2 single stranded HIV RNA each containing entire copy of the virus genes. The 3 structural genes present in HIV namely gag, pol and env which have all informations required to make new structural proteins. Also the 6 regulatory genes (tat, rev, nef, vif, vpr and vpu) contains message for development of proteins to control the activity of HIV to enter into cells, produce more replicates of the virus and hence causes the disease, Figure 1.1.\textsuperscript{13}

1.2.1. Steps of HIV replication process

1. **Step i:** binding of HIV with the surface of the host cell to a specific type of CD4 receptor and a co-receptor (CCRS) on the surface of the CD4 cell.

2. **Step ii:** Entry of HIV RNA, reverse transcriptase, integrase and other viral proteins into the host cell and modify the genetic material of the virus.

3. **Step iii:** Formation of viral DNA by reverse transcription.
4. **Step iv:** Transportation of the viral DNA across the nucleus hence integration into the host DNA.

5. **Step v:** Use of the newly generated viral RNA as the genomic RNA and produce viral proteins.

6. **Step vi:** Transportation of the newer viral RNA to the surface of the cell, followed by the formation of new immature HIV viruses.

7. **Step vii:** Finally the virus matures and cause the disease.\textsuperscript{14,15}

Figure 1.1. Replication cycle of Human immune deficiency virus
1.2.2. AIDS/ HIV Drugs and Its Limitations

Infections with HIV remains an incurable condition.13,14 Existing system of classification of anti-retroviral can be summarized as nucleoside reverse transcriptase inhibitor (NRTI), nucleotide reverse transcriptase inhibitor (NtRTI), non- nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), and the latest being fusion and integrase inhibitors.15 The role of drugs categorized under various classes with its half life (t_{1/2}), bioavailability as well as available dosage forms are shown in table 1.16-20

The combination of these drugs are under prescription practice, which is indicated as highly active anti-retroviral therapy (HAART).21 The latest classes of drugs under exploration are the group of up-coming potential inhibitors and the zinc finger inhibition including the HIV-1 capsid (CA) protein and human cyclophilin A (CyPA).22-24 But, the main disadvantages of these drugs are higher biotransformation in liver and GIT, with low elimination half life chiefly causing reduced and inconsistent bioavailability and poor targeting, and the development of multidrug resistance.25-27 These molecules are also put up with certain physicochemical challenges starting from insolubility and leading to erratic formulation issues.28,29
Table 1.1. Detail of Approved Antiretroviral Drugs for the Treatment of HIV Infection, including its date of approval, half life and available dosage form.

<table>
<thead>
<tr>
<th>Drug name/ Category</th>
<th>Approved date</th>
<th>Half- lives (h)</th>
<th>Available Dosage form</th>
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<tr>
<td><strong>Entry inhibitors</strong></td>
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<tr>
<td>Maraviroc (UK-427,857, Selzentry®)</td>
<td>06 Aug 2007</td>
<td>14-18</td>
<td>Tablet</td>
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<tr>
<td><strong>Fusion inhibitors</strong></td>
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<tr>
<td>Enfuvirtide (T20, Fuzeon®)</td>
<td>13 Mar 2003</td>
<td>3.8</td>
<td>Powder for SC Injection</td>
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<td><strong>Integrase inhibitors</strong></td>
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<td>Raltegravir (MK-0518, Isentress®)</td>
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<td>Tablet</td>
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<td><strong>Reverse transcriptase inhibitors</strong></td>
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<td>Nucleoside/nucleotide analogues</td>
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<tr>
<td>Abacavir (ABC, Ziagen®)</td>
<td>17 Dec 1998</td>
<td>1-2</td>
<td>Tablet, liquid</td>
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<tr>
<td>Didanonise (ddI, Videx®)</td>
<td>09 Oct 1991</td>
<td>1.3-1.6</td>
<td>Tablet, Capsule, solution</td>
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<tr>
<td>Emtricitabine (FTC, Emtriva®)</td>
<td>02 July 2003</td>
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<td>Capsule</td>
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<td>Stavudine (d4T, Zerit®)</td>
<td>24 June 1994</td>
<td>1-1.6</td>
<td>Tablet, Powder</td>
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<tr>
<td>Lamivudine (3TC, Epivir®)</td>
<td>17 Nov 1995</td>
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<td>Tenofovir (DF, Viread®)</td>
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<td>Tablet</td>
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<td>Efavirenz (EFV, Sustiva®)</td>
<td>17 Sep 1998</td>
<td>40-50</td>
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### 1.3. Role of Novel Drug Delivery Systems for Delivery of Anti-HIV Drugs

