CHAPTER-1

INTRODUCTION
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1.1 Introduction to infection and disease

Disease and death have always attracted the attention of the human mind. Varo and Columella in the first century BC postulated that diseases were caused by invisible beings, inhaled or ingested. As microbes are invisible to the unaided eye, definitive knowledge about them had awaited the development of microscope. The credit for having first observed and described bacteria goes to Anton van Leeuwenhoek. Infectious diseases can be caused by bacteria, viruses, fungi, protozoa or parasitic worms.

There are large numbers of anti infective drugs that are currently available in the market those are against

1. Viral infectious diseases- AIDS, Chickenpox, common cold etc.

2. Bacterial infectious diseases- Tuberculosis, Anthrax, Cholera, Typhoid.

3. Parasitic infectious diseases- Amoebiasis, Trypansomiasis etc.

4. Fungal infectious diseases – Blastomycosis, Candidiasis etc.


Bacterial infections are treated with antibiotics. There are many antibiotics available, but they fall into three major groups based on their mode of action: inhibitors of bacterial nucleic acid synthesis; inhibitors of cell wall synthesis; and inhibitors of bacterial protein synthesis.
Viral infections are normally overcome by the patient’s immune system. However, the advent of HIV infections and AIDS has led to the development of several new antiviral drugs.

In case of chronic infections long term drug therapy is required. In drugs having the shorter biological half life, it is required to take the drug more number of times per day. Long term exposure of antiviral drugs to the microorganisms and to the body tissue leads to the development of drug resistance, toxicity and some other adverse reactions.

Controlled release of the anti infective drug will reduce the exposure of higher concentrations of the drug to the microorganisms. This will reduce the bacterial resistance, tissue toxicity and other adverse reactions.

1.2 Introduction to controlled drug delivery systems

Ideally, a drug should arrive rapidly at the site of action (receptor) in the optimum concentration, remain for the desired time, be excluded from other sites, and be rapidly removed from the site when indicated. Generally, the time course of a dosage form (Pharmacokinetic) in man considered to be controlled by the chemical structure of the drug. Decreasing the rate of absorption and/or changing the dosage form provide a useful adjunct. When it is not feasible or desirable to modify the drug compound at molecular level, often sought is a product that will require less frequent administration to obtain the required biologic activity time profile; for example, a tablet that has the same clinical effect when administered every twelve
hours. In another instance it may be desirable to decrease the absorption rate in order to obtain a more acceptable clinical response. The goal in designing sustained or controlled delivery systems is to reduce the frequency of dosing or to increase the effectiveness of the drug by localization at the site of action, reducing the dose required, or providing uniform drug delivery.

Oral route is the most popular route of drug administration and is universally acceptable route. Indeed, for sustained release systems, oral route of administration has received most of the attention with respect to research, physiological and drug constraints as well as to design and testing products. This is because of the fact that there is more feasibility in dosage form design for oral route than for parenteral or any other route.

New drug entities have increased with concomitant recognition of the therapeutic advantages of sustained release drug delivery. Greater attention has been focused on development of sustained released drug delivery systems. ‘Sustained release’ constitutes any dosage form that provides medication over an extended period of time. Sustained release drug products are designed for different routes of administration based on physicochemical, pharmacological and pharmacokinetic properties of the drug and upon the properties of the materials used in the dosage form.
1.2.1 Classification of oral sustained release drug delivery systems:

**Based on techniques used in formulation**

Matrix tablets, Microencapsulation, Enteric coated beads or spheres in capsules, Ion exchange resin preparations and Osmotic pumps.

**Based on release mechanism**

Dissolution controlled release systems, Diffusion controlled release systems, Diffusion and dissolution controlled release systems, Ion exchange resins, pH - independent formulations and Osmotically controlled release systems.

1.3 Human immunodeficiency virus (HIV) and Acquired Immunodeficiency syndrome (AIDS)

HIV defined as the human immunodeficiency virus type 1 (HIV-1), the well known human pathogen and the main causative agent of AIDS infecting more than 40 million people. Till today there is no vaccine or permanent cure for HIV and AIDS. Anti retroviral drugs show effective treatment for the disease. The drug resistance strains of virus show greater impact and more treatment options. This further showed more impact on the identification and development of new drugs with improved safety and efficacy. The basic biological property of HIV-1 is rapid evolvement and greater genetic diversity. There are two factors that are responsible for HIV-1 to generate this genetic variability. (1) The error-prone nature of the HIV-1 polymerase and the rapid replication of HIV-1. The HIV recombination also increases the
drug resistance strains during reverse transcription\textsuperscript{10,11}. The quasi-species in viral population have more number of drug resistant strains\textsuperscript{12}. Suboptimal concentration of the antiretroviral drugs leads to the drug resistant strains. In vitro study of the suboptimal drug concentrations tool to study the drug resistance strains and this data is useful to study the strains developed in in-vivo \textsuperscript{13, 14}.

