# CONTROLLED RELEASE FORMULATIONS OF ANTIRETROVIRAL (ARV) DRUGS USING POLYMETHACRYLATE POLYMERS

**BY**

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**SEPTEMBER, 2010**

## Declaration

I hereby declare that the work reported in this dissertation entitled 'CONTROLLED RELEASE FORMULATIONS OF ANTIRETROVIRAL (ARV) DRUGS USING POLYMETHACRYLATE POLYMERS' has been performed by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University Zaria, Nigeria, under the supervision of Prof. Y.K.E Ibrahim, Dr. R.A Oyi, Dr. A.B Isah, Prof. P. F Olurinola and Late Dr. J.E Ojile. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at any university.

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# Certification

This Thesis titled 'CONTROLLED RELEASE FORMULATIONS OF ANTIRETROVIRAL (ARV) DRUGS USING POLYMETHACRYLATE POLYMERS' by Mahmud Sani-Gwarzo meets the regulations governing the award of the degree of Doctor of Philosophy (Pharmaceutics) of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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## copyright Statement

# Dedication

In the name of Allah, the compassionate, the merciful.

This work is dedicated to my creator (ALLAH), my parents, my teachers, my extended family, my wife, my children, my grandson and my friends.

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Mahmud Sani Gwarzo

**ABSTRACT**

To improve patient compliance and ensure better success in the management of HIV, spansule technology was employed to formulate controlled release dosage drug. Generic forms of seven of the most commonly used Anti-retro viral (ARV) drugs (didanosine, indinavir, lamivudine, nelfinavir, nevirapine, stavudine and zidovudine) and their clinically approved combinations were used in this study. Calculated daily dose was divided into one loading and four maintainace doses. Granules of the drugs, prepared using wet granulation method, were divided into five batches and spray coated with various grades of polymethacrylate polymers (Eudragit brand). Using different combinations/ratios, each ARV drug and their combinations were filled into capsules of sizes 00 to 000. Parameters such as solubility, disintegration, coating thickness and dissolution were determined. In-vitro testing for drug release was conducted to mimic various pH conditions of the G.I.T using disodium hydrogen orthophosphate and potassium dihydrogen phosphate buffers. Spectrophotometric method was used to determine drug release. To serve as control, conventional forms of the seven ARVs obtained from a major manufacturer were tested for drug release.

Conventional didanosine achieved a maximum drug release of 25% within ten minutes, as against 97% of the spansule controlled release capsule, over a period of ten hrs. In contrast, drug released from the

conventional indinavir formulation produced 100% drug release within fourty minutes, while the formulated spansule released only 19% of the drug. Similarly lamivudine conventional formulation released 90% in ten min, while the controlled release spansule formulation took eight hours to reach the same level. Similar results were obtained with nelfinavir, nevirapine, stavudine and zidovudine. Drug release pattern of the binary mixture of stavudine with didanosine and the triple (combinations nevirapine + zidovudine + didanosine**;** stavudine + lamivudine + nevirapine and zidovudine + lamivudine + nevirapine) were generally similar to each individual component release profile. It was observed that the release pattern of each component was not affected by the presence of other components in the formulations.

Except for indinavir, all other sustained release coatings were able to maintain drug levels for extended period of time.

Generally granules coated with polymethacrylate, Eudragit L 100 released their drug content at pH >5 very quickly, while granules coated with Eudragit S 100 released their drugs predominantly at pH >7.5, akin to the small intestinal pH; formulations containing Eudragit RL, RS and NE released their drug contents gradually over time.

The spansule formulations containing polymethacrylate polymers as controlled release agents could reduce pill burden of HIV/AIDS therapy, as dosage regimen compliance is improved from four times daily dosing to once daily dosing.

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## ABBREVIATIONS

A.I.D.S..... Acquired Immune Deficiency Syndrome

ACTG..... AIDS Clinical Trial Group

ARV .....Anti Retro Viral

BID.....Twice a day (bis in die)

CAPS.....Capsules

CDDS.....Controlled Drug Delivery System

CDER.....Center for Drug Evaluation and Research

DHHS.....Department of Health and Human Services

FDC.....Fixed Dose Combination

HAART.....Highly Active Anti Retroviral Therapy

H.I.V .....Human Immunodeficiency Virus

MTCT.....Mother To Child Transmission

O.D.....Once a Day

P.P.R.P..... Polymethacrylate Polymer Rohm Pharma

QID (QDS).....Four times a day (quart in die)

RSD.....Relative Standard Deviation

STI**.**..... Sexually Transmitted Infection

SD.....Standard Deviation

TABS.....Tablets

TID.....Three times a day (ter in die)

UNAIDS.....Joint United Nations Programme on AIDS

USAID.....United States Agency for International Development

USP……United States Pharmacopoeia

WHO……World Health Organization

# CHAPTER ONE

## 1. INTRODUCTION

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) commonly referred to as HIV/AIDS, constitute one of the most serious infectious disease challenges to public health globally, and has had a crippling effect in certain parts of the world especially in Sub-Saharan Africa (UNAIDS Update, 2007; Naidoo, 2006). There are currently about 33.2 million people living with HIV/AIDS globally. Of this total number, an overwhelming 22.5 million people are HIV positive in Sub-Saharan Africa specifically; representing 67.8% of the global number (HIV/AIDS drug information, DHHS 2007). Interventions such as AIDS counseling, educational tools and antiretroviral drug therapy have contributed to transforming HIV infection, from a fatal, to a manageable chronic infectious disease (Chinen, 2008). Despite the availability of these measures, the above statistics indicate that much remains to be accomplished as the number of newly reported HIV infections still remains unacceptably high.

One of the measures in the management of the infection is drug therapy. A variety of drugs which belong to different physico-chemical and pharmacological classes are currently being employed in the drug management of the infection. These drugs are administered as several combinations, and taken several times per day.

Although ARV drug therapy has contributed significantly to improved patient/disease management, its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patients. Many ARV drug undergo extensive first-pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decrease in patient compliance (Vyas *et.al,* 2006). The sub-therapeutic drug concentrations and short residence time at the required sites of action contribute significantly to the failure of eliminating HIV and the development of multi drug-resistance against the ARVs (Sanchez-Lafuente *et.al,* 2002). These drugs also suffer from physicochemical problems such as poor stability and solubility, which may lead to formulation difficulties (Xiang and Fang, 2002). Strategies currently being investigated to overcome these limitations include the identification of new drugs and chemical modifications of existing drugs, the examination of various dosing regimens as well as the design and development of controlled drug delivery systems (CDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade, there has been an explosion of interest in the development of CDDS for the incorporation of ARV drug as a way of circumventing the problems described above and optimizing the treatment of HIV/AIDS patients (Naido, 2006). Controlled drug delivery systems present an opportunity for formulation scientists to overcome

the many challenges associated with antiretroviral (ARV) drug therapy, thereby improving the management of patients with human immunodeficiency/acquired immune deficiency syndrome (HIV/AIDS). When Zidovudine was first approved in March 1987, it was taken six times a day. A few years ago, combination ARV drug treatment became the standard care; this involves HIV positive patients swallowing as many as twenty (20) pills a day.

Current ARV drug combinations are geared towards once or twice a day dosing. Example is the July 2006 approval of Atripla$^{R}$ containing a day's dose of efavirenz, tenofovir and emtricitabine. Researchers (Gately, 1986; Graham, 1992; Paterson, 2000; Gaya 2007 and Lima 2008) have shown that near perfect adherence is the leading predictor of treatment success or failure. United Nations Programme on AIDS (UN AIDS publication, 2002) has strongly recommended simplification of HIV treatment by:

1) Reducing the total number of times that medication needs to be taken each day and

2) Reducing the total number of pills that need to be swallowed each time medication needs to be taken. To achieve these goals, 'Once-A-Day Dosing' has been found to be most appropriate for simplification of the life long treatment. This allows a complete drug regimen in one pill, once a day. Similar simplification improvements have been made with older drugs as well and it is the aim of this project to follow suit.

### 1.1. Importance of Treatment Adherence

As had been stated earlier, near perfect adherence has been found to be the leading predictor of treatment success or failure. Lima (2008) found that HIV patients who miss more than five (5%) percent of their ARV drug doses were much more likely to experience treatment failure. Numerous studies have shown that strict adherence was necessary to keep the HIV virus fully suppressed and to avoid developing drug resistance (PETRA study team 2002). To determine the impact of adherence on treatment success, Lima and her colleagues (Lima *et. al*, 2008) studied 878 HIV patients. Their results showed that only 41% had greater than 95% adherence and had good response to treatment. The study concluded that patients who miss more than 20% of their doses had less than 11% chance of treatment success.

## 1.2 THEORETICAL CONSIDERATIONS

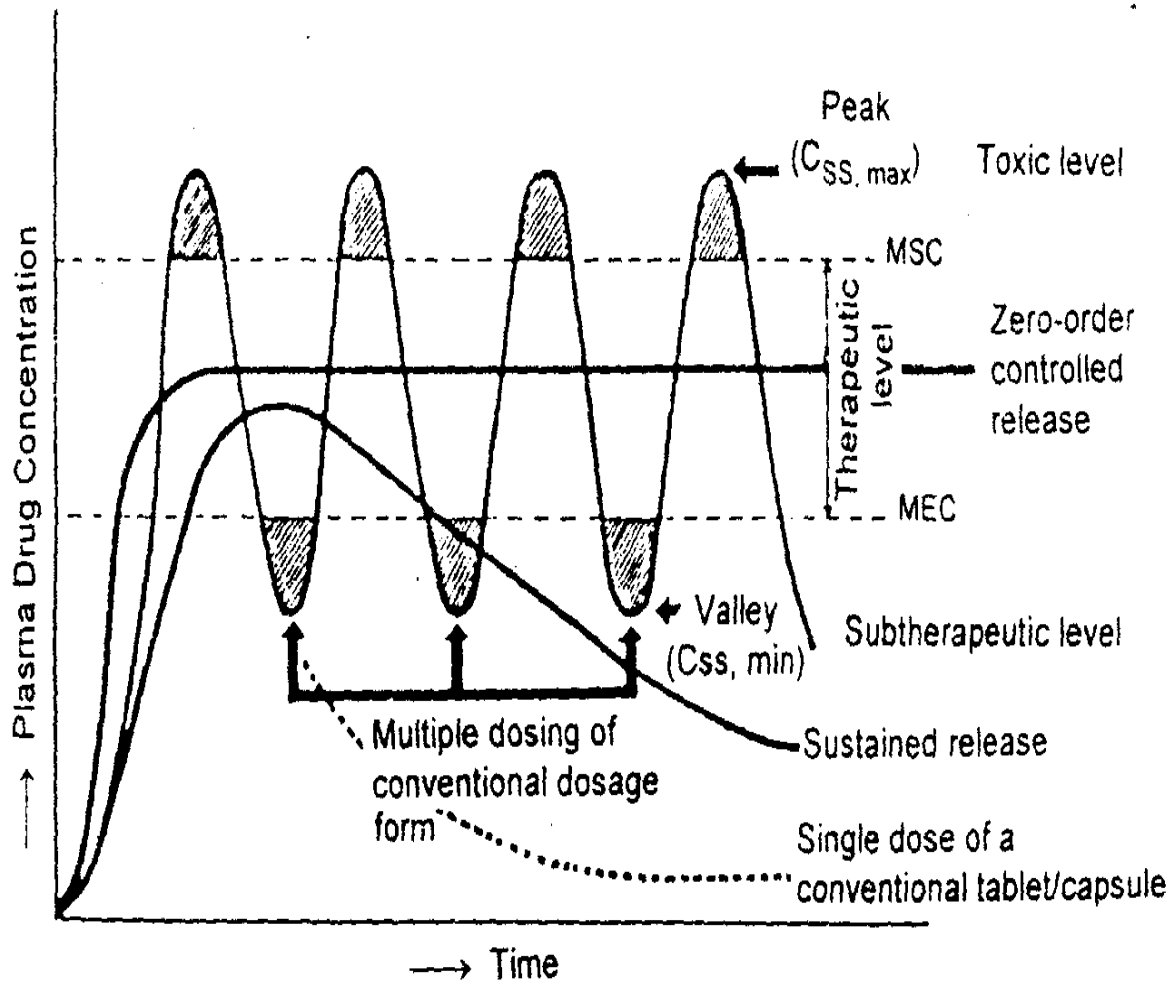



**Figure 1.1**: A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulation

Adapted from (Brahmankar and Sunil 2000)

KEY: $C_{SS.}$ Steady State Concentration maximum and minimum.

MSC: Maximum Safe Concentration

MEC: Minimum Effective Concentration

An ideal dosage regimen in the drug therapy of any disease is the one which immediately attains the desired therapeutic concentration of drug in plasma (or at the site of action) and maintains it constant for the entire duration of treatment. This is possible through administration of a conventional dosage form in a particular dose and at a particular frequency. The frequency of administration or the dosing interval of any drug depends upon its half-life or mean residence time (MRT) and its therapeutic index. In most cases, the dosing interval is much shorter than the half-life of the drug resulting in a number of limitations associated with such a conventional dosage form:-

a) Poor patient compliance; increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.
b) A typical peak-valley plasma concentration-time profile is obtained, which makes attainment of steady-state condition difficult (Fig 1.1: A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations)
c) The unavoidable fluctuations in the drug concentration may lead to under medication or overmedication as the $C_{SS}$ values fall or rise beyond the therapeutic range.

d) The fluctuating drug levels may lead to precipitation of adverse effects, especially of a drug with small therapeutic index whenever overmedication occurs (Brahmankar and Sunil 2000).

There are two ways to overcome such a situation:

i) Development of new, better and safer drugs with long half-lives and large therapeutic indices, and

ii) Effective and safer use of existing drugs through concepts and techniques of controlled and targeted delivery systems.

The first approach is rather utopian and hardly realizable which therefore, resulted in increased interest in the second approach. An ideal controlled drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Thus, unlike conventional immediate release systems, the rate of appearance of drug in the body with such a system is not controlled by absorption process alone. Following absorption of drug from such a system, there is no control over its fate. An ideal targeted drug delivery system is the one which delivers the drug only to its site of action and not to the non-target organs or tissue.