Commercially available products for HIV treatment are solid oral dosage forms, topical semi solids and few as parenterals, which requires higher dose administration and provide significantly lesser therapeutic action at the required site. Oral route of administration lags in achieving the desired bioavailability due to high rate of biotransformation, variable absorption and degradation process, instability in extreme pH conditions, barriers for drug transport to target site. Hence, novel drug delivery approach would be reasonable to overcome these challenges for the effective treatment of HIV infections. One of the significant features of controlled delivery systems for anti-HIV drugs is
maintenance of suitable plasma concentration that improves the potential activity of the drugs thereby reducing the dose and cost of treatment, and provides better patient compliance, Figure 1.2.

1.4. Advanced Techniques

1.4.1. Sustained release dosage forms

Sustained release systems play important role to accomplish desired level of drug at predetermined kinetics for prolonged period of time in the systemic circulation. This reduces the frequency of dosing and overall dose of administered drug, which ultimately leads to improved patient compliance\textsuperscript{30, 31}. This can be achieved by using the bio-adhesive polymer which can interact with mucin.\textsuperscript{32} Model drug which have been formulated as sustained release formulation is Didanosine (ddI)\textsuperscript{33-37}

1.4.2. Ceramic implants

The survey of literatures have shown a tremendous scope in drug delivery utilizing the ceramic implants to alter the release pattern for anti retroviral drugs such as deoxynucleoside.\textsuperscript{38, 39}

1.4.3. Vesicular systems

1.4.3.1. Liposomes

The liposome encapsulation potency for Azidothymidine (AZT) was established early using mice models.\textsuperscript{40, 41} Unlike free AZT injection, there was no bone marrow toxicity observed in normal erythrocytes and leukocytes profile for the drug encapsulated in
liposomes. Also these liposomes were extremely localized in organs like spleen, liver and lungs. They have been demonstrated to show superior anti-retroviral activity in mice models by reducing the haematopoietic toxicity especially through transdermal route\textsuperscript{42} and there was an increase in bioavailability and targeting reticuloendothelial system (RES) for the optimized liposomal formulation.

The DDI containing liposomes showed increase in half-life and having efficient lymphatic targeting and macrophage rich tissue. Moreover, through successive studies, the half life of ddI in plasma was extended further from 3 – 4 h (conventional liposomes) to 14.5 h by loading into sterically stabilized liposomes. IV administration of these liposomes showed high amount of drug in spleen reaching the maximum concentration at end of 24 h.\textsuperscript{43}

Zalcitabine was examined for its encapsulation effect into liposomes.\textsuperscript{44, 45} These liposomes were also found to be quickly absorbed by the cell lines of mouse macrophages, as compared to that of the free ddI. The elevated level of intracellular uptake of ddI was concluded as due to the facilitated transport of the anionic natured liposomes.

To overcome limitations like impermeability of phosphorylated form to cell membrane\textsuperscript{46} and achieve targeted delivery, the anti-retroviral effects of ddI, ddI triphosphate (ddi-tp) and liposome encapsulated ddi-tp were compared in cultured HIV-1 infected human monocytes macrophages.\textsuperscript{47} Newly synthesized pro-drugs has also been explored through liposomal encapsulation technique.\textsuperscript{48} The liposomes surface attached with ligands which could specifically improve the receptor interactions were also identified for the active
targeting of HIV infected cells.\textsuperscript{49} Mannoxylated liposomes show high drug content and maximum cellular uptake.\textsuperscript{50} Together, these studies\textsuperscript{51,52} confirmed superior tissue targeting effect in galactose specific receptors and proved their ability to offer sustained drug release phenomenon. Hence, various ligands have been reported for the active targeting of antiretroviral loaded liposomes, for their potential properties towards formulations development.\textsuperscript{53} One of the disadvantages of liposomes is the reduced stability, especially for the encapsulation and retention of drugs, and this was chiefly influenced by the lipid composition in it.\textsuperscript{54} In another study, authors developed Acyclovir as non-liposomes and mono-niosomes obtained vesicles in the size range of <100 nm-1µm. It releases for longer period of time with longer residence time in systemic circulation which could help them reach targeted tissues. Saquinavir and nevirapine stealth liposomes developed by Lakshmi \textit{et al.} showed enhanced efficiency with low toxicity.\textsuperscript{55,56}