The most effective treatment for the HIV is highly active antiretroviral therapy (HAART) in which three or four drugs were combined and administered to the HIV patient\textsuperscript{13, 14}. In HAART, combination of reverse transcriptase and protease inhibitors were used for the effective HIV therapy and it takes years long for the suppression of viral load \textsuperscript{15, 16}. This can lead to the development of the resistant viral strains. The basic idea of the drug resistance strains is useful for the development of new drugs and new formulations.

The steps involved in the replication of HIV -1 virus is (1) Entry (2) Integration (3) Mutation. These three steps are the main focus for the scientists for the development of the new drugs. The knowledge on the development of the HIV cycle is valuable in conforming the drugs safety, efficacy, identifying the new drug targets and predicting the resistance in the patients \textsuperscript{17}. Figure 1.1 shows the HIV life cycle in the cell. The following table shows the list of different antiretroviral drugs that are currently used in the treatment of HIV infection.
<table>
<thead>
<tr>
<th>Name of the drug</th>
<th>Classification</th>
<th>Elimination Half life (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>NRTI</td>
<td>1.1</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>NRTI</td>
<td>3–6</td>
</tr>
<tr>
<td>Didanosine</td>
<td>NRTI</td>
<td>1.3–1.6</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>NRTI</td>
<td>1–3</td>
</tr>
<tr>
<td>Stavudine</td>
<td>NRTI</td>
<td>1–1.6</td>
</tr>
<tr>
<td>Emtricitabine</td>
<td>NRTI</td>
<td>10</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>NtRTI</td>
<td>17</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>NNRTI</td>
<td>25–30</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>NNRTI</td>
<td>40–50</td>
</tr>
<tr>
<td>Delavirdine</td>
<td>NNRTI</td>
<td>5.8</td>
</tr>
<tr>
<td>Etravirine</td>
<td>NNRTI</td>
<td>30-40</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>PI</td>
<td>7-10</td>
</tr>
<tr>
<td>Indinivir</td>
<td>PI</td>
<td>1.2-2</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>PI</td>
<td>1.5-2</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>PI</td>
<td>3.5-5</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>PI</td>
<td>3-5</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>PI</td>
<td>7</td>
</tr>
<tr>
<td>Darunavir</td>
<td>PI</td>
<td>15</td>
</tr>
<tr>
<td>Enfuvirtide</td>
<td>FI</td>
<td>3.8</td>
</tr>
<tr>
<td>Maraviroc</td>
<td>FI</td>
<td>14-18</td>
</tr>
<tr>
<td>Raltegravir</td>
<td>II</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1.1 List of antiretroviral drugs currently in the market

NRTI, Nucleoside reverse transcriptase inhibitors
NtRTI, Nucleotide reverse transcriptase inhibitors
NNRTI, Non-nucleoside reverse transcriptase inhibitors
PI, Protease inhibitors
FI, Fusion inhibitors
II, Integrase inhibitors
Fig. 1.1 HIV life cycle inside the cell after infection (Photograph from internet source (http://img.thebody.com/nmai/cycle.jpg)}
1.3.1 Drawbacks of conventional antiretroviral drugs

At present there are so many antiretroviral drugs that are commercially available in the market as solid oral dosage forms such as tablets and capsules, liquid oral dosage forms such as solutions and suspensions. The oral dosage forms have several advantages like convenience, oral delivery of drugs have some disadvantages also such as first pass effect, absorption variation and enzymatic degradation of the drug in the GI tract. For example, the first antiretroviral drug approved for HIV treatment such as zidovudine shows rapid elimination half life of 1 hour and hepatic first pass metabolism and looses 40 % of the administered drug.

In the conventional dosage forms the duration of the drug’s pharmacological action is very short and limited because the Mean residence time of the drug depends on the elimination half life and there by the absorption of the drug. And also, many of the antiretroviral drugs show poor or low bioavailability due to various physicochemical factors such as dissolution, solubility and permeability (didanosine).

The drug’s performance in in-vivo manly depends on its physicochemical property such as drug stability and solubility. Research scientists today face so many formulation problems because of drug stability and GI tract liability. This can lead to the poor bioavailability and absorption. This can be overcome by the various studies on the drug physicochemical properties during preformulation
study. For example, the bioavailability is rate limiting in Non NRTI due to their low water solubility. Thus the variation in oral bioavailability of many antiretroviral drugs may be a significant factor for failure of some drug regimens. Even though the drug is absorbed from the GI tract and enter in to the blood circulation, metabolism, elimination and transport of the drug will affect the drug to reach the target tissue/site. In order to make the successive therapy in AIDS, it is required to maintain the drug at constant and optimum concentration in the blood and also to the target tissue through out the treatment.