There are several terms used interchangeably to describe such products. These include controlled release, programmed release, sustained release, prolonged release, timed release, slow release, extended release, et cetera. Controlled release differs from sustained release systems. The latter simply prolong the drug release and hence plasma drug levels for

an extended period of time (i.e. not necessarily at a predetermined rate). Thus, the chief objective of most products should be controlled delivery to reduce dosing frequency to an extent that once daily dose is sufficient for therapeutic management through a uniform plasma concentration at steady-state (Mithal 2000).

Ching *et al*, 2008 explains the several advantages of a controlled drug delivery system over a conventional dosage form are:

a) Improved patient convenience and compliance due to less frequent drug administration;

b) Reduction in fluctuation in steady-state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects (Figure 1.1);

c) Increased safety margin of high potency drugs due to better control of plasma levels;

d) Maximum utilization of drug enabling reduction in total amount of dose administered;

e) Reduction in health care cost through improved therapy, shorter treatment period less frequency of dosing and reduction in personnel time to dispense, administer and monitor patients.

## 1.3 SPANSULE FORMULATION

The term 'Spansule' can be defined as hard gelatin capsule filled with coated granules or pellets. A spansule consists of a capsule containing a large number of pellets coated with various thicknesses of a slowly dispersible substance as well as uncoated pellets or powdered drug to provide initial drug concentration. Each group of the coated pellets, usually 100 per group, contains an equal amount of the drug. The total amount of drug in the sustained form is from 2 to 4 times the dose given in a conventional table or capsule (Osol 1980).

### 1.3.1 Types

Two different types of spansule formulations are in vogue:

**a) Coated Slow-Release Beads-** This type of spansule refers to a formulation containing 10 parts of a medicinal part, each part coated with different coating materials and thickness. The coated pellets are mixed and taken as the dose. Coating materials used are cellulose esters and ethers with or without added resins, fats, keratin, and gluten (Banker 1987).

**This type of Spansule formulation which contains coated pellets intended to provide sustained release of contained drugs. In this system, the total daily dose is divided into 3 to 9 parts. One part of any given dose is divided such that it consist of drug intended to establish initial therapeutic level and the remaining parts, being the sustained release dose. A part may contain 50 to 500 small pellets or beads of drug and excipients. It has been judged practical to divide the dose into 4 equal**

**parts; one part consisting of uncoated beads, the next part with a coating which should on the average resist disintegration for 3 hours, the next with a 6 hour coating and the last with a 9 h coating (Osol 1980).**

A number of materials have variously been found as satisfactory for coatings. Among this are mixtures of beeswax, carnauba wax, or bayberry wax with glyceryl monostearate. Others are: stearic acid, palmitic acid, glyceryl myistrate, acetyl alcohol, and similar substances that could be expected to be slowly dissolved or digested or to act as semi-permeable membranes through which drug can diffuse when the preparations are ingested (Banker 1987).

Leon (1997) suggested a method for applying coatings is to prepare 3% to 25% by weight, solutions of the materials, dissolve it in carbon tetrachloride and spray them on the granules while the coating mixture is being heated to about $60^0$c.

Preparation of prolonged release beads has been described wherein a coating as such is not applied over the drug, but rather the drug is mixed with a materials such as shellac in order to provide a mass from which drug is leached out when the beads are in contact with fluid at the absorption site. Small cylindrically shaped slow release pellets have been prepared by extruding mixtures of drug and materials for encapsulation such as Zein or Kafirin. Also, small beads with a pH-sensitive coating have been used to prepare long-acting pellets (Banker 1987).

There are many products consisting of coated beads/granules, or pellets contained in hard gelatin capsules or compressed into tablets that are presently being marketed. The coating materials used in these products are usually fats and waxes, polymeric substances sensitive to small changes in pH of gastrointestinal fluids, shellac, or various mixed or independently applied mixtures of these materials or others mentioned.

This principle which involves dividing a dose of drug into many small bodies is theoretically sound. Gastrointestinal absorption of drugs is, in general, quite erratic. In many cases, erratic absorption is due to variations in release of drug from the dosage form. Division of the dose into many parts increases the probability that an effective dose of the drug will be made available for absorption, and hence, properly designed long acting products can be expected to make gastrointestinal absorption more regular and predictable. Some new long acting products employ the principle of micro encapsulation, where small quantities of solids, liquids, or gases may be coated. Example of materials for such coating products are gelatin, polyvinyl alcohol and ethylcellulose. An example of this type of product is a “timed-release” aspirin, which is claimed to be effective up to 8 hours (Osol, 1980).

**b) Tablets with slow Release Cores-** This consists of a core containing the therapeutically active materials evenly mixed in a mixture of substances which are non-absorbable from the gastrointestinal tract.

For examples:-

(i) Fortespan$^{R}$, which contains Vitamin A, $B_1$, $B_2$ $B_6$ $B_{12}$, C, D and Nicotinamide, Manufactured by SK&F.

(ii) Measurin$^{R}$ Tablet, containing Acetylsalicylic acid, by Breon.

(iii) Mol-Iron-Chronosule$^{R}$ containing Ferrous sulphate with vitamins C by Schering, and

(iv) Prydon$^{R}$ Spansule containing Belladonna alkaloids made by SK&F.

Coatings applied to granules can produce characteristics approximating ideal sustained release. Spansule is the best known example of controlled release dosage form which depends on different materials and thicknesses of the coating on numbers of granules to produce various disintegration and dissolution times (Ansel 2005).

### 1.3.2 Considerations in Spansule formulation

**If the dose of the drug is large, the starting granules of material may be composed of the drug itself. Some granules may remain uncoated to provide immediate drug release. Other granules (about two-thirds to three-fourths) receive varying coats of polymers. Then granules of different coatings are blended to achieve a mix having the desired drug release characteristics. Typically the coated granules are about 1 mm in diameter. They are combined to have three or four release groups among the more than 100 granules contained in the dosing unit. This provides the different**

**desired rates of sustained or extended release and targeting of granules to the desired segments of the gastrointestinal tract (Ansel 2005)**

If all of the granules in each coated group were covered with a uniformly thick coat, the medication would be released after the initial production; there is some variation of thickness of the coat within each coated group. The variance of thickness within each group permits some granules to disintegrate sooner than those with the median thickness of that group. The drug so released overlaps the drug remaining from the previous group. Some granules within a group are slightly thicker than the median thickness and do not disintegrate until later so that the drug released overlaps with the next groups. This overlapping between groups provides a smoother and more uniform release, which approaches a continuous type of release.

Spansule formulation of orally administered drugs, extended drug action can be achieved by affecting the rate at which the drug is released from the dosage form. The rate of drug release from solid dosage forms can be modified by the technology employed in spansule, which is based on modifying drug dissolution by controlling access of biologic fluids to the drug through the use of barrier coatings (Bugner, 1997).

Micro encapsulation is a process by which solids are enclosed in microscopic particles by formation of thin coating material around the drug (Drug delivery technologies script report 2003)

Spansules disintegrate independent of pH with the release mechanism primarily one of moisture vapor pressure permeability of the lipid film. The drug, the composition of the coating and the thickness of the coating determine the rate of moisture permeability (Howard *et al,* 2000).

In addition to promoting a sustained release effort, spansule provide a more uniform distribution of the drug in the gastrointestinal tract. If a single tablet fails to disintegrate, the benefit of the entire dose is lost. If a few pellets of a Spansule fail to disintegrate, the loss of a small amount of the total drug will not greatly affect the over-all dose. Advantages of using controlled drug delivery of ARV drugs may be summarized as follows:-

1. Increased patient compliance resulting from four times daily dosing to above once daily dosage regimen. The implication of this for successful treatment has been emphasized by many workers (Lima, 2008; Peterson, 2000; WHO report, 2007).

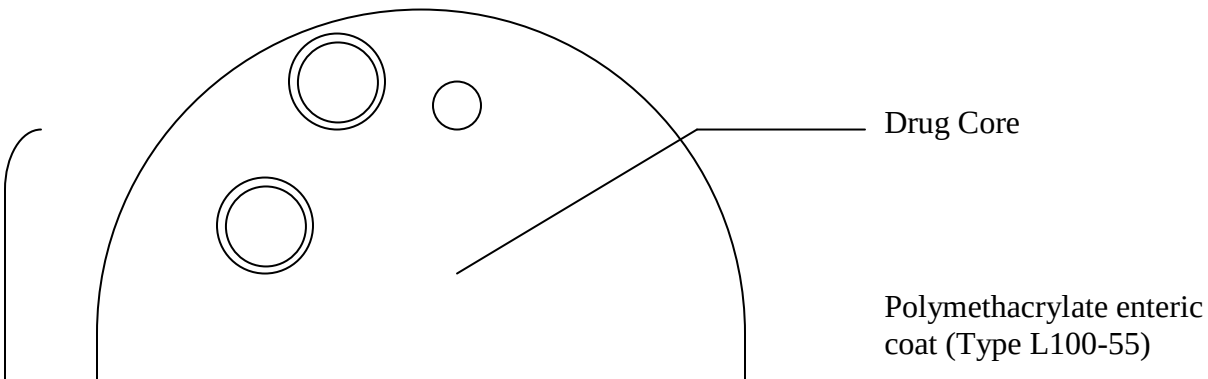
2. The prevention of breeding resistant strain of the virus because of the expected leveling-off of peaks and valleys (produced by conventional release) in the drug-release pattern and by deduction, of the plasma-during levels, into a smoothened, even plateau (in the spansule formulation). The HIV virus is no longer exposed to sub-therapeutic drug levels, which have been documented to cause drug resistance (Boden, 1998).

3. A significant reduction in symptomatic side-effects is to be expected, for those ARVs known to have dose dependant side effects such as Didanosine (Katlama, 1996).

4. The controlled release formulations using polymethacrylate polymers are expected to have a reduced bias, as regards to food in-take. This is due to the well documented, non reactive nature of the polymers to drugs, foods, and physiological fluids of the g.i.t (Lehmann, 2001). Many of the ARV'S have restrictions of food and liquid intake, as well as the timing intervals between food and drug intake. The removal of this constrains should have clinical and compliance implications.

5. Because of the protective and non-reactive nature of the polymethacrylate polymers (Lehmann, 2002) on the active ingredients, stability and hence the shelf-life of the drugs is expected to be greatly enhanced. The implications of this for Africa (3/4 of all HIV cases) are enormous, due to lack of electricity to maintain optimum temperature and humidity, needed for ARV drug storage.

6. Studies (UNAIDS up-date, 2007; Thompson, 2004 and Rathbun, 2006) have shown substantial reduction in cost from $660 per patient for multiple single drugs to $140 for two fixed dose combinations. Further reduction of these fixed dose combinations to once daily, should continue the down-wards cost-reduction. This has been achieved by the spansule formulation design of this project. The implication for poverty and disease stricken Africans can be enormous.


Drug Core
Polymethacrylate enteric
coat (Type L100-55)