\textbf{1.4.3.2. Ethosomes}

Ethosomes was reported by Touitou \textit{et al.} as one of the successful vesicular carriers for the delivery of anti HIV drugs.\textsuperscript{57} They were developed using phospholipids in combination with relatively higher level of alcohol like ethanol / isopropyl alcohol along with water. These systems could be designed with size of vesicles ranging from nano to micron dimensions, hence suitable for many route of application including transdermal delivery. Ethosomes were distinctly identified to exhibit higher transdermal permeation and flux through the stratum corneum layer of skin, that possessed significant activity than the classical liposomes.\textsuperscript{58,59}
1.4.4. Emulsions and dispersions

1.4.4.1. Micelles and Microemulsions

The anti-retroviral molecules with decreased bioavailability and increased enterohepatic metabolism were successfully bypassed from the portal blood to the HIV rich intestinal lymph circulation through the novel microemulsion type of formulation approach, which could ultimately result in its enhanced bioavailability. Using oleic acid, three different microemulsions were formulated and studied in rat models for targeted intestinal lymphatic transport mechanism. The microemulsions resulted in greater mesenteric lymph levels compared to the micellar formulation of cremophore-oleic acid mixed micelles, D-alpha tocopheryl-PEG6000- succinate oleic acid mixed micelles.

1.4.4.2. Suspensions

Nanosuspensions intended for pharmaceutical application contains finely dispersed solid particles in an aqueous base vehicle for oral or topical use. Administration through parenteral or pulmonary route is also recommended, for which the product should be sterile or non-toxic and developed using biodegradable or non-biodegradable polymers. The suspension formulated with lipid related complexes for subcutaneous administration have been reported with enhanced localization in lymphoid tissue and also reduces viral load, in the HIV-2287 diseased macrophages. The concentration in peripheral region and the visceral lymph nodes was in the range of 2250-2270%, that was higher compared to placebo as 35% lipid free drug given in individuals. This lipid complex of drug reduced the viral load and increased the CD4 T-cell count.
1.4.5. Drug delivery through skin

1.4.5.1. Transdermal

Delivery of drugs through skin via transdermal absorption has gained interest of many researchers especially due to its significant merits such as an alternative for invasive injections, by-passing biotransformation in hepatic and gut wall, GI degradation, decreased side effects by reducing fluctuation in plasma, excellent targeting of drug for improved patient compliance\textsuperscript{62,63}. The most challenging issue and drawback with transdermal route of absorption is the uncertain and low cutaneous transport for the uptake of molecules. Majority of the studies involved in the enhancement of penetration of drugs with help of salt formation, solvent and co-solvent addition, iontophoresis or anodal current application, by which litho simple or combination helps to enhance the permeation of ARV drugs. The transdermal gels and patches have been developed for AZT\textsuperscript{64, 65} in addition with polymeric ingredients like gum matrix.\textsuperscript{66, 67} They both exhibited better permeation of ARV, moreover the gel was more stable compared to the drug solution. The foremost transdermal delivery with vesicular system of AZT was Aquasomes, in which the vesicles were designed with mixture of ascorbyl palmitate (ASP), cholesterol along with addition of a negatively charged lipid, dicetyl phosphate. It enhances the drug absorption through transdermal than AZT solution form, and it shows higher skin permeation than solution, because the ascorbyl palmitate could augment the permeability effect through skin. The transdermal permeability flux and site specific targeting of 3-AZT was found to be better with an elastic liposomal formulation which also provided sustained release of drug. Oleic acid was utilized as a potential penetration
enhancer to study the penetration of various drugs such as ddC, 3TC and several N-acyl lamivudine esters, because of its nature to disrupt the interconnected lipid membrane. Different *in-vivo* and *ex-vivo* studies conducted on ARV drugs like ddI, ddC and AZT using animal’s skin like rat, mouse, pig and human cadaver have proved the efficacy of these ARV drugs via the transdermal route.