Most of the antiretroviral drugs have shorter biological half life. However, because of their short biological half life these drugs needed to administer frequently. Hence with do not maintain the drug concentrations constantly for longer period of time. Due to the HIV’s virustatic nature these drugs should be administered for the life of the patient. All most all antiretroviral drugs exhibit toxic effects such as hyperglycaemia, hepatotoxicity, hyperlipidemia, lactic acidosis, lipodystrophy, osteonecrosis, osteoporosis, osteopenia, skin rashes, due to higher blood concentration of the drugs. In such conditions dose reduction and some times cessation of treatment because lactic acidosis may even be sometimes fatal. So the benefit and risk from the treatment is same with the use of these antiretroviral drugs but the treatment should be continued to increase the survival rate of the HIV infected person. And also with the continuation of the therapy resulted in frequent administration and there by increased the Pill
burden. These problems can be overcome by design of novel drug delivery systems\textsuperscript{20}.

**1.3.2 Need for novel and controlled drug delivery of anti retrovirals**

To succeed in the HIV therapy for long term treatment with the anti HIV drugs, where the patients suffer from the problems associated with the plasma fluctuations, dose frequency; it is required to have an effective dosage form in the form of sustained and controlled release formulations to improve the therapeutic benefit and ideal therapy. With the help of the controlled and sustained drug delivery, effective plasma concentration was achieved without any fluctuations. It is also possible to avoid toxic plasma concentrations where it is a problem with conventional formulations and also possible to achieve effective therapy with low dosage of the drug, and to avoid the frequency of the dose administration.

Percutaneous delivery of most antiretroviral drugs has been studied. The study indicates a challenging future of this route for antiretroviral drugs\textsuperscript{21-24}. Novel delivery systems such like liposomes, microparticles and encapsulated erythrocytes are also under investigation. Liposomal drug delivery is one of the best deliveries to achieve the target and site specific drug delivery of various molecules. Scientists focused on the liposomal drug delivery, and they studied various drugs via liposomal drug delivery, studies show that the liposomal drug delivery systems are showing less toxic effects than the conventional formulations of the same drugs. For example liposomes
of doxorubicin and amphotericin B were less toxic when compared with free drug. Haematopoietic toxicity study was conducted on the zidovudine loaded liposomes on mice. The study showed that the drug loaded liposomes are more active than the convention zidovudine formulations. Thus the zidovudine liposome showed significant reduction in toxicity, and also increased the antiviral activity and enhanced drug localization in the liver and spleen.

Further, it was clearly observed that liposomes of dideoxycytidine-5'-triphosphate (ddCTP) exhibited better chemical stability of the drug molecule. The results of the encapsulated liposomes with dideoxycytidine-5'-triphosphate in murine AIDS model indicate that ddCTP encapsulated- liposomes reduced proviral DNA in cells of the mononuclear phagocyte system (MPS) in both bone marrow and spleen. And also the liposome drug delivery increases the efficacy of the drugs, reduces the adverse and toxic effects and also increases the drug’s elimination half life $^{25-27}$. 

1.4 LITERATURE REVIEW ON DRUGS

1.4.1 Lamivudine

The literature review of various anti retroviral drugs showed that limited work was done on the anti retroviral drugs. Punna Rao et al \(^28\) prepared the controlled release matrix tablets of lamivudine using various viscosity grades of hydroxypropyl methylcellulose (HPMC) as the retardant polymer.

Himadri Sen et al \(^29\) studied the extended release formulations containing lamivudine, zidovudine and combination of lamivudine and zidovudine or their pharmaceutically acceptable derivatives. Amitava Ghosh et al \(^30\) studied lamivudine incorporated microspheres composed of ethyl cellulose as release controlling polymeric material.

Prathiba V \(^31\) studied lamivudine microspheres using Eudragit polymers.

1.4.2 Zidovudine

Punna Rao et al \(^32\) \(^33\) prepared the controlled release (CR) matrix tablets of zidovudine (AZT) using HPMC, ethyl cellulose EC and carbopol-971P CP.

Kuksal A\(^34\), prepared the matrix tablets of zidovudine using hydrophilic Eudragit RLPO and RSPO alone or their combination with hydrophobic ethyl cellulose. Zidovudine microcapsules prepared with PLGA \(^35\)-36.
Diana P 37 prepared the parental nanospheres of zidovudine. Rama Rao et al 38 39 prepared zidovudine microcapsules with ethyl cellulose.

Abu-Izza K 40 studied the in vivo bioavailability studies of zidovudine extended release formulations in beagle dogs. Jones, Harry P 41 invented discrete extended release formulation containing zidovudine.