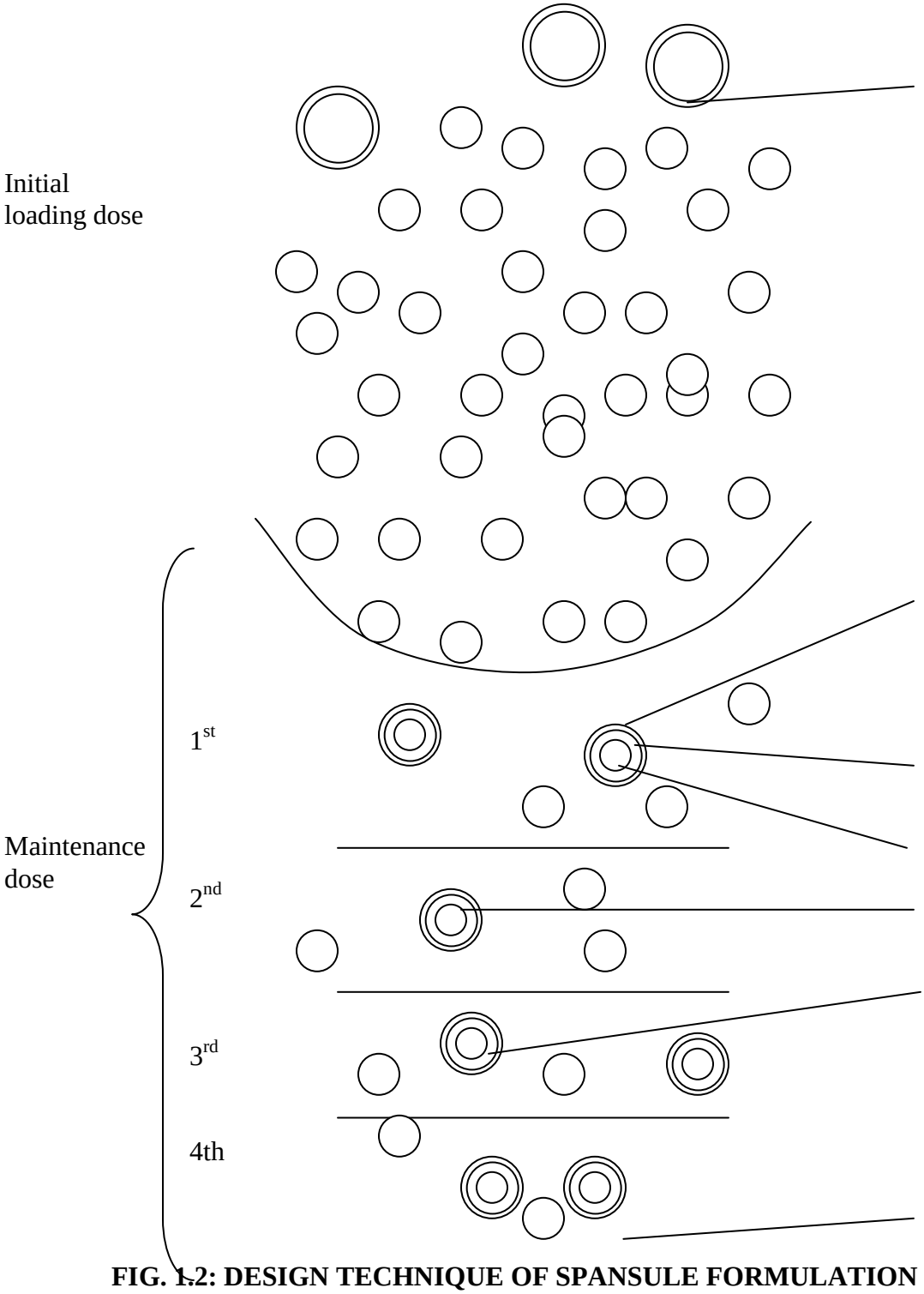



**FIG. 1.2: DESIGN TECHNIQUE OF SPANSULE FORMULATION (M.S Gwarzo)**

Sprayed coat layer

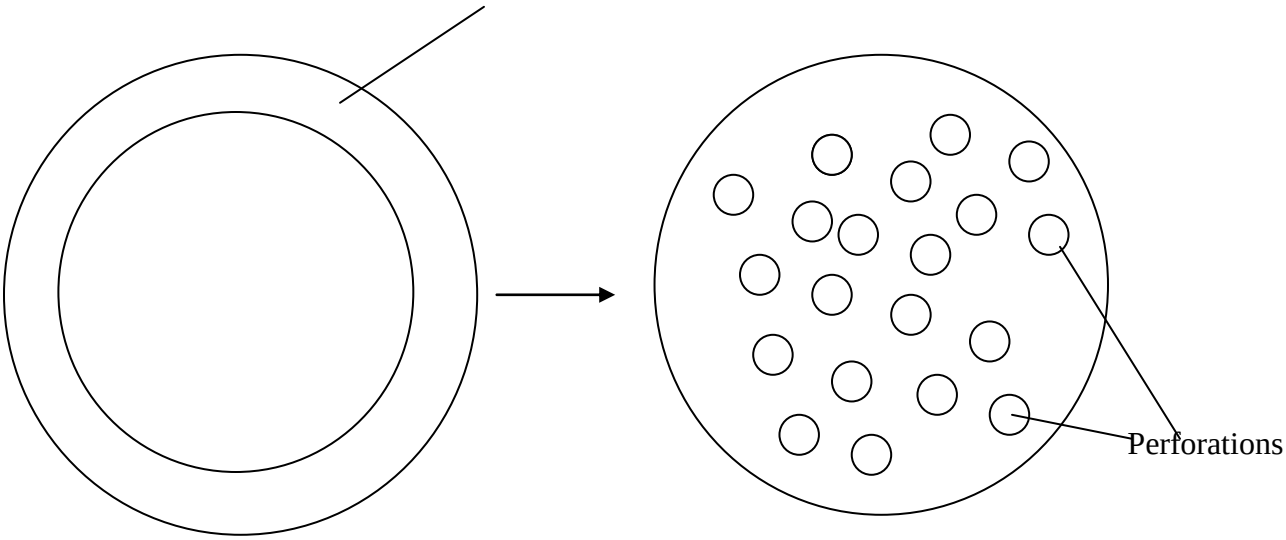



**Figure 1.3: Schematic diagram of controlled release Spansule formulation capsule (Lehmann 2000).**

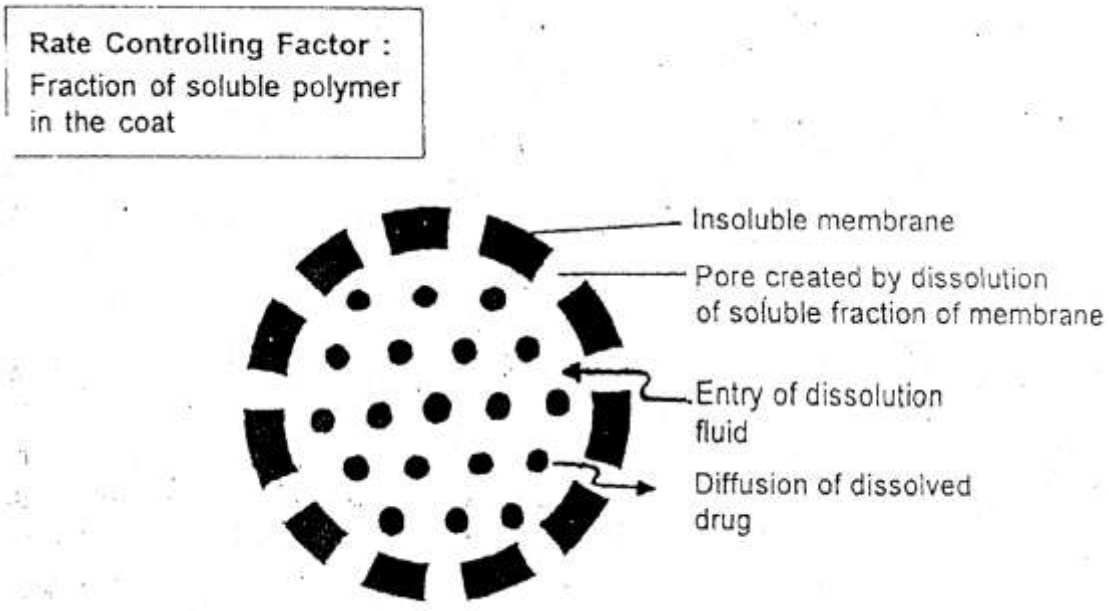



**Figure 1.4: Rate Controlling Factor, Portions of Soluble Polymer in the coat (Lehmann 2000)**

## 1.4 STATEMENT OF RESEARCH PROBLEM

No other disease condition known to man has a higher "pill-burden" (and treatment duration is life-long), than HIV/AIDS. To improve patient's compliance and therefore achieve higher success in the therapy/management of HIV/AIDS, scientists are directing their focus on how to simplify drug administration. While use of drug combination therapy have been found to enhance patient's compliance, research on developing a single daily dose regimen for treating HIV infection continues. Patients not only consume ARV drugs several times a day, they are often faced with consuming about three or four different drugs at a time. On average, a HIV/AIDS patient may take some 12 tablets of ARV drugs 3- 4 times daily, thus creating pill burden for the patient with its attendant effect on compliance.

Documented records have shown that even the most dedicated patients are hard-pressed to maintain compliance. Gately (1986) reported patient compliance of 22% for four times daily intake; 44% for three times daily intake); 50% for twice daily in take and 70% for once daily in take. This finding was confirmed by Gaya (2007) in his survey of HIV/AIDS patient on triple HAART (Highly Active Anti Retroviral Therapy). He found 88% compliance on HAART and 11% on monotherapy. He also found that 60% of the patients have missed doses in the treatment schedule.

Other researchers, such as Graham (1992) documented that the missing of doses lead to failure in treatment and breeding of resistant

strains of the virus. Paterson (2000) and Lima (2008) showed that less than 95% adherence is the number one leading cause of treatment failure in HIV/AIDS drug treatment. For these reasons, highly complex therapeutic regimens were reserved for individuals who are capable of adhering to the rigorous demand of taking multiple medications and having these monitored. In the case of the protease inhibitors, patients have to pass a screening test before they can be placed on these drugs.

## 1.5 JUSTIFICATION FOR THE RESEARCH

A number of ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half life for several ARV drugs is short, requiring frequent administration of doses leading to decreased patient compliance (Li, 1999).

Current drug management of HIV/AIDS involves combination of several drugs using the HAART system. The problem poised to patients is enormous and any measure that will alleviate it, is highly welcome.

In order to improve treatment out-come, both UNAIDS and WHO have recommended the simplification of HAART by combining the ARV drugs to once daily dosing or maximum of twice daily dosing.

In order to meet the objective of pill-burden reduction, seven of the most commonly used ARV drugs as well as their clinically approved combinations, are to be formulated into controlled release formulations.

Selecting one or two ARV drugs for controlled formulation will not enable the project to achieve sufficient pill-burden reduction "as is" under HAART programme. Fixed dose combinations to be formulated will be based on this programme.

Damle (2002) reported that didanosine is rapidly hydrolyzed in the acid medium of the stomach. Current didanosine formulation contains magnesium or aluminum antacids to neutralize the stomach acid in order to protect the drug. The controlled release formulation will contain no antacid that give rise to drug-drug interaction (e.g. with ketaconazole, HIV-protease inhibitors, dapsone and tetracyclines) and drug food interactions (e.g. with fruit juice and milk). The tablets have to be thoroughly chewed or crushed in water. This leads to poor taste in the mouth reducing patient compliance, as well as causing nausea. Indinavir contain large quantities of desiccants in its original container and is only stable for only three days when removed (Damle et al, 2002).

Spansule formulation of the selected ARV drugs will not only ensure that the drugs are administered as once a day regimen, Problems associated with some of the ARV DRUGS that often lead to low compliance, poor bioavailability and low therapeutic efficacy will be obviated. In essence, the desired goal of simplifying therapy management of HIV/AIDS will have been achieved. The methacrylate polymers selected for use as coating materials for the formulation of controlled release ARV have been

proven to possess the required properties that will enable achievement of the desired goal.

The typical peak-valley plasma concentration vs time profile, associated with conventional dosing of ARV drugs, is caused by the unavoidable fluctuations in the drug concentration of conventional dosage forms. This often leads to either under medication, which encourages viral resistance and over medication, which precipitates adverse side effects. It is expected that by controlling the drug release, fluctuations in drug levels become minimized resulting in reductions of side effects and development of resistance.

Thompson (2004) gave the cost of $660 (N100, 000) per patient, for six conventional drugs, as compared to $140 (N21, 212) for the same drugs in two fixed dose combinations. It can therefore be expected, that formulation into one single fixed dose combination, should lower the cost further.

Lehmann (2002) proved that the polymethacrylate polymers for pharmaceutical use have exceptional decade-long stability to air, light and water. This provides “good basis" for the shelf life of coated pharmaceutical dosage forms. In the case of Indinavir, the drug is only stable for three days out side its original container, which must contain a big sachet of desiccant. Because of the non-reactive and protective nature of the polymethacrylate polymers (MacGinity 1983, Cameron 1987), new ARV drugs formulations with these protective coatings, are

expected to offer less restrictions on food intake with regards to dosing time, for example, take the drug on an empty stomach, one hour before or two hours after a meal. Some ARV drugs like didanosine will be protected from acidic degradation.

## 1.6 AIMS AND OBJECTIVES

**T**he main aim of this project is to reduce the "pill-burden" of the HIV/AIDS patient, by means of controlling the release of the ARV drugs, from a spansule dosage formulation, using polymethacrylate polymers as the release controlling agents.

The seven most commonly used ARV drugs and five of the most commonly used combinations are to be formulated into once daily controlled release capsule dosage forms, with a view of increasing compliance. Specific Objectives are:-

- Selection of ARV drugs which are associated with unfavorable physico-chemical or bioavailability characteristics for formulation into controlled release dosage form.
- Production of granules of the selected ARV drugs with appropriate sizes for micro-encapsulation.
- Production of enteric coated granules of different drug release profiles for the different regions of the G.I.T, using different grades of polymethacrylate polymers.

- Encapsulation of the enteric coated granules into spansule dosage form.
- Design a method of quantifying the content of the ARV spansule formulations with those of conventional ARV formulations.
- Evaluation of the drug release profiles of the formulated spansules, using the in-vitro controlled release dissolution form system.

## 1.7 SCOPE OF THE STUDY

- Seven of the commonly employed ARV drugs namely (didanosine, indinavir, lamivudine, nelfinavir, nevirapine, stavudine and zidovudine) and their clinically approved combinations, will be formulated into controlled release spansule dosage forms.
- Five grades of polymethacrylate polymers manufactured by Rohm Pharma Polymers namely: Eudragit L 100 which is pH dependant for drug delivery in duodenum and jejunum; Eudragit S 100 which is pH dependent for drug delivery in ileum; Eudragit RL 100 which is insoluble high permeability, to coat granules for immediate sustained-release; Eudragit NE 100 which is insoluble, low permeability, for intermediate sustained release and Eudragit RS for long term sustained release in colon region.
- Drug release in four regions of the G.I.T will be studied.
- In-vitro drug release evaluation technique will be employed.
- Granules of average size 1.0mm will be used for coating.

- Coating of granules will be by the gun spray method.

## 1.8 LIMITATIONS

The project design is limited to the formulation and spectrophotometric analysis of seven of the most commonly used ARV drugs and four clinically approved HAART formulations, into controlled release spansule dosage forms, using polymethacrylate polymer grades of Rohm Pharma. Therefore, results obtained may not necessarily apply to grades of polymers irrespective of the manufacturer.

Drug release evaluation is by in-vitro evaluation techniques in medium simulating the different regions of the G.I.T. Drug release pattern may not accurately reflect the situation in animal or human body systems.

# CHAPTER TWO

## LITERETURE REVIEW

## 2.1 DESIGN OF CONTROLLED DRUG DELIVERY SYSTEMS

The basic rationale of a controlled drug delivery system is to optimize the biopharmaceutical, pharmacokinetic and pharmacodynamic properties of the drug in question, in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using the smallest quantity of drug, administered through the most suitable route (Brahmankar and Sunil, 2000).

### 2.1.1 Biopharmaceutical Characteristics of the Drug.

The performance of a drug presented as a controlled release system depends upon its:

i) Release from the formulation;

ii) Movement within the body during its passage to the site of action.

The release depends upon the fabrication of the formulation and the physicochemical properties of the drug, while the movement is dependent upon pharmacokinetics of the drug. In comparison with conventional dosage form where the rate-limiting step in drug availability is usually absorption through the biomembrane, the rate-determining step in the availability of a drug from controlled delivery system is the rate of release of drug from the dosage form, which is much smaller than the intrinsic absorption rate for the drug (Figure 2.1).

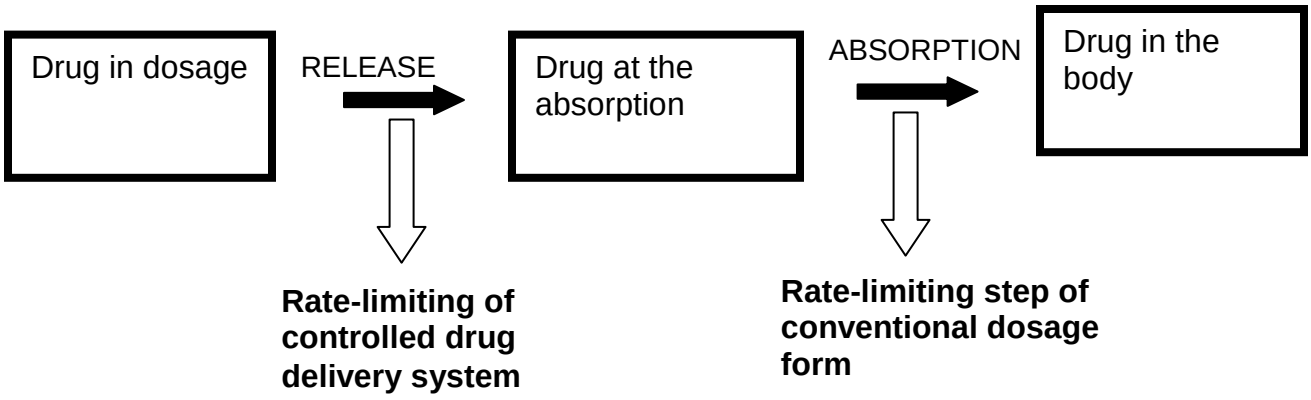



**Figure 2.1: Schematic Representation of Rate-Limiting Step in the Design of Controlled Drug Delivery System**

(Adapted from Brahmankar and Sunil, 2000).