**1.4.5.2. Buccal delivery**

Buccal delivery of drugs can bypass the enterohepatic circulation and neglect gastrointestinal degradation, which struck many researchers to choose it over the traditional conventional routes for providing superior bioavailability of drugs. It shows greater transmembrane penetration compared to skin drug administration and also provides several advantages over other mucosal routes of delivery like nasal, rectal and vaginal mucosa, which includes its larger surface area, easy accessibility for application, enormous capillary blood supply. The ARV drugs are highly benefited through buccal mucosal drug delivery as preferable choice. Shojaei *et. al* used ddC as model drug and investigated by using the safe and effective permeation enhancer method through buccal route. In this study 1-menthol shows increase in permeation of ddC with enhancement factor of 2.02 and *t*\(_{1/2}\) of 6 h. It was proved that its not concentration dependant, by varying the concentration as 0.1, 0.2 and 0.3 mg/ml of 1-menthol. In other study it was found that the basal lamina present within the buccal mucosal epithelial layer work as the essential membrane wall for the penetration of ddC. As well, they concluded the SGC (sodium glycodeoxy cholate) enhancing anti-retroviral drug therapy.
1.4.5.3. Rectal delivery

Rectal administration of drugs has been recognized as successful eternal route for such drugs, exhibiting high enterohepatic metabolic reaction and gastro intestinal decomposition. Sustained release AZT HPC suppositories were assessed in rats.\textsuperscript{71} Suppositories of AZT in the dose range of 10 mg/kg maintain constant plasma concentration above 1mg/kg for more than 6 h. Certain research works have also reported highlighted results with AZT suppository delivery systems. Also studies revealed that the absorption data and other pharmacokinetic parameters similar to sustained release device could be achieved by rectal administration of AZT.\textsuperscript{72}

1.4.6. Vaginal drug delivery

1.4.6.1. Vaginal Creams and Gels

Even though a large number of semisolid formulations (ointments, creams, gels) are commercially available for the topical intra-vaginal drug delivery of microbicides, they are not patient reliable in most cases due to its unavoidable demerits such as greasy nature, leakage, inaccurate dose and poor spreading and circulation.\textsuperscript{73} The recent research has focused remarkably on the improvement of controlled drug delivery through novel hydrogel systems.\textsuperscript{74-79} The 93% alginate gel of nanoxynol-G has been productively investigated for intra-vaginal spermicidal activity. Modification in the pH and osmolarity of the product showed a considerable difference in the diffusion and spermicidal activity of the drug.\textsuperscript{78} An innovative microemulsion based gel formulation containing phenyl phosphate derivative of zidovudine was produced with superior and sustained anti-HIV effects.\textsuperscript{80}
1.4.6.2. Vaginal Tablets and Suppositories

The large number of intra-vaginal delivery systems is also available in the variety of solid dosage forms like tablets, pessaries and suppositories. These formulations with programmed or timed release mechanism are also been used as an alternative to the conventional vaginal tablets.81,82

1.4.6.3. Vaginal rings

A circular ring type delivery device containing two layers has been developed to insert into the vaginal cavity which release the drugs in controlled rate.83-85 There are systems fabricated with a third layer (drug free - rate controlling elastomer membrane) which plays excellent role in minimizing the drug load and release.86 The fabrication of such device is merited with the usage and position control by the patient in a convenient manner to avoid interference with coitus and also providing a continuous delivery of the drugs.

1.4.6.4. Bioadhesive intra-vaginal systems

To overcome the demerits of the conventional intra-vaginal dosage forms such as poor retention, improper dose administration and leakage of the formulations, the new-fangled bioadhesive drug delivery systems are being launched in the market.87 The bio-adhesive polymers that have been used for intra-vaginal formulations includes polycarbophil, hydroxypropyl cellulose and polyacrylic acids.88 The first formulation worked on this principle was bio-adhesive tablets of Bleomycin for the treatment of cancer.89-92 There are systems used for delivering microbicides using mucoadhesive microparticulate
vaginal systems. This shall be multi-phase liquid or semisolid containing systems that have been designed to avoid slipping from the vaginal cavity.  

Figure 1.2. Different novel drug delivery systems used for the treatment of AIDS

1.4.7. Nanodrug delivery systems

1.4.7.1. Nano-containers

The concept of ARV targeting using the carriers like dendrimers based systems has also been explored well. Dendrimers are macromolecules synthetically designed as spherical and highly branched structures. These macromolecules have come out into the sight as thrived tool among the existing drug carriers for targeted delivery, due to their uniqueness in structural design. Hence, predictably they have been identified for targeting of anti-retroviral drugs. The poly (propylene imine) dendrimer based nanocontainers was used for targeting Efavirenz (EFV) to Mo/Mac. These molecules are referred as
nanocontainers since they behave like closed nanosize vessel with entrapped drug inside. Moreover, the mannosylated PPI dendrimers have been declared as a valuable carrier system for site specific delivery of anti-retroviral drug like EFV.