1.4.3 Stavudine

F Feleke et al 42 prepared the matrix tablets of stavudine using HPMC. Robert et al 43 prepared the extended release bead-lets of stavudine. S K Sahoo et al 44 prepared the microcapsules of stavudine using combination of ethyl cellulose and PVP.

Suresh et al 45 studied PLGA microcapsules of stavudine. Recently US FDA approved the long acting stavudine capsule of stavudine with brand name of ZERIT XR46.

1.5 LITERATURE REVIEW ON POLYMERS

Many attempts were made using cellulose polymers. Nochida et al prepared the microcapsules with CAP and EC 47,48. Biju et al prepared the erodable microcapsules of diclofenac sodium 49. Lin et al attempted matrix tablets with CAP 50. Joseph et al studied the factors affecting the microcapsules of paracetamol with CAP 51.

Gheorghe et al attempted the cellulose acetate butyrate(CAB) microcapsules 52. Sayed et al studied the compressed tablets with CAB microcapsules 53. Shivakumar et al prepared the paracetmol
microcapsules with CAB\textsuperscript{54}. Jinghua \textit{et al} studied the film properties and permeability properties of CAB \textsuperscript{55, 56}. Yasunori \textit{et al} studied the mucoadhesive properties of CAB \textsuperscript{57}.

Zandi \textit{et al} attempted ethyl cellulose microcapsules and studied the physicochemical characteristics \textsuperscript{58}. George \textit{et al} prepared theophylline ethyl cellulose micro capsules \textsuperscript{59}. Amri \textit{et al} studied the effect of viscosity grades on the drug release from the ethyl cellulose microcapsules\textsuperscript{60}. Anjali \textit{et al} studied the feasibility of wet granulation process with ethyl cellulose indomethacin matrix tablets \textsuperscript{61}. Katikaneni \textit{et al} studied the ethyl cellulose matrix tablets with water soluble drugs. \textsuperscript{62}.

Hydroxypropyl methyl cellulose (HPMC) is universally accepted polymer that was presently used in the many formulations even in many anti retrovirals, as binder and release retardant. A vast literature was available on the HPMC polymer.

Bhaskar \textit{et al} studied the release behaviour of ambroxil from the HPMC matrix tablets\textsuperscript{63}. Cabella \textit{et al} stdied the feasibility of direct compression with HPMC\textsuperscript{64}. Halena \textit{et al} effect of HPMC and Hydrogenated castrol on the naproxen release \textsuperscript{65}. Thomas \textit{et al} studied the effect of tablet surface area and Surface area/Volume on the drug release from the HPMC matrix tablets\textsuperscript{66}.

Hydroxypropyl methyl cellulose phthalate (HPMCP) is widely used polymer in the controlled release formulation as well as enteric coating. Palmierie \textit{et al} studied the matrix tablets of HPMCP \textsuperscript{67}. Gergorio \textit{et al} prepared the microcapsules with HPMCP\textsuperscript{68}. 
Abu et al studied the microcapsules of indomethacin with HPMCP\textsuperscript{69}. Navneet et al used HPMCP in colon targeting\textsuperscript{70}. Torris et al studied the microcapsules of drug resin complex with HPMCP\textsuperscript{71}.

Crowley et al studied the stability of Poly ethylene oxide (PEO) in the matrix system\textsuperscript{72}. Robert et al prepared the matrix tablets with PEO by roller compaction technique\textsuperscript{73}. Joao et al studied the feasibility of PEO as extrudate forming material\textsuperscript{74}.

Eudragits are the most commonly used enteric coated and sustained release polymers. Many attempts were made with the use of eudragits.

Ndesendo et al prepared the microcapsules with use of Eudragits RS 100\textsuperscript{75}. Abdelkader et al prepared the matrix tablets of baclofenac with Eudragit L 100\textsuperscript{76}. Hua-Pin Huang et al studied the acrylic matrix system for diphenhydramine HCl with Eudragits\textsuperscript{77}.

Carbopols are also the most popularly used in the controlled release formulations. Lacramioara et al studied the matrix systems of carbopols with alendronate for controlled release\textsuperscript{78}. Sanz Taberner et al studied the gel strength of the carbopol\textsuperscript{79}.
1.6 LITERATURE REVIEW ON ANALYTICAL METHODS

Kano et al developed the HPLC method for the determination of lamivudine in human plasma 80. Namitha et al simultaneously determined the lamivudine and stavudine in antiretroviral fixed dose combination 81. Bin et al determined the zidovudine, lamivudine and nevirapine from human plasma 82. Emilla et al determined the zidovudine and lamivudine by RP-HPLC method 83. Ashanefi et al determined the zidovudine using RP-HPLC method 84.