For a drug to be successful as oral controlled release formulation it must get absorbed through the entire length of G.I.T (Ching *et.al,* 2008). Since the main limitation of this route is the transit time (a mean of 14 hours) the duration of action can be extended for 12 to 24 h. The route is suitable for drugs given in dose as high as 1000mg. The main determinants in deciding a route for administration of a controlled release system are physicochemical properties of the drug, dose size, absorption efficiency and desired duration of action.

### 2.1.2 Pharmacokinetic Characteristics of the Drug.

A detailed knowledge of the ADME (Absorption, Distribution, Metabolism, and Elimination) characteristic of a drug is essential in the design of a controlled release product. An optimum range of a given pharmacokinetic

parameter of a drug is necessary beyond which controlled delivery is difficult or impossible (Corrigan, 2009).

i) **Absorption Rate:** For a drug to be administered as controlled release formulation, its absorption must be efficient since the desired rate-limiting step is rate of drug release ($K_r$) i.e. $K_r << K_a$ ($K_a$ is rate of absorption). A drug with slow absorption is a poor candidate for such dosage forms since continuous release will result in a pool of unabsorbed drug, iron is a good example. Aqueous soluble, but poorly absorbed potent drugs, like decamethonium are also unsuitable candidates since a slight variation in the absorption may precipitate potential toxicity (Butler *et al,* 1998).

ii) **Elimination Half-Life**: The smaller the $t_{1/2}$ the larger the amount of drug to be incorporated in the controlled release dosage form. For a drug with $t_{1/2,}$ less than 1 hour, a very large dose may be required to maintain the repetitive dosing. Drugs with half-life in the range of 1 to 4 hours make good candidates for such a system, e.g. zidovudine. Drugs with long half-Life need not be presented in such a formulation, e.g. amilodipine. For some drugs such as MAO inhibitors, the duration of action is longer than that predicted by their half-lives. A candidate drug must have $t_{1/2}$ that can be correlated with its pharmacologic response (Leon *et al*, 2000).

iii) **Rate of Metabolism:** A drug which is extensively metabolized is suitable for controlled release system as long as the rate of metabolism is not too rapid. The extent of metabolism should be identical and predictable when the drug is administered by different routes. A drug capable of inducing or inhibiting metabolism is a poor candidate for such a product since steady-state blood levels would be difficult to maintain.

iv) **Dosage Form Index (DI):** It is defined as the ratio of $C_{ss\ max}$ (maximum steady state concentration) to $C_{ss\ min}$ (minimum steady state concentration). Since the goal of controlled release formulation is to improve therapy by reducing the dosage form index while maintaining the plasma drug levels within the therapeutic window, ideally its value should be as close to one as possible (Leoan *et al*, 2000).

### 2.1.3 Pharmacodynamic Characteristics of the Drug.

i) **Therapeutic Range:** A candidate drug for controlled delivery system should have a therapeutic range wide enough to accommodate variations in the release rate with in the range, such that, the variations do not result in a concentration beyond this level (Wang *et.al.* 2007)

ii) **Therapeutic Index (TI):** The release rate of a drug with narrow therapeutic index should be such that the plasma concentration attained is within the therapeutically safe and

effective range. This is necessary because such drugs have toxic concentration nearer to their therapeutic range. Precise control of release rate of a potent drug with narrow margin of safety is difficult. A drug with short half-life and narrow therapeutic index should be administered more frequently than twice a day. One must also consider the activity of drug metabolites since controlled delivery system controls only the release of parent drug but not its metabolism.

iii) **Plasma Concentration-Response Relationship:** Drugs such as reserpine whose pharmacological activity is independent of its concentration are poor candidates for controlled released system (Wang *et al*, 2007).

### 2.1.4 Pharmacokinetic principles in the design and fabrication of controlled drug delivery systems.

The controlled release dosage forms are so designed that they release the medicament over a prolonged period of time usually longer than the typical dosing interval for a conventional formulation. The drug release rate should be so monitored that a steady plasma concentration is attained by reducing the ratio $C_{ssmax}$ /$C_{ssmin}$ while maintaining the drug levels within the therapeutic window (Fig. 2.1). The rate –controlling step in the drug input should be determined not by the absorption rate but by the rate of release from the formulation which should be slower than the rate of absorption (Fig. 2.2). In most cases, the release rate is so slow that if the drug exhibits two-compartment kinetics with delayed

distribution and one can, thus, collapse the plasma concentration-time profile in such instances into a one-compartment model i.e. a one-compartment model is suitable and applicable for the design of controlled drug delivery systems (Jantratid *et.al.* 2009). Assuming that the ADME of a drug are first-order processes to achieve a steady, no fluctuating plasma concentration, the rate of release and hence rate of input of drug from the controlled release dosage form should be identical to that from constant rate intravenous infusion. In other words, the rate of drug release from such a system should ideally be zero-order or near zero-order. One can thus treat the desired release rate $R_0$ of controlled drug delivery system according to constant rate I.V infusion. In order to maintain the desired steady-state concentration ($C_{ss,}$) the rate of drug input, which is zero-order release rate ($R_0$) must be equal to the rate of output. Thus according to Brahmankar and Sunil (2000):

$$R_0 = R_{output} \qquad (1)$$

The rate of drug output is given as the product of maintenance dose ($D_M$) and first-order elimination rate constant ($K_E$)

$$R_{output} = D_M K_E \qquad (2)$$

For a zero-order constant rate infusion, the rate output is also given as:

$$R_{output} = K_E C_{ss} V_d \qquad (3)$$

(were $V_d$ is volume of distribution and $CI_r$ is the clearance rate).

Since $CI_r = K_E V_d$ the above equation can also be written as:

$$R_{output} = C_{ss} CI_r \quad (4)$$

$$R_0 = C_{ss} CI_r \quad (5))$$

The dosing interval for a drug following one-compartment kinetics with linear disposition is related to elimination half-life and therapeutic index (TI) according to equation 6.

$$T < t_i = In. (t_{1/2}) \quad (6)$$

KEY:

$C_{SS.}$ Steady State Concentration (maximum and minimum).

t = dosing interval.

$R_0$ = zero order release rate.

$R_{output}$ = rate of output.

$V_d$ = volume of distribution.

$CI_r$ = clearance rate.

$D_M$ = maintenance dose.

$K_E$ = first-order elimination rate constant.

TI = therapeutic index.

$T_{1/2}$ = elimination half-life.

## 2.2 HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

The goals of therapy for HIV/AIDS are to provide the optimal and individualized treatment for persons infected with HIV at all stages of disease. Antiviral therapy for HIV became available in 1987 with the approval of zidovudine (AZT), a reverse transcriptase inhibitor and a

nucleoside analogue. While life was prolonged by zidovudine monotherapy, the beneficial effects were short-lived and within months the disease progressed due to development of resistance. Combination therapy with two nucleoside analogues offered some improvement, however, the benefits were again time-limited regardless of the specific combination. It was not until the non-nucleoside reverse transcriptase and protease inhibitors became available and were used in combination with two nucleosides, that sustained clinical results were achieved. The use of three antiretroviral agents from two drug classes has been termed "highly active antiretroviral therapy" or HAART. This therapy is associated with sustained suppression of plasma HIV RNA (viral load) as measured by PCR and significant improvement in immune status as measured by absolute and percentage CD4+ cell counts. These results have translated into a proven increase in survival, reduced morbidity, decreased vertical and sexual transmission, and prevention of infection following inadvertent exposure (Winters, 2003).

### 2.2.1: Guidelines to HIV/ AIDS Therapy

While the results of HAART are dramatic, the task of taking such medications is not easy. HAART regimens can be difficult to administer for many patients. Obstacles to taking such medications include high pill burden, frequent dosing, acute and chronic drug-related adverse effects, drug-drug interactions, and food effects. Other properties of antiretroviral drugs that should be taken into consideration include ability to suppress

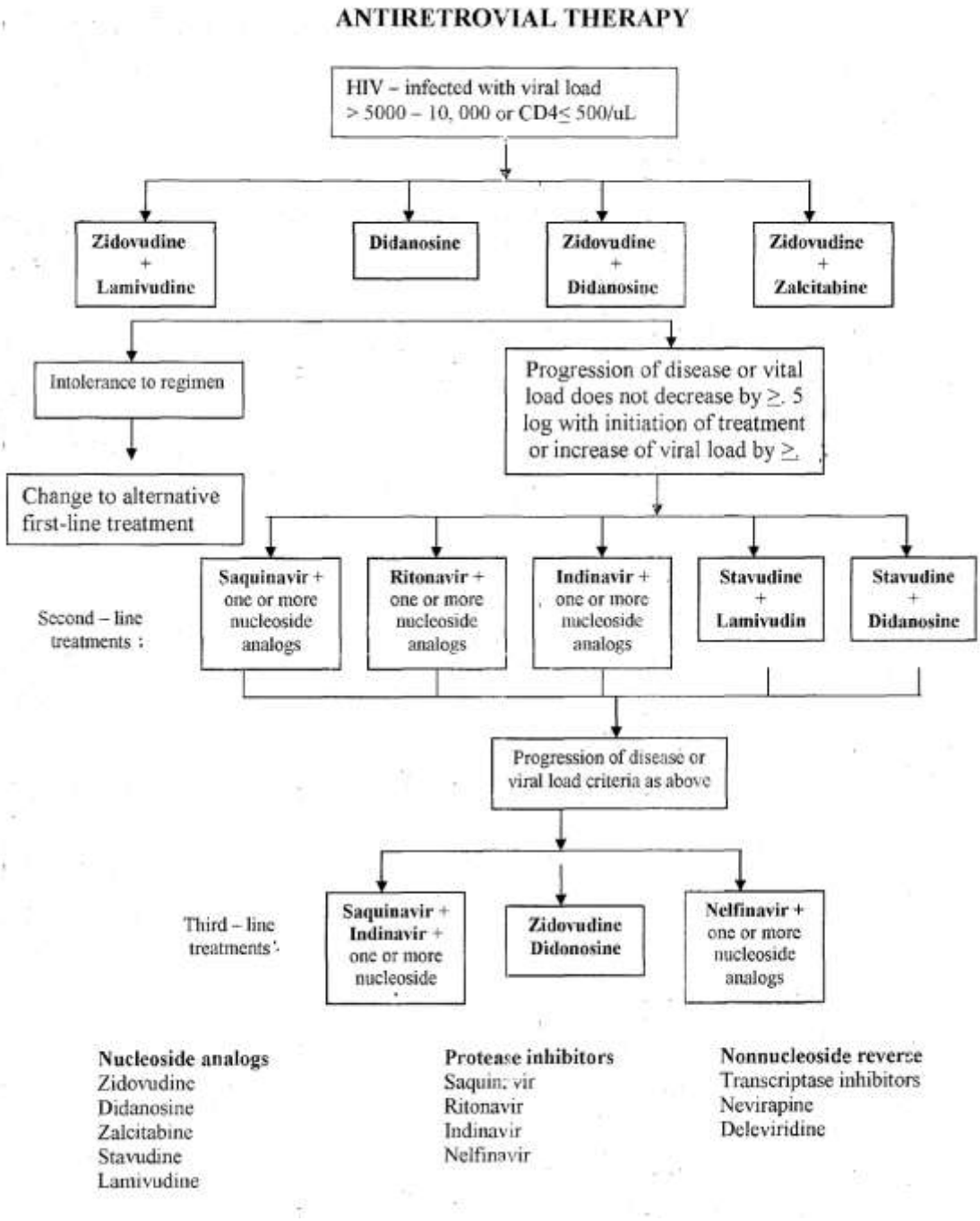



**Figure 2.2: Guidelines to HIV/ AIDS Therapy (Lawrence *et al*, 2000).**

HIV under suboptimal conditions, such as when a patient misses a dose, has an unexpected and unfavorable drug interaction, or takes a dose without regard to potential food effects. Such is the reality of taking a three drug treatment "cocktail" for the remainder of one's life (Souleymane, 2002). Whatever the drug regimen that is ad0pted, the goals of ARV therapy do not change – they are:

a. Prolong life.
b. Reduce morbidity.
c. Enhance the quality of life.
d. Reduce the transmission of HIV to infants and sexual partners.
e. Maximally suppress plasma HIV RNA (viral load)
f. Enhance immunity (increase CD4 cell count)
g. Provide the most convenient HAART regimen by choosing one with a low pill burden, few food effects, and infrequent dosing schedule.
h. Select a regimen with the least acute and chronic adverse effects, and
i. Choose the most "forgiving" regimen, one that has favorable pharmacokinetic properties and a high threshold for the development of resistance such as Nevirapine + Zidovudine + Didanosine.

### 2.2.2 Initial Strategies.

**While there are many effective HAART regimen that are used to treat HIV/AIDS, the initial strategy must be based on proven potency, ease of administration, potential drug toxicities, pharmacokinetics, resistance threshold, expense and availability (HIV/AIDS drug information 2007). At this time, initial regimen should include a dual combination of nucleoside reverse transcriptase inhibitors plus one non-nucleoside reverse transcriptase inhibitor or a protease inhibitor. As an initial strategy, if a protease inhibitor is chosen, most can be effectively administered without Ritonavir$^{R}$ for pharmacokinetic enhancement. However, Ritonavir$^{R}$ enhancement is likely to be more effective but may have more side effects.**

The initial choice of dual nucleoside reverse transcriptase inhibitors is typically limited to one of two options: stavudine plus lamivudine or zidovudine plus lamivudine. Both are effective but their side effect profiles are different. Stavudine plus lamivudine is the best tolerated acutely, however, over time, some patients may develop peripheral neuropathy and/or peripheral and facial lipoatrophy, a form of lipodystrophy. Zidovudine plus lamivudine is associated with more gastrointestinal side effects, anemia, and neutropenia. It is not associated with peripheral neuropathy with less peripheral and facial lipoatrophy. Other than these differences, the regimens are essentially equivalent.