1.4.7.2. Nanopowder

Nanopowders have been utilized efficiently through peroral route of drug delivery for the augmentation of solubility and drug release rate of many hydrophobic drugs.\textsuperscript{101} When Loviridine nanopowder morphology was analyzed, plate resembling features were observed whereas the unmodified (free drug) substance showed crystal structures.

1.4.7.3. Nanoparticles

Nanoparticles can exist as either solid colloidal particles or suspended in liquid media, the particles being in the size range of 1-100 nm.\textsuperscript{102} Depending on the polymer type and ratio in the formulation designed, the size of these particles can be varied and effectively launched for site specific and sustained release of drugs.\textsuperscript{103} This concept works better with molecules showing poor physicochemical strategies like insolubility and instability.\textsuperscript{104} In all aspects, nanoparticles investigation is promptly suited for ARV drugs especially to target infected cells and prolong the duration of action. The success of such delivery system loaded with ARV drugs have been proved with improved encapsulation, greater efficacy, high targeting to macrophages, decreased drug resistance, retarded systemic toxicity, lower dose and side effects, finally betterment in patient compliance. In former studies, AZT had been loaded with polymers like poly alkyl cyanoacrylate,\textsuperscript{105} poly methyl methacrylate and human serum albumin. When these nanoparticles were treated with macrophages secluded from HIV infected people, their
uptake was superior to pure drug. In the same way, when Saquinavir and DDC nanoparticles were formulated using poly (hexacyanoacrylate) through emulsion polymerization technique, a drastic improvement in efficiency was seen for the nanoparticles than the pure drug suspension. An *in-vivo* study was conducted by Loberberg, Ananjo and Kruter, using rat models to explore the performance of oral delivery of AZT bound hexacyanoacrylate nanoparticles for targeting the reticulo endothelial cells. In a latest *in-vitro* study, the uptake of AZT nanoparticles by pronuclear leukocytes was demonstrated, to show the effect of the nanoparticles prepared with poly (lactic acid) poly (ethylene glycol) polymer was found to be reliant based on PEG ratio.

The targeting of ARV drugs into CNS is also a major issue, because of the possible mechanism of migration, multiplication and localization of HIV in brain resulting in unpredicted neurological symptoms. The tightly packed lipid astrocytes layer of blood brain barrier (BBB) and existence of reflux transporters on the surface of cells, averts the direct entry of ARV drugs to the brain. Whereas the nano systems get easy access to the brain through the mechanism of endocytosis, which can also move away from the locality of efflux pumps.

The polymeric systems identified for enhanced permeability of various drugs are all being reported with smaller particle size. Ligand based nanoparticles have also emerged out for receptor mediated targeting approach of ARV drugs. Certain approaches were also utilized for targeting other sites such as GI mucosa and its interconnected lymph tissues. Apart from targeting approach, the ARV nanoparticles were
paid attention for formulation modification to improve the drug loading and reduce the systemic toxicity and also raise its absorption rate, as like facilitated pH sensitive drug release.\textsuperscript{115,116}

1.5. Choice of Drug - Efavirenz

Efvaverinz comes under the non nucleoside reverse transcriptase inhibitor (NNRIT)\textsuperscript{117,118} and used in HIV treatment type I, combined with other antiretroviral drug like protease inhibitor and nucleoside reverse transcriptase inhibitor, approved by the FDA in the year of 1998 and used as first line drug in HIV treatment. Efavirenz is practically insoluble in water (< 10 µg/ml) with molecular weight of 315.675 g/mol and chemically known as (4S)-6- chloro-4-(2-cyclopropylethynyl) -4-(trifluoromethyl) -2,4-dihydro- 1H-3,1-benzoxazin-2-one\textsuperscript{119,120} (Figure 1.3). Due to its poor solubility in water and chemical stability the drug is almost administered as solid dosage forms like tablets and capsules, which are well accepted for oral administration, but undergoes multiple stages of metabolism which leads to less availability of drug at the site of action, essentially termed as less bioavailability ( < 45%). Efavirenz has high potency that inhibits the reverse transcriptase enzyme present in HIV-1 but of ineffective on HIV-2 and it shows a half life of 45-55 h with variable bioavailability\textsuperscript{121-123}. Recent researches trust Efavirenz, as a promising candidate for HAART in the treatment of HIV infection due, to its higher level of efficiency and low dosage requirement. Physical observation confirms it to be a yellowish/ white crystalline powder and the solubility tests reveal its insoluble nature in water but, soluble in organic solvents like methanol and dichloromethane. It belongs to Biopharmaceutical classification system (BCS) class-II category which ushers a high
lipophilicity (log P=5.4), limited oral bioavailability (40–50%), and high intersubject variability. Debilitation occurs at low level of therapeutic dosage and an adverse effect is noted at higher dose level\textsuperscript{124,125}. Efavirenz is commercially available as 600 mg tablet, 50 mg or 200 mg capsules (Sustiva) and oral suspension. Administration of this drug causes potential side effects and hence its intake is seized by 4% of patients\textsuperscript{126}, but it is prescribed reiteratedly as the first line drug in antiretroviral therapy (ART). This drug is also prescribed in combination with nucleoside reverse transcriptase inhibitor (NRTI) and protease inhibitors (PI) as a highly active antiretroviral therapy. It shows an effective reduction (>80%) of viral load in HIV patients and hence put forward as a better treatment option. It has been postulated that the Efavirenz functions by inducing cytochrome P450 enzyme but its relative dosage level is still not clear. It is expected that solubility enhancement can pave the way for an improvised bioavailability because, dissolution step is considered to be the rate determining step (RDS) in its absorption. In the similar lines we proposed to propel our work using Polymeric nanoparticles\textsuperscript{127}.

![Structure of Efavirenz](image)
To increase the bioavailability, various techniques such as micronization, solid dispersion, novel drug delivery systems like vesicular systems, solid lipid nanoparticles, micellar and micro emulsion systems, transdermal delivery, buccal and rectal delivery, nanopowders, nanosuspensions and nanoparticles are being employed\textsuperscript{128-131}. Nanotechnology has been used more widely in recent times, in the treatment of many life threatening diseases through modified and targeted drug delivery systems which helps in overcoming limitations of many conventional dosage forms like short half life, instability (physical / chemical / conformational), poor solubility, etc of many conventional dosage forms\textsuperscript{132,133}. It also helps to enhance the therapeutic activity of the drugs\textsuperscript{134}. Polymer encapsulated nanoparticle administered through vaginal route enhances the permeation of drug particles in the vaginal mucosa which allows the drug for easy targeting of the HIV infected cells\textsuperscript{135}.

As Efavirenz is poorly soluble in water and possess low bioavailability (<45%), the dose required for the therapeutic activity is high and hence recommended as 800 mg once daily. Many literatures support the methods for improvement of its bioavailability, which reduces the unnecessary dose dumping and consecutive complications\textsuperscript{136-140}. Efavirenz has been found to have maximum stability at pH 4 (vaginal pH 4-5) and also have optimal stability in the vaginal environment if it is developed as microbicide. Also it could be useful for achieving the long term pre-exposure prophylaxis of HIV-1\textsuperscript{141}.

1.6. Bioavailability

The survey by Repusaki and Redaki revealed that around 95% of the drugs developed and available in the market are in poorly soluble category. Dissolution is the rate limiting
step in the bioavailability of these poorly soluble compounds and the drug absorption, its therapeutic activity is dependent upon the dissolution rate\textsuperscript{142, 143}.

Based on the dissolution and its absorptive permeability Amidon \textit{et al.}, classified drugs into four classes (Class I, II, III and IV). The drugs possessing high solubility and high permeability characteristics placed under class I, the class II drugs of low dissolution and high permeability for which the rate limiting step is dissolution, class III drugs defined as drugs with high solubility and low permeability where the rate limiting step is permeability, the last category class IV drugs in the nature of low solubility and low permeability. The class II and class IV drugs are particularly facing the problem of poor dissolution needing the carrier or system for effective drug delivery\textsuperscript{144}.

On increasing the pore volume of matrix system of hydrophilic materials and incorporation of superdisintegrants in the dosage form development are the common techniques applied for enhancement of the dissolution or solubility of particles insoluble or slightly water soluble drugs in pharmaceutical industry\textsuperscript{145, 146}.

Many studies have revealed that focused on different techniques imposing to enhance the dissolution of Efaverinz nanoparticles by Destche \textit{et al}\textsuperscript{147}, using PLGA with combination of other anti viral drugs. The other study by Yang \textit{et al}\textsuperscript{148}, converted as amorphous dispersion with Efaverinz and PVP as solubility enhancing polymer. The solid solution was prepared by spray drying technique and the material used as spraying solution, the solid dispersion by different techniques like solvent evaporation and physical mixture developed. PEG used as a hydrophilic carrier for Efaverinz to enhance the dissolution. Enhancement of solubility and bioavailability of Efavirenz was achieved through various
techniques such as solid dispersion, microparticles, nanoparticles, dendrimers, etc. using different polymers, as shown in the table.