In addition to dual nucleoside reverse transcriptase inhibitors, the third drug of a HAART regimen is a critical choice and should be chosen based

on potency, pharmacokinetics, adverse event profile and availability. The most common third drug added to a HAART regimen is a non-nucleoside, either nevirapine or efavinez. Both drugs have favorable pharmacokinetic profiles, are dosed infrequently, can be administered once daily, and have been shown to be effective. Both drugs are inducers of the P450 cytochrome system and may increase the metabolism of hepatically metabolized drugs and lower their effective concentrations in blood. What differentiate these drugs are their safety profiles. Delavirdine, a third approved non-nucleoside is infrequently used because of higher pill burden and lack of central nervous system penetration. Delavirdine is an inhibitor of cytochrome P450 (Sande *et. al*, 1994).

Nevirapine, the first non-nucleoside to be approved, is associated with rash in approximately 17% of patients, one of which 0.5% can be quite serious with potentially fatal Stevens-Johnson Syndrome or toxic epidermal necrolysis. One half of the rashes developed are actually quite mild, self-limited and do not require discontinuation of drug. Fatal hepatic failure has been reported but is rare. These effects appear to be correlated with CD4+ counts, the higher the CD4 count, the higher the rate of hepatoxicity as well as other adverse reactions. These adverse effects typically occur within the first few weeks or months of therapy and are unlikely to occur after this time period. A distinct advantage of Nevirapine is the lack of adverse affects on the fetus and newborn, hence the use for prevention of maternal-fetal transmission. A potential

disadvantage is an interaction with rifampin. The drugs should not be used together (Cerber *et al,* 2008).

An effective alternative to the non-nucleoside approach is the addition of a protease inhibitor as the third drug in a HAART regimen. The most effective and practical protease inhibitors to be considered for first line therapy include lopinavir/ritonavir (Kaletra$^{R}$), Indinavir with or without ritonavir, and atazanavir with or without ritonavir. Nelfinavir is an option but it has been demonstrated to be less effective than lopinavir/ritonavir-based therapy. Pharmacokinetic enhancement of nelfinavir with ritonavir is less effective than with the other protease inhibitors and is associated with unacceptable gastrointestinal intolerance. Saquinavir hard gel (Invirase$^{R}$) is also an alternative but only when co-administered with ritonavir. Saquinavir soft gel (Fortovase$^{R}$) is an alternative but the pill burden is excessive and when administered with Ritonavir is associated with excessive gastrointestinal side effects. Amprenavir should only be administered with Ritonavir, but the side effect profile is unfavorable.

The nucleoside combinations have also been studied as first line HAART treatment. However, although convenient and with fewer drug interactions", potency has been inferior to non-nucleoside and protease inhibitor-based regimens. Therefore, these regimens should only be used in circumstances where the non-nucleosides and protease inhibitors are not available or tolerated. The choice of the third and key component of

an optimal HAART regimen should be based on potency, tolerability, case of administration and availability (Anon, 2002).

### 2.2.3 Adherence.

**HIV infection is one of the most difficult chronic diseases to manage effectively. There are multiple drugs to be administered, pill burden is too high, the regimen may be complicated, toxicities are common and drug interactions may occur. The therapy is lifelong and expensive which carries enormous social and psychological burdens too many. True, HAART therapy is lifesaving, however it is anything but easy. Besides the difficulty in treatment, HAART therapy is very unforgiving. Less than 95% adherence to a regimen can lead to viral resistance and ultimately treatment failure. It has been estimated that for every 10% decrease in adherence, there is a corresponding 16% increase in mortality (Lima *et al*, 2008).**

Improving patient adherence is possible. Basic knowledge about antiviral drugs and HIV/AIDS disease is imperative and will stress the overall importance of treatment to the patient. This is helped by having an experienced physician and support staff that have a high level of competence about HIV/AIDS and antiretroviral drugs. Other factors that have been associated with optimal adherence include prescribing regimes with low pill burden, infrequent dosing schedule, minimal toxicities and no food interactions. Correlation between adherence and virological success was given by Paterson (2000) as follows:-

Table 2.1: Correlation between Adherence and Virological Success:

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| ADHERENCE LEVEL | VIROLOGICAL SUCCESS |
|---|---|
| >95% | 88% |
| 90% - 95% | 45% |
| 80% - 90% | 33% |
| 70% - 80% | 29% |
| < 70% | 18% |

In response to virological treatment failure due to resistance, second line treatment options approve the use of six (6) or more drugs as follows:- Tenofovir + Didanosine **+** Indinavir + Enfuvirtide + Nevirapine + Zidovudine.

This however, represents a last stand option (ARV DRUG GUIDE, 2003)

## 2.3 FIXED DOSE COMBINATION (FDC) FOR HIV/AIDS TREATMENT

Combination therapy is essential for the treatment of HIV/AIDS. The goals of HIV therapy are to maximally and durably suppress virus to allow recovery of the immune system and reduce the emergence of HIV resistance. At least three active drugs, usually from two different classes, are required to suppress the virus, allow recovery of the immune system, and reduce the emergence of HIV resistance. Simplified HIV regiments in the form Fixed Dose Combinations (FDCs) have been found to facilitate distribution and improve patient adherence. Triple FDCs are most useful for treatment of naïve patients. (CAESAR, 1997)

Although there are more than 20 unique antiretroviral drugs approved in the United States under, only a few are approved for use as FDC products, and none is approved as a co-packaged product. Some antiretroviral should not be combined due to overlapping toxicities and potential viral antagonism. Other antiviral drugs should not be used in pregnant women and other special populations. It is important, therefore, that possible combinations of these products be evaluated for safety and efficacy in the various populations that may have need of them.

Recently, newer FDCs have received attention, and some are being promoted for use in resource poor nations where HIV-1 has reached epidemic proportion (UN AIDS publication, 2007). These FDCs have been shown to offer cost advantages and allow simplified dosing because two or three drugs are combined in one pill. The Food and Drug Administration (FDA) believes that adequate evidence of safety and efficacy already exists for the use of certain individually approved HIV drugs in combination.

### 2.3.1 Desired Characteristics of Potential Regimens for FDC in HIV/AIDS Therapy

The goal of having FDC HIV/AIDS products is to simplify regimens to allow for easier distribution and improved patient adherence, particularly

in resource poor settings. Proposed combination products should be relatively well tolerated and easy to administer while providing potency and a sufficient barrier to the emergence of drug resistance. When developing FDCs product, it is recommended that the products have the following important characteristics:

- Contain two or more components of a fully suppressive regimen.
- Require a once or twice daily administration.
- Be recommended as a preferred or alternate regimen (or regimen component) in treatment guideline.
- Have clinical efficacy and safety data that support use of the combination
- Be commonly used in treatment-native patients
- Have drug interaction and toxicity profiles that allow for concomitant dosing.

It is recommended that when considering proposed FDCs or co-packaged products, sponsors should take into account the required dosing frequency of each of the components. Each of the components of an FDC should have an identical dosing frequency and similar food instructions. Co-packaged products may include products with different dosing frequencies (once or twice daily). Studies by (Yeni *et al,* 2002), International AIDS Society USA Panel (2002) and several other treatment guidelines, recommended preferred and alternate HIV treatment regimens for initial therapy. In general, recommended triple-treatment

regimens consist of two drugs from the nucleoside (or nucleotide) reverse transcriptase inhibitor (NRTI) class and one drug from either the non-nucleoside reverse transcriptase inhibitor (NNRTI) class or protease inhibitor class.

To encourage development of FDCs, FDA created a list of examples of regimens and regimen components for which the clinical safety and efficacy of concomitant use have been evaluated and described in product labels or peer reviewed literature. FDA expects that developing FDCs on this list could be accomplished without conducting new clinical efficacy and safety studies and that FDCs consisting of combinations on the attached list will satisfy the principles underpinning 21 CFR 300.50 with regard to their safe and effective use in combination. The rule states ‘ Two or more drugs may be combined in a single dosage form when each component makes a contribution to the claimed effects and the dosage of each component (amount, frequency, duration) is such that the combination is safe and effective for a significant patient population requiring such concurrent therapy as defined in the labeling for the drug’.

There are antiretroviral drugs that should not be combined due to viral antagonism and overlapping toxicities. In addition, there are triple-combination regimens that have shown poor virological efficacy, likely due to an inadequate mutational barrier against the emergence of resistance (Yeni *et al*, 2002). Drugs and regimens that would not be

acceptable for FDCs or co-packaged because of known viral antagonism, poor virological efficacy, or toxicity, are listed.

Combinations of two or more active antiretroviral drugs in the FDA list are not the only type of FDC product suitable for combinations. For example, Kaletra (lopinavir/ritonavir), an approved FDC, is an antiretroviral combined with a metabolic booster. In this combination, a low dose of ritonavir (an inhibitor of cytochrome p450 4A) is used to increase plasma concentrations of Lopinavir, the component responsible for the antiviral efficacy. Other HIV protease inhibitors are often administered with low doses of Ritonavir and may be suitable for or co-formulation.

### 2.3.2 Clinical Considerations in Fixed Dose Combinations

For many potential FDCs FDA states that where adequate clinical studies confirming safety and efficacy of the combination have already been conducted, there is no need for new clinical studies. Applicants for FDC are required to provide clinical efficacy and safety information by one or more of the following mechanisms:

- Referencing their own relevant NDA or IND submission
- Cross-referencing another applicant's submission for which they have been given right of reference.
- Submitting peer-reviewed literature describing relevant clinical studies other scientific information and a summary that

synthesizes the information and provides the rationale for the combination.

- Relying on FDA's findings of safety and effectiveness for approved drug products, subject to U.S. intellectual property rights.

In general, clinical support for a FDC should include efficacy and safety data from at least one well-controlled study for at least 48 weeks in duration evaluating changes in HIV-RNA and $CD_4$ cell counts. Optimally, the study should have been designed to demonstrate statistical no inferiority, or superiority, of the regimen to an accepted control regimen (at the time study was conducted). In addition, other clinical studies evaluating components of the proposed regimen used in various triple combinations may help to support the efficacy of the proposed triple regimen. In some cases, clinical support for a regimen may be based on a collection of well-controlled triple-combination studies that, when evaluated together, provide a convincing rationale for the proposed combination (CEASAR, 1997).

**2.3.3 Dissolution Testing.**

A discriminating dissolution method should be developed, with limits set, for each active pharmaceutical ingredient in a drug product. The dissolution method should be incorporated into the stability and quality control programs. Dissolution testing should ensure that the presence of two or more drugs does not affect the dissolution performance testing. Additional details are given in the

guidance for 'dissolution testing of immediate release solid oral dosage forms" (BP, 2008).

### 2.3.4 Assurance of reproducible drug release from the dosage form.

It is important to establish that each manufactured lot of drug product will release all active ingredients at an appropriate rate. This is typically monitored by a dissolution test performed as part of the drug product specification. This test should use a physiologically relevant medium, one that can be correlated to an in vivo study, or a scientific justification for the dissolution medium (e.g. pH, composition) should be provided in the application (Emami, 2006). This factor has been strictly adhered to in this study.

### 2.3.5 Microbiological and Virological Considerations.

- Mechanism of action of the individual components
- Antiviral activity in vitro against standard laboratory strains and clinical isolates (including a variety of the most common HIV clades from diverse geographic regions), and effects of serum protein binding on antiviral activity.
- Cytotoxicity for dividing cells, including mitochondrial toxicity
- In vitro combination activity studies of the antiviral components to rule out antagonistic effects.
- In vitro selection of resistant virus and phenotypic/genotypic characterization of the isolates. When components of the

combination have the same target protein, selection of resistant virus in vitro should be carried out in the presence of the combination at concentrations equivalent to the in vivo concentrations. The genotypic and phenotypic nature of the resultant resistant isolates should be characterized to identify common resistance pathways.

FDCs and co-packaged products should contain drugs that together impose a significant mutational barrier for the development of resistance. In clinical studies, some triple-nucleoside regimens have been shown to have high virological failure rates associated with high rates of drug resistance. The cause of the high failure rates appears to be associated with the emergence of single or dual cross-resistant mutations that confer resistance to all three components (US FDA, 2002).

**2.4 COMBINATIONS FOR TREATMENT OF HIV/AIDS SUPPORTED BY CURRENT CLINICAL DATA FOR FDC.** (Guidance for Industry: Fixed dose combination and co-packaged drug products for treatment of HIV. May 2004)

**Two-drug combinations (to be used in combination with a third drug)**

- Abacavir + Lamivudine
- Didanosine + Lamivudine
- *****Didanosine + Stavudine *****
- Stavudine + Lamivudine
- Tenofovir + Emtricitabine
- Tenofovir + Lamivudine
- Zidovudine +Lamivudine (approved FDC, trade name Combivir$^{R}$)

**Three-drug regimens**

- Abacavir + Lamivudine + Lopinavir/Ritonavir
- Abacavir + Lamivudine + Nevirapine
- Abacavir + Lamivudine + Efavirenz
- Didanosine + Emtricitabine + Efavirenz
- Didanosine + Lamivudine + Efavirenz
- *****Didanosine + Zidovudine + Nevirapine*****
- Stavudine + Lamivudine + Efavirenz
- Stavudine + Lamivudine + Lopinavir/Ritonavir
- Stavudine + Lamivudine + Nelfinavir
- *****Stavudine + Lamivudine + Nevirapine *****
- Tenofovir + emtricitabine + Efavirenz
- Tenofovir + Lamivudine + Efavirenz
- Zidovudine +Lamivudine + Abacavir (approved FDC, trade name Trizivir$^{R}$)
- Zidovudine + Lamivudine + Efavirenz
- Zidovudine + Lamivudine + Lopinavir/Ritonavir

- Zidovudine +Lamivudine + Nelfinavir
- *****Zidovudine + Lamivudine + Nevirapine *****

**KEY: *** Fixed Dose Combinations undertaken in this study.

**Combinations with Viral Antagonism or Overlapping Toxicity:**

- Stavudine + zidovudine
- Stavudine + zalcitabine
- Didanosine + zalcitabine

**Combination with Inadequate Efficacy:**

- Abacavir + Lamivudine (or Emtricitabine) + Tenofovir
- Didanosine + Lamivudine (or Emtricitabine) + Tenofovir

Previous successful formulations of FDC antiretroviral drugs include the following:-

- FCD tablets of Nevirapine + Lamivudine + Stavudine, (Srinarong *et.al*. 2004).

Similar products which have recently been approved and are coming into the market are:-

- Combivir$^{R}$ (Zidovudine + Lamivudine),
- Trizivir$^{R}$ (Abacavir + Lamivudine + Zidovudine)

Combinations of:-

- Stavudine + Nevirapine + Lamivudine and
- Zidovudine + Lamivudine + Nevirapine are currently being marketed by Rambaxy.

## 2.5 EVALUATION OF CONTROLLED RELEASE DOSAGE FORMS

Controlled action dosage forms are evaluated *in vivo* only at the development stages but once data is obtained by clinical evaluation; the routine testing is done *in vitro* only.

### 2.5.1 *In-Vitro* evaluation

The fundamentals of such *in-vitro* evaluation involve measured quantities of aliquots, adjustments of pH, toxicity, enzymes levels, temperature and similarity movements. Since a sustained action dosage form may release drug both conditions to the gastric and intestinal fluids, measurements ought to be done in both to obtain a complete time release profile. The most commonly used method is one in which a single tablet/capsule is placed in 60 ml of the fluid contained in 90 ml cylindrical screw capped vials which are rotated end-over-end at $37^0$C (Brahmankan and Sunil 2000) . The quantity of drug released is estimated after ½ hour to bottle No. 1 Their estimation is done in bottle No. 2 after 1 hour After 1 to 2 hours the simulated gastric fluid is replaced by simulated intestinal fluid in the remaining vials and testing continued in consecutive vials up to 8 hour.

In another version of this method (Kendall *et.al.* 2009), an apparatus similar to the one used for dissolution time test of tablets has been suggested, from which aliquot samples are taken at predetermined time period, replacing the quantity of fluid samples by fresh simulated gastric/intestinal fluids as the case may be. The Food and Drug Administration of U.S.A. recommends a method in which 100 ml of gastric intestinal fluids are circulated with the help of a pump across the tablet and 5.0 ml of the fluid removed after every hour for estimation is replaced by fresh gastric/intestinal fluids.

In both the methods, the overriding consideration is to simulate body situations as precisely as possible.

### 2.5.2 *In-Vivo* Evaluation

*In vivo* evaluation can be a very complicated and involving affair. It is nothing short of a clinical trial (Emami, 2006). The first step in the in vivo testing of controlled action dosage forms is accumulation of sizeable data on animal systems. Then clinical trials are to be undertaken in collaboration with a physician, since animal data cannot be verbatim superimposed in man due to in built differences in the systems of animals and man. Statisticians may also be involved, since to provide for the inherent variation between one human being and another, a large number of individuals must be involved in the test to get a statistically valid picture. The fundamental principle in the in vivo testing is to administer the dosage form to volunteer patients and then at intervals of time evaluate either the drug levels in body fluids such as plasma or urine or to measure some pronounced and measurable pharmacological action, such as “degree of cough” or “sleep time”, etc. from this, a time concentration profile can be plotted.

## 2.6 POLYMETHACRYLATE POLYMERS

They provide unique, innovative solutions, for controlled drug delivery technology.

### 2.6.1 Reasons for Selecting the Polymethacrylate Polymers.

Polymethacrylate polymers, as manufactured by Rohm Pharma (P.P.R.P.) Germany, offers, a broad product spectrum of polymethacrylate-based pharmaceutical polymers, (Lehmann, 2001). The quality and the wide assortment of the polymers permit customized formulations for controlled drug release that is not found with any other type. The Rohm polymers have the following advantages:-

- *Targeted drug release into the various portions of the GI tract*
- Gastro resistant coatings
- Colon delivery
- Controlled-release in all portions of the GI tract

The polymethacrylate polymers feature high versatility in their applications. Their physicochemical properties open up a wide range of uses that make them ideal for use in various processing techniques. With these polymers, an optimum solution can be found for practically any oral solid dosing problem. The outstanding features of pharmaceutical dosage forms that are formulated with polymethacrylate polymers are:-

- Targeted release and accurate reproducibility
- Reliable function of the film formed
- High binding capacity for pigments
- High stability toward chemical influences during manufacture and storage.

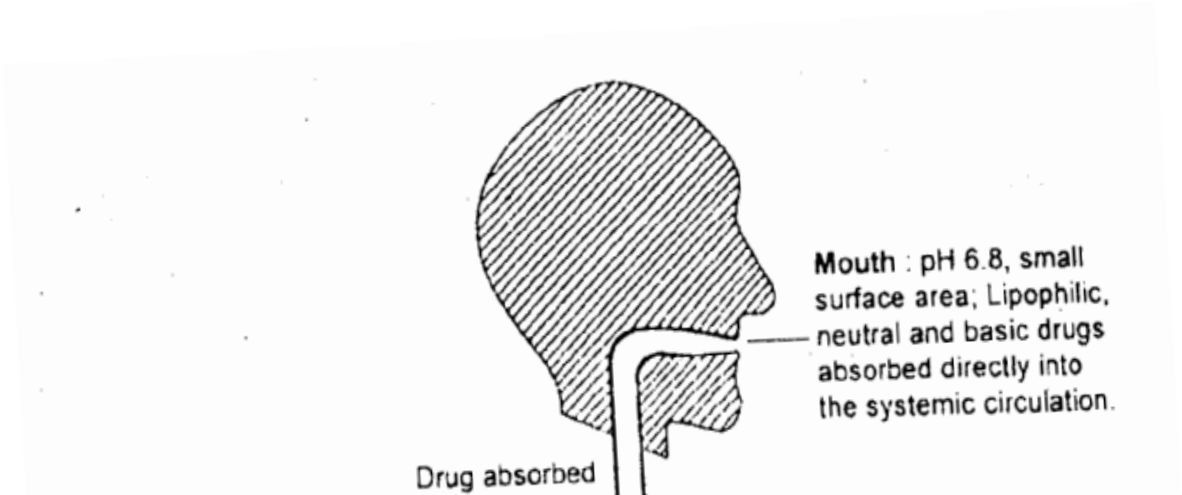


**Figure 2.3: Schematic Representation of the GIT And Different Sites of Drug Absorption (Howard 2000)**

**Table 2.2: Anatomical and functional differences between the important regions of the G. I. T (Mithal, 2000).**

| | Stomach | Small intestine | Large intestine | Rectum |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **pH range** | 1—3 | 5—7.5 | 7.9—8.0 | 7.5—8.0 |
| **Length** (cms) | 20 | 285 | 110 | 20 |
| **Diameter** (cms) | 15 | *2.5* | 5 | 2.5 |
| **Surface area** (sq.m) | 0.1 — 0.2 | 200 | 0.15 | 0.02 |
| **Blood flow** (l/mm) | 0.15 | 1.0 | 0.02 | - |
| **Transit time** (hrs) | 1 — *5* | 3.6 | 6-12 | 6-12 |
| **Absorptive role** | Lipophilic acidic and neutral drugs | All types of drugs | Some drugs water and electrolytes | All types of drugs |
| **Mechanisms of absorption** | Passive diffusion<br><br>Convection transport | All mechanisms of absorption | Passive diffusion<br>Convection transport | Passive diffusion<br><br>Convection transport.<br>Endocytosis |

McGinity and Cameron (1987), Lehman (1994), List (1982) and Sanchez-Lafuonte (2002), have demonstrated the possibility of using other types of polymers in their works, using various ARV drugs and other active ingredients, in controlled drug release formulations. They stated that the material properties of some of these polymers can be varied widely with additives and excipients. In this way, the polymers afford not only functional films (important for controlled-release capsules and granule coatings) and matrix structures, but also adhesive coatings with

precisely adjusted adhesive and cold flow properties. This implies economic advantages in terms of:-

- Film coatings, because generally, only minor layer thicknesses and polymer weights are needed to ensure the desired function. In some film coats, only about one hundredth of a millimeter (0.01mm) is needed to exert an effect (Lehmann 2001)

The polymethacrylate polymers, under the brand name EURAGIT are copolymers of esters of acrylic acid and methacrylic acid, the properties of which are determined by functional monomers. The individual polymethacrylate grades differ in the proportion of neutral, acidic or basic groups and hence in their physicochemical properties (Lehmann 2002).

### 2.6.2 Chemical Structure and Properties of Polymethacrylates.

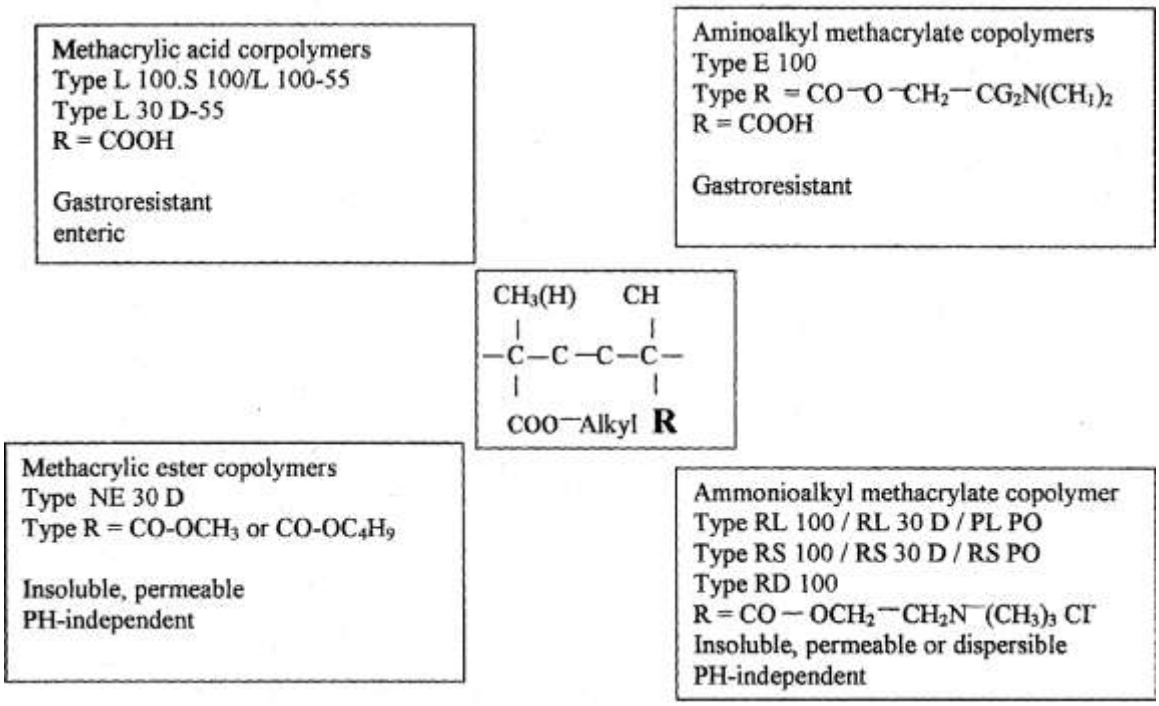



**Figure 2.4: Chemical Structure of the Polymethacrylate Polymers (Lehmann 2001).**

The line of polymers for oral dosage forms comprises two basic grades:

a) Polymethacrylates rendered soluble in gastric fluid by salt formation. These polymer grades contain graduated numbers of acidic or basic groups. They permit pH-dependent release of the active. Their scope of application extends from simple taste-masking to gastro resistant formulations with targeted drug release in all intestinal regions.

b) Polymethacrylates insoluble in digestive fluid: These grades permit time-dependent control of drug release and hence development of release formulations. These are water insoluble

swellable film formers based on neutral methacrylic esters or with a small proportion of trimetlhylammonioethyl methacrylate chloride. Formulations with these polymers provide for delayed drug release independently of pH, fewer quaternary ammonium groups are contained in the copolymer, the lower the permeability of the coating is, the more sustained release properties are achieved.

Coatings of anionic Polymethacrylates also permit G.I targeting. For this purpose, polymer grades with different carboxyl group contents which are also miscible with each other can be used, as shown by Sanchez (2002). In this way the pH at which the coating goes into solution, can be adjusted precisely. Because the pH increases along the intestine, the site of drug release can be controlled with such polymethacrylate coating.

Further, a gastro resistant (enteric) coating due to the insulating effect, provides for storage stability and increases patient compliance.

Drug release depends not only on the polymer used, but also on the thickness of the film coating and on the dissolution properties of the active agent under physiological conditions. This project is designed to take full advantage of this property of the polymethacrylate Polymers.

The special characteristics of gastro resistant polymethacrylate are as follows:-

- Protection of active ingredients sensitive to gastric fluid
- Protection of the gastric mucosa from aggressive drug

- pH-dependent drug release
- GI targeting (e.g. in the colon)
- Good storage stability

Examples of oral dosage forms, using time controlled drug release, independently of the pH of gastric fluids, have been made by Deshmukh *et al* (2003) and Munasir *et al* (2006).

Oral preparations with controlled time release of active can be formulated using swellable permeable polymethacrylate polymers. There are two possible ways of doing this:-

1. Polymethacrylate polymer is used as a coating material. Typically pellets or micro particles are coasted with a polymer film and filled into capsules. This method was selected for use in formulating ARV drugs.

In the digestive tract, the coated pellets or micro-particles act as diffusion cells and release a constant drug quantity per unit of time (multi-unit dosage forms).

2. Alternatively, the polymethacrylate polymers can serve as the matrix in which the drug is embedded. The matrix structure can be produced by direct compression or wet granulation.

Such as the characteristics of controlled release formulations with polymethacrylate polymers are:-

- Controlled time release;
- High reliability due to reproducible drug release;
- Generation of therapeutically optimized drug release profiles;

- Enhanced compliance with a single daily dose, and
- Economical processing.