Table 1.2. List of polymers used to enhance the solubility and bioavailability of Efavirenz by different techniques

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Technique</th>
<th>Polymers / technique used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Solid dispersion</td>
<td>PVP – β-cyclodextrin\textsuperscript{149,150}, PEG 6000\textsuperscript{151,152}, modified starch (starch citrate and phosphate)\textsuperscript{153}, Hydroxy propyl β-cyclodextrin – PVP K30 – SLS\textsuperscript{154}, Cyclodextrin inclusion complex\textsuperscript{155}, PEGylation\textsuperscript{156}, β- cyclodextrin, maltodextrin, Plasdone\textsuperscript{32} \textsuperscript{157}, β-cyclodextrin – Hydroxy propyl β-cyclodextrin, Carrageenan\textsuperscript{158}, Eudragit E100\textsuperscript{159}, HPMC – PVP, PVP – Plosdone, PVP – HPMC</td>
</tr>
<tr>
<td>2.</td>
<td>Nanoparticles</td>
<td>PLGA\textsuperscript{160}, PCL\textsuperscript{161}, Poly ethylene oxide Poly propylene oxide copolymer, Eudragit RS 100 – PCL\textsuperscript{162}, PLGA nanoparticles in vaginal gel\textsuperscript{163,164}, PCL spray dried\textsuperscript{165,166}, drug nanoparticles (non-polymeric) by wet milling, homogenization, sonication</td>
</tr>
<tr>
<td>3.</td>
<td>Dendrimers</td>
<td>Tufstn dendrimers\textsuperscript{167,168}, citric acid dendrimeric archives\textsuperscript{169}</td>
</tr>
</tbody>
</table>
1.7. Vaginal bioavailability

The administration of drugs through vagina for effective pharmacological activity is in active form, from 1920 when insulin was administered into dog vaginal cavity for its therapeutic activity assessment\textsuperscript{170}. The drug administration through human vaginal cavity is known for several decades and improved therapeutic activity was achieved by vaginal mucosal absorption of suppositories and pessaries\textsuperscript{171}. Several other agents were reported for vaginal administration like steroids, peptides and drugs for local action (antifungal, antibacterial, spermicidal etc..) and systemic action (hormones, contraceptives, etc.)\textsuperscript{172}.

The vaginal route of administration of drugs explores advantages over other route of administration, in the section of bioavailability and controlled drug release. Improvement in bioavailability was achieved in many cases such as prostaglandins, steroids, sulphonamides etc. The other relative advantages for this improved bioavailability are its larger surface area, avoidance of first pass metabolism, high peak plasma concentration achieved through vena cava absorption, metabolizing agents of vaginal cavity differs from the gut that influence the stability of drug at the site of administration which ultimately helps for better absorption\textsuperscript{173}.

The bioavailability of drug is also influenced by various physicochemical properties like pH and pKa of the drug, lipid solubility, particle size and partition coefficient. The drugs with smaller particle size and higher partition coefficient greater than 5 and also existing in the unionized form in the vaginal cavity showed improved bioavailability\textsuperscript{174}. The type of dosage form and its composition also play significant role in increasing the
bioavailability through vaginal cavity. The excipients such as surfactants (tween, polozamer) and solubilising agents (Beta CD, citric acid, etc.) enhances the solubility which ultimately results in improved bioavailability. The highly retained formulations on the vaginal mucosa such as mucoadhesive gels and foams are forced to increase the bioavailability than the other formulations. The permeability of drugs through vaginal cavity is high compared to the other mucosal route of drug delivery like rectum, buccal and transdermal. Also the permeability of drugs can be influenced by the penetration enhancer which disturb the epithelial tight junctions and allows the drug to penetrate through intra cellular pathway\textsuperscript{175}.

1.8. Carriers

The solubility and bioavailability generally increased using various carriers for poorly soluble drugs. The carriers can be of natural sources (HPMC, HPC, Chitosan etc.) and synthetic (PVP, mannitol, urea, PEG, Polymethacrylates etc..). The Eudragit is a copolymer prepared by polymerization process with combination of different ratios of monomers acrylic and methacrylic acids and their esters. The physical and chemical properties are varied based on the functional groups. Its ease of availability in various forms such as aqueous dispersion, granules, powders and organic solutions make it as a highly usable polymer\textsuperscript{176}. This polymethyl acrylate polymer is well established in pharmaceutical industry with the trade name of Eudragit marketed by Rohm Gmg H & Co., Germany since 1950.

Eudragit E100, a cationic polymer is composed of dimethyl amine ethyl methacrylate, butyl methacrylate and methyl methacrylate with average molecular weight of 150000D
approximately, available in the form of granules with colourless to yellow colour nature produce amine like odour. It is soluble in wide range of solvents and acidic aqueous environment (pH < 5) and it acts as swellable polymer above pH 5\textsuperscript{177}. The solubility takes place through protonation of amine groups which leads to repulsion action in backbone of polymer chain, and proton diffusion and counter ion action disassociates the secondary interactions. Also the columbic force is involved in complexation of positively charged Eudragit E100 reacts with negatively charged alcohols\textsuperscript{178}.

Eudragit polymer is generally used for various applications such as masking the taste or odour, protection of drug from external environment, modified release system, solubility and bioavailability enhancement etc., Using the Eudragit polymers, solubility and bioavailability of poorly soluble drugs could be enhanced through various techniques like solid dispersion (Itraconazole, Ibuprofen, Diclofenac, Flurbrofen, Indomethacin, Ketoprofen, Neprotan, Chlordiazepoxide, Chlorpheneramine maleate, Efaverinz etc.), polymeric microparticles, nanoparticles (Genitein, Beclomethasone, Cyclosporin, Meloxicam, Quercetin, etc.), complexation, etc\textsuperscript{176-177, 179-180}.

1.9. Conclusion

The Human Immunodeficiency Virus (HIV) is a pandemic disease spreading very rapidly all over the world, causing approximately 15,000 or more new infections every day and the community acquiring sexually transmitted infections (STIs) is prone to easily acquire this HIV infections. Various techniques have been innovated to deliver the drugs to targeted site to advance the efficiency of anti-HIV drugs. Novel drug delivery system gives an opportunity to surmount many challenges associated with anti-retroviral drug
therapy. It helps in addressing the formulation difficulties such as poor solubility, stability and entrapment. Studies on the various systems for alternative routes of ARV drug administration includes intravaginal, transdermal, buccal, rectal and through lymph, along with nano particulate systems such as liposomes, ethosomes, nanoparticles, emulsomes, micelles etc., This chapter highlights the potential of novel drug delivery systems in preventing the transmission and treatment of HIV/AIDS and its role in improving the bioavailability of poorly soluble drugs.

1.10. Objective and Scope of Work

The goal of the present study is to develop polymeric nanoparticle formulation of Efaverinz to enhance its dissolution, and carry out in-vivo biodistribution studies to select the suitable site of application for its effective delivery and better therapeutic activity.

To achieve the above objective the following specific aims has been identified.

1.11. Specific Aims

**Specific Aim-I:** Formulation and Characterization of Nanoparticles

Hypothesis: We believe that loading of the drug in nanocarriers will improve the solubility and enhance the bioavailability.

**Specific Aim-II:** Cell uptake, *in-vitro* Cytotoxicity and Anti-HIV activity of Efavirenz loaded nanoparticles.
Hypothesis: We hope that the drug encapsulation in the polymeric carrier will reduce the toxicity, improve cell specific uptake and predominantly reduce the viral load that would help to overcome the undesirable side effects.

**Specific Aim-III: In-vivo Bio-distribution study of Nanoparticles**

Hypothesis: We believe that the drug encapsulated into the polymeric carriers will improve the bio-distribution pattern and other activity *in-vivo* conditions.

**Specific Aim-IV: Selection of Vaginal route of administration and development of Aerosol Foam formulation containing Nanoparticles and its evaluation.**

Hypothesis: We believe that converting the polymeric nanoparticles into Aerosol foam formulation improve the site specific drug delivery for intra vaginal application and its effective drug release and absorption.

### 1.12 Rationale for choice of the carriers and solubility enhancing strategy

Nanoparticle: The polymeric nanoparticle preparation for encapsulating the poorly soluble drugs is convenient and is also well established technology for size reduction.

Eudragit E-100 (Methyl methacrylate co-polymer) is used as a carrier material for enhancing the solubility of poorly soluble anti-retroviral drug.

Aerosol foam: The foam formulations for vaginal route of administration were prepared by compression filling method and it has been used for local and systemic activity as modified drug delivery.
1.13. References


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