Researchers, who have successfully used these polymethacrylate polymers, includes List and Kassis (1982) in the controlled release formulation of potassium chloride; Cameron and McGinity (1989) in the controlled release formulation of Theophylline and Lehman *et.al* (1994) in the controlled release formulation of Lithium Citrate.

## 2.7 PROCESSING OF POLYMETHACRYLATE POLYMERS

The physicochemical properties of polymethacrylates not only open up a wide spectrum of applications but also confer high flexibility in terms of processing. Generally the polymethacrylates can be processed as follows:-

- As an organic solution
- As an aqueous dispersion (latex dispersion)
- Completely solvent free by the hot-melt process and
- As a powder (direct compression)

The Rohm grade polymethacrylate polymer products are chemically stable polymers and are quite compatible with many other pharmaceutical excipients and process aids. Before processing, the addition of functional aids such as plasticizers or glidants is necessary. Wide variation possibilities in terms of grade and amount make it possible to develop individual dosage forms and optimize the formulation processes. The coating of cores (pellets, micro-particles granules and

tablets is accomplished by spraying to solutions or dispersion and is possible in all film coating or fluidized bed systems commonly used.

### 2.7.1 Controlled Release Formulations for Oral Dosage Forms with Polymethacrylate Polymers:

The acrylic polymers types RL, type RS and type NE were developed for pH-independent, delayed release of active ingredients from oral dosage forms. For coatings of graded permeability, type RL and type RS can be mixed in any desired ratio. The drug release can be continually controlled through all sections of the digestive tract, from the stomach to the colon. Anionic type L and type S grades permit the development of pH-dependent systems to achieve linear release profiles or to balance pH-dependent drug solubility. As the coating thickness increase, the typical properties of the polymer exert an ever greater influence on the dosage form (Lehmann 2000).

### 2.7.2 Mechanism of action of Polymethacrylates.

i) Eudragit RL, RS and NE act through diffusion controlled drug release.

ii) Eudragit RL and RS are water-insoluble, and act through swellable film formers based on neutral methacrylic acid esters with a small proportion of trimethylammonioethyl methacrylate chloride.

With Eudragit RL, the molar ratio of the quaternary ammonium groups to the neutral ester groups is 1:20 (corresponding to about 50 meq/100g). With Eudragit RS this ratio is 1:40 (corresponding to roughly 25 meq./100g). Since quaternary ammonium groups determine the

swellability and the permeability of the films into water, dissolved salts and medicinal substance, Eudragit RL, which contains more of these groups, forms highly permeable films with little delaying action. By contrast and owing to the reduced content in quaternary ammonium groups, films of Eudragit RS swell less easily and are only slightly permeable to active ingredients. Given coherent film coatings and an adequate layer thickness, it is therefore possible to slow down drug diffusion very noticeably.

iii) Eudragit NE, grade 30 D is neutral ester dispersion without any functional groups that forms water-insoluble films. This soft polymer is particularly suitable for granulation processes in the manufacture of matrix tablets and controlled-release coatings without any plasticizer addition (Lehmann 2001).

**2.7.3 Miscibility between Polymethacrylate Polymers.**

In the form of solutions or aqueous dispersions, Eudragit RL and RS polymers are freely miscible. Depending on whether it is the highly permeable (Eudragit RL or the slightly permeable Eudragit RS) component that predominates, the mixtures form films of varying permeability. The diffusion of active substance decreases progressively with increasing coating thickness.

By adding Eudragit L and/or S to RL or RS, the release profile in neutral to alkaline medium can be further influenced. In the case of thin film coats, release of the active ingredient can be effected through pores.

Their number and size depend mainly on the application technique and on the addition of soluble core constituents as well as on the excipients used. Given proper coating application, pore –free films are obtained at a thickness of about 1 to 3 mm. Drug diffusion then occurs through swellable, hydrophilic regions formed in the polymer matrix by quaternary ammonium groups.

In order to obtain films of adequate flexibility, 10 to 20% plasticizer on polymer has to be added to the organic solutions and 20% plasticizer on to the dispersions of Eudragit RL and RS. Glidants like talc, micronised amorphous silica gel and glycerol monostearate facilitate spray application to the cores. Opaque white or colored coatings can be obtained by adding pigments such as titanium dioxide, food colors and iron oxide (Lehmann 2002).

**2.7.4 Physico-Chemical Properties of Polymethacrylate Polymers.**

The quaternary ammonium groups, present as chlorides in Eudragit RL/RS polymers, are completely dissociated in the physiological pH range of about 1 to 8. In the initially formed film, the permeability of the coating is therefore independent of pH. Since welling and permeability are influenced by electrolytes, however, the release profiles observed in pure water are often not transferable to buffer solutions of different ionic strength. Furthermore, the permeability of the film coatings may be affected by ion exchange processes with buffer salts or medicinal agents.

Apart from neutral methacrylic acid esters, polymer Eudragit NE 30 D contains no further functional units. Even without the addition of plasticizer the product forms highly flexible, elastic films. Their permeability is comparable with that of polymer Eudragit RS and variable more or less exclusively, via the film thickness. For this reason, Eudragit NE 30 D is above all recommended for granulation in the manufacture of matrix tablets (Lehmann 2002).

The simulated digestive fluids specified in Pharmacopoeias are recommended for normal in-vitro release testing, whereas the approximately isotonic phosphate buffers according to USP/NF are suggested for testing in intermediate pH environments.

## 2.8 FORMULATION OF CONTROLLED RELEASE DOSAGE FORMS WITH POLYMETHACRYLATE POLYMERS.

The polymethacrylate polymer systems are suitable for the formulation of pellets, micro tablets, solid granules, compact crystals and various controlled-release dosage forms. If the active ingredient is to be released in dissolved form, this is usually affected by diffusion through polymer structures. With disintegrating dosage forms, release of the active ingredient is accelerated by an enlarged surface area. In the case of poorly soluble active ingredients, the release rate is frequently determined by the disintegration pattern of the dosage form (Lehmann 2001).

If the active ingredient is coated by a largely pore-free, only slightly permeable membrane, its delayed release can be controlled very effectively. However, it is not the degree of permeability of the coating membrane alone which determines the drug diffusion, the solubility of the drug in the buffer solutions and its molecular weight or molar volume in solvated form also play a part.

If the active ingredients are present as salts, much thought has to be given to their solubility as a function of pH and the variation of their properties, depending on whether they are neutral molecules or ions. Polymer films form diffusion cells. High concentrations of active ingredient in the core often lead to the formation of saturated solutions in these cells. Drug release then initially occurs linearly via zero-order kinetics, i.e. a constant amount of active ingredient is set free per unit of time. Each active ingredient and drug formulation requires a special release profile, which must be established by optimization of the applied coating of polymer Eudragit NE 30 D or of the mixing ratio of Type RL and Type RS to a reproducible coating layer of adequate thickness (Lehmann 1994).

Polymethacrylate polymer matrices provide dosage forms of good mechanical strength and control the diffusion of embedded active ingredients through pores and channels. Release of the active ingredients from matrix structures often occurs proportionally to square root of time ($\sqrt{t}$), i.e. an initial steep rise is followed by a gentler slope. In any case, it

is the type and quantity of polymethacrylate used which dictates the release pattern of the final dosage form.

**i) Enteric Coatings-pH Control with the Polymethacrylate Polymers**

Many pharmaceutical dosage forms irritate the stomach due to their chemical properties. Others undergo chemical changes in gastric acid and through the action of enzymes. Didanosine is an example of such a drug. Specific acrylic polymers have been developed for oral dosage forms, with step-wise release of active ingredients in the digestive tract.

Coatings which dissolved at rising pH values:

- Release of active ingredients in the duodenum with Eudragit L 100-55 or the aqueous dispersion Type L 30 D-55 at pH values over 5.5.
- Release of active ingredients in the jejunum to ileum with Eudragit L 100 at pH values over 6.0 or with mixtures of Type L 100 and Type S 100 in a pH range from 6.0 to 6.5
- Release of active ingredients near the colon with Type S 100 in a pH rang from 6.5 to 7.5.

Eudragit L and S**:** These Types are anionic polymers based on ethacrylic acid esters. The films are insoluble below pH 5 and thus resistant to gastric fluid. By salt formation in the neutral to weakly alkaline medium of intestinal fluid, the films dissolve step-wise at pH values above 5.5 (Lehmann 2003).

**ii) Polymethacrylate polymer properties:**

Of decisive importance for the controlled release of enteric-coated active ingredients is the dissolution profile of the Type L/S film formers in the intestinal pH range form 5.5 to 7.0. Previous studies (Lehmann 2001; 2002) have shown how the film coatings dissolve in the intestine. In the duodenum, a pH range of 5.5 – 6.0 is to be expected; in the lower sections of the intestine, the pH value normally increases gradually to about pH 6.5 –7.0 near the colon. However, the release of active ingredients also depends on the thickness of the film coatings and the solubility characteristics of the active ingredient under physiological conditions. All polymer types can be mixed with each other in any desired ratio, thus making it possible to adjust for intermediate values. The release values established in vitro must be confirmed in pharmacological and clinical tests.

Use in Various Applications**:** The polymers can be applied as coatings to all conventional, solid oral dosage forms such as tablets, capsules, small particles. These polymers can also be used to manufacture pellets, granules and controlled-release tablets and capsules (Dahlberg et al, 2010).

**iii) Polymers processing form**

- Solution in organic solvents (alcohols, acetone)
- Mixtures of organic solutions with water

- Purely aqueous latex dispersions

The coatings can also be processed with ease in all film-coating pans or fluidized-bed equipment.

Eudragit L/S coatings show an excellent sealing effect even at very thin layers. This enables the following formulation effects to be obtained:

- Protection against atmospheric humidity
- Isolating mutually incompatible particles in combination products
- Masking of cores with an unpleasant odour or taste
- Granulation of active ingredients in powder form

The sealing effect of the film coatings naturally increases in proportion to the film thickness. In that case however, the release of active ingredients in digestive fluids with a pH of less than 5 is also delayed. Compromises can; however be found (Lehmann 2000).

**iv) Addable excipients to the polymers:**

**a) Plasticizers**:

Films of polymethacrylate polymers tend to become brittle (cracking) below 10%. To improve the elasticity up to 25% can be added. Type L 100 and type S 100 in aqueous formulations require a much higher proportion of plasticizer. In all formulations, triethyl citrate has proved its worth as a plasticizer.

**b) Solvents**:

Acetone and alcohols are preferentially used for manufacturing polymer solutions. The average dissolution time in minutes, for the most common solvents or solvent/water mixture ranges from one to five minutes.

**c) Glidants**:

Polymer solutions and dispersions go through a tacky phase during drying. To avoid agglomeration of the cores, glidants are added to the spray suspensions. At critical points of manufactures, these can also be added in the form of a powder (Lehmann 2001):

- Talc and Kaolin are often used in combination with pigments.
- Glycerol monostearate e.g. Imwitor(R) 900 is a good alternative to talc as a glidant in all the aqueous formulations mentioned.
- Micronised silic acid can be used in quantities of 10-30% on polymer and does have a matting effect and increases the permeability of film coatings.
- Magnesium stearate is somewhat more effective than talc and often provides good sealing of the film coatings and low permeability. However, it can only be used in organic polymer solutions, since coagulation or thickening may occur in aqueous dispersions.
- Pigments for film coating processes, both aluminium colour lakes and iron oxides are suitable. Water soluble dyes usually cause inhomogeneous colouring of the coatings, which rub off during handling.

- Titanium dioxide can be used to adjust the intensity of the color coatings, which moreover does not rub off during handling.

## 2.9 PRODUCT EVALUATION AND TESTING: *IN-VITRO* MEASUREMENT OF DRUG RELEASE

Lehmann (1991) set the following parameters on *in-vitro* measurement of drug release: it is not possible to simulate in a single in vitro test system, the range of variables that affect drug release during the passage of controlled release medication through the GI tract. Properly designed in vitro tests for drug release serve two important functions. First, data from such tests are required as a guide to formulation during the development stage, prior to clinical testing. Second, in vitro testing is necessary to ensure batch-to-batch uniformity in the production of a proven dosage form design. Different methods are usually required by these two distinctly different testing objectives. Although attempts to correlate in vitro release profiles with clinical performance are useful once sufficient clinical testing has been completed, *in-vitro/in-vivo* correlation must not be assumed. *In vitro* studies are not sufficient to establish the efficacy of a new preparation.

Tests developed for the purpose of quality control are generally limited to USP dissolution testing methods, using the rotating basket (Apparatus 1), the paddle (Apparatus 2), or the modified dissolution testing apparatus (Apparatus 3). In many instances in which USP test

procedures are followed, upper and lower limits are specified for drug release in simulated gastric and/or intestinal fluid. Measurements are made at specified time intervals appropriate to the specific product. Complete release profiles are not measurable, unless automated techniques are used. Procedures are determined by nature of the dosage form (e.g. tablet or capsule), the principle utilized to control drug release (e.g., disintegrating or non-disintegrating), and the maintenance period.

During formulation development testing methods should be designed to provide answers to the following questions (Fan *et al*, 2009).

1. Does the product "dump" maintenance dose before the maintenance period is complete? Controlled release products are subject to either of two modes of failure: Insufficient dose is released, or too much drug is made available too quickly.
2. What fraction of the dose remains unavailable, i.e., what fraction will not be released in the projected time of transit in the GI tract?
3. What is the effect of physiologic variables on drug release?
4. Is the loading dose (if present) released immediately? Is release of the maintenance dose delayed? If so, is the delay time within the desired range?
5. What is the unit-to-unit variation? How predicable is the release profile?

6. What is the stability of the formulation with respect to its drug release profile?
7. In short, does the observed release profile fit expectations?

The methods used to measure drug release profile should have the following characteristics. Allowance should be made for changing the release media from simulated gastric to simulated intestinal fluid at variable programmed time intervals, to establish the effect of retention of the dosage form in gastric fluid as well as to approximate more closely the pH shifts that the dosage form is likely to encounter *in vivo*.

## 2.10 TYPES OF CONTROLLED DRUG DELIVERY SYSTEMS (CDDS) IN ARV THERAPY:

### 2.10.1 Oral delivery

Controlled drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimization of drug related side effects due to controlled therapeutic blood levels instead of oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered (Gates 1994). Bioadhesive drug delivery systems are designed for prolonged retention on the mucosa to facilitate drug absorption over a prolonged period of time by interacting with mucin (Kamath and Park, 1994). Hence, the

combination of both controlled release and bioadhesive properties in a delivery system would further enhance therapeutic efficacy. ARVs such as Didanosine (ddl) would be an ideal candidate for controlled drug release due to its short half-life of 1.3-1.6h, necessitating frequent administration of doses, as well as its severe dose dependent side effects (Li and Chan, 1999). In an attempt to improve the oral absorption of Didanosine by delivering it over a prolonged period of time, as well as prolonging retention on the mucosa, Betageri *et al* (2001) prepared a sustained release bioadhesive tablet formulation of Didanosine, containing polyox WSRN-303. Carbopol 974P-NF and Methocel K4M as polymeric matrix materials. Hydrogel forming tablet formulations with 10% and 30% polyox WSRN-303 were able to extend the release of Didanosine, while 30% methocel K4M was required for extending the drug release in other formulations. Preparations with Carbopol 934P prevented complete release of didanosine from the tablet during the test period, and the authors attributed this to drug-polymer interactions. The bioadhesivity also increased with an increase in polymer concentration. These researchers concluded that a single polymer could be used for the preparation of hydrogel matrix didanosine tablets, designed to provide both sustained release and bioadhesivity. However, while a single polymer may provide both bioadhesivity and sustained drug release, it has since become well recognized in the literature, via various in vitro drug release and bioadhesivity tests, during formulation studies, that

simulations optimization of both these properties will require the blending of various polymers (Betageri *et al* 2001; Munasur *et al* 2006 and Govender *et al*, 2005) for both single and multiple unit systems. These systems are yet to be investigated for their clinical applicability.

Didanosine controlled release matrix tablets containing methacrylic (Eudragit RSPM) and ethyl cellulose (Ethocel 100) polymers have also been prepared by Sanchez-Lafuente *et al* (2002). A Doehlert design was applied to evaluate the influence of variables and possible interactions among such variables on Didanosine release from the directly compressed matrix tablets based on the blends of the two insoluble polymers, Eudragit RSPM and Ethocel 100 (Sanchez-Lafuente et.al, 2002), the drug content and the polymers had the most significant effect on drug release, while the compression force had no significant effect. The optimum formulation conditions identified in the studied experimental design for a formulation with optimum drug release were Eudragit-Ethocel ratio of 83/17 (w/w) and a drug content of 13 % w/w. the experimental values obtained from the optimized formulation highly agreed with the predicted values, thereby validating the mathematical model used in the preparation of Didanosine tablets.

Didanosine also undergoes acid degradation in the gastric medium (Anderson *et al,* 1988). An enteric coated matrix tablet formulation that combines sustained drug release, bioadhesivity and an enteric coating to resist acid degradation to maximize therapeutic efficacy has also been

reported. Deshmukh *et al*, (2003) reported the preparation of enteric coated sustained release bioadhesive matrix tablets of didanosine comprising polyox, WSRN-303 and Methocel K4M with hydroxypropylmethylcellulose phthalate (HPMCP 5.5). The formulation was shown to be resistant to dissolution in 0.1N HCI but dissolved within 10 min in PBS of pH 7.4. Furthermore, the stability of the formulation for 6 months at varying storage conditions was confirmed. Permeation studies on the matrix tablets showed that polyox WSRN-303 containing tablets demonstrated higher Didanosine permeability across live intestinal tissue compared with conventional tablets.

While the above tablets sought to prove sustained drug release, bioadhesion and resistance to gastric acid degradation, a possible limitation could be the fact that it would still undergo extensive first pass degradation since it is meant for oral administration.

### *2.10.2 Buccal delivery*

Delivery of drugs via the buccal mucosa has received increased attention in the literature as an attractive alternative to the traditional oral and other conventional routes of drug administration. Use of the buccal mucosal route presents several advantages, such as the bypass of first pass hepatic metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs (Rossi *et.al* 2005): higher permeability than that of the other routes such as the skin (Squire and Hall, 1985): large surface area for drug

application, and good accessibility compared to other mucosal surfaces such as nasal, rectal and vaginal mucosa (Rathbone *et al*, 1994). ARV drugs may therefore benefit from buccal mucosal administration instead of traditional oral administration.

Studies investigating the feasibility of the systemic buccal delivery of anti-HIV drugs have emerged. (Shojael and Berner 1998) initially investigated the use of a safe and effective permeation enhancer, i.e., menthol, on the buccal permeation of didanosine. This study showed that the in vitro trans-buccal permeation of didanosine increased significantly in the presence of 1-menthol with an enhancement factor of 2.02 and a $t_{tag}$ of 6 h. The permeation enhancement was not concentration dependent as no significant difference was observed between the permeation enhancement of didanosine in the presence of 0.1, 0.2 and 0.3 mg/ml of 1-menthol (Shojael and Berner 1998). Latter, Xiang *et al* (2002) also studied the feasibility of trans-buccal delivery of Didanosine using Mclivaine buffer solution (MB). Their study focused on identifying the major permeation barrier within the epithelium of the buccal mucosa, the influence of sodium glycodeoxycholate (GDC) as a perbuccal mucosa. These researchers reported that the basal lamina layer within the epithelium of buccal mucosa acted as an important barrier to the permeation of didanosine. They also found that the permeability of didanosine was significantly enhanced by GDC up to 32 times. Histological studies revealed that the basal lamina remained

intact, and no nucleated cell leakage was found within 24h. These studies also showed that the thickness of epithelium was greatly reduced after buccal tissues were immersed in IMB solution for 12 and 24 h and no difference was observed between the tissue samples incubated in the IMB and Didanosine-IMP solutions. These two research groups concluded that trans-buccal delivery is a potential route of administration of Didanosine, and hence for enhancing antiretroviral drug therapy.

Unlike the transdermal route, the buccal route for ARV permeation potential has not been comprehensively investigated. The reported studies to date have focused only on two different permeation enhancers, and no studies on the formulation and assessment of buccal delivery systems of ARVs could be found.

**2.10.3 Rectal delivery**

The rectal route has also been considered for effective delivery of ARV drugs that undergo first pass hepatic metabolism and/or extensive GI degradation. Two studies were found to have been reported in the literature. Sustained release Zidovudine suppositories were prepared by (Kawaguchi *et al* 1991) using hydroxypropyl cellulose (HPC), and were assessed in rats. It was found that zidovudine suppositories at 10 mg/kg maintained constant plasma levels about 1 μM for more than 6 h and they subsequently proposed suppositories as an alternative drug delivery system for zidovudine. A further study of rectal administration of

zidovudine (Wintergerst *et al* 1997) showed that the drug was considerably absorbed after rectal administration, with a pharmacokinetic profile that resembles that of a sustained release delivery device. No further studies on this approach have since been identified in the literature. The work in this area appears to be limited, most probably due to patient inconvenience, as well as to the fact that HIV/AIDS patients often suffer from diarrhea.

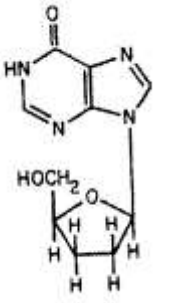
## 2 .11 PROPERTIES OF SOME ARV DRUGS

Source of ARV chemical structures: Martindale (2007) pharmacopoeia.

### 2.11.1. Didanosine

2', 3'-Dideoxyinosine

$C_{10}H_{12}N_4O_3$ = 236.2




Didanosine is a nucleoside reverse transcriptase inhibitor structurally related to inosine with activity against retroviruses including HIV. It is used in the treatment of HIV infection, usually with other antiretrovirals as part of combination therapy (Deshmukh *et al*, 2003).

Didanosine is given by mouth, as buffered chewable/dispersible tablets, or enteric-coated capsules, or oral solution. The tablets have a bioavailability 20 to 25% greater than that of the solution. Doses should be taken at least 30 minutes before, or 2 hours after, a meal. The total daily dose may be given as either a single dose or as two divided doses, the choice being dependent upon both the formulation and the strength used. Doses for adults are greater than 60kg body-weight, 400mg (tablets or capsules) or 500mg (oral solution) daily; under 60kg, 250mg (tablets or capsules) or 334 mg (oral solution) daily (Elion *et al*, 2006).

Didanosine is generally taken with an antacid (often included in the formulation), drugs that could be affected by an increased gastric pH (for example, Protease inhibitors, ketaconazole, fluoroquinolone antibacterial, and dapsone) should be given at least 2 hours before didanosine. Didanosine preparations containing magnesium or aluminium antacids should not be given with tetracycline.

Absorption of some HIV-protease inhibitors may be reduced by the antacids in didanosine formulations and doses should be separated by at least 2 hours (Elion *et al*, 2006).

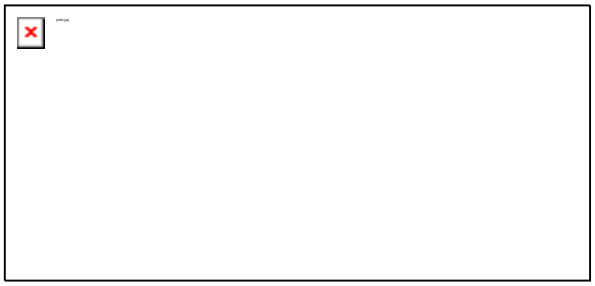
Didanosine is rapidly hydrolyzed in the acid medium of the stomach and is therefore given by mouth with pH buffers or antacids. Bioavailability is reported to range from 20 to 40% depending on the formulation used; bioavailability is substantially reduced by administration with or after food. Maximum plasma concentrations are achieved about 1 hour after

oral administration. Binding to plasma proteins is reported to be less than 5%. Didanosine has been reported not to cross the blood brain barrier. It is metabolized intracellularly to the active antiviral metabolite dideoxyadenosine triphosphate. The plasma elimination half-life is reported to about 1.5 hours. Renal clearance is by glomerular filtration and active tubular secretion; about 20% of an oral dose is recorded in the urine. Didanosine is partially cleared by haemodialysis but not by peritoneal dialysis (Cimoch 1998).

### 2.11.2 Indinavir Sulfate

(αR, γS,25)- α-Benzyl-2-(tert-butylcarbomoyl)-γ-hydroxy-N-[(IS,2R)-2-hydroxy-l-indanyl] -4-(3-pyridylmethy)-l- sulfate (1:1).

$C_{36}H_{47}N_5O_4,H_2SO_4$ = 711.9.



Indinavir is a protease inhibitor with antiviral activity against HIV. It is used with nucleoside reverse transcriptase inhibitors for combination therapy of HIV infection.

Indinavir is given by mouth as the sulfate, but doses are expressed in terms of the base. 116 mg of Indinavir sulfate is approximately equivalent to 100 mg of Indinavir. It is given in a usual adult dose of 800 mg every 8 h. It should be given either an hour before or two hours after

meals, or with a light, low-fat meal. Adequate hydration should be maintained. Treatment may have to be interrupted if acute episodes of nephrolithiasis occur (Flexner 1998).

Indinavir has also been recommended as part of the chemoprophylactic regimen with zidovudine and lamivudine in patients at high risk of HIV infection following occupational percutaneous exposure (Harris 1998).

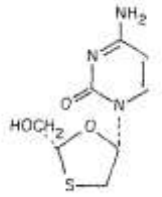
Indinavir is rapidly absorbed following oral administration procuring peak plasma concentrations in 0.8 hours. Bioavailability is about 65% following a single dose. Absorption is reduced by administration with a meal high in calories, fat, and protein but is less affected by a light meal. At doses up to 1g, increases in plasma concentration are proportionately greater than increases in dose. Plasma protein binding is about 60%. Indinavir is reported to cross the blood-brain barrier. It undergoes oxidative metabolism by cytochrome P450 isoenzyme CYP3A4 and glucuronidation. The elimination half-life is 1.8 hours. Less than 20% of the absorbed dose is excreted in the urine, about half of this as unchanged drug. The remainder is excreted in the faeces (Hammer 1998).

### 2.11.3. Lamivudine

3TC;L (-)-2'-Deoxy-3'-thiacytidine;

$C_8H_{11}N_3O_3S$ = 229.3.

(Lamivudine). A white or off-white solid. Soluble in water. Protect from light.




Lamivudine is rapidly absorbed following oral administration and peak plasma concentrations are achieved in about 1 hour. Absorption is delayed, but not reduced, by ingestion with food. Bioavailability is between 80 % and 87 %. Binding to plasma protein is reported to be up to 36 %. Lamivudine crosses the blood-brain barrier with a ratio of CSF to serum concentrations of about 0.12. It crosses the placenta and is distributed into breast milk. (Mueller *et al.* 1998)

Lamivudine is metabolized intracellularly to the active antiviral triphosphate. Hepatic metabolism is low and it is cleared mainly unchanged by active renal excretion. An elimination half-life of 5 to 7 hours has been reported following a single dose. (Bruno *et al. 2001*)

Lamivudine is a nucleoside reverse transcriptase inhibitor structurally related to cytosine with activity against retroviruses including HIV. It is used, usually with other antiretrovirals, for combination therapy of HIV infection. It is also used for the treatment of chronic hepatitis B.

For HIV infection, the dose of lamivudine for adults is 300mg by mouth daily as a single dose or in two divided doses (Eron 1995).

Lamivudine is a potent inhibitor of HIV-1 and HIV-2 in vitro, including variants resistant to zidovudine (WHO Drug Inf., 1996). Resistance emerges rapidly when lamivudine is given alone to patients with HIV

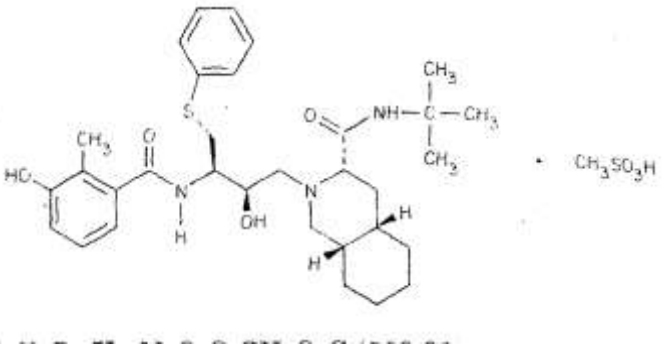
infections (Wainberg *et al*, 1999) although sustained responses have been reported despite the emergence of resistance (Ingrand *et al*, 1995). Combination therapy with lamivudine delays, and may even reverse the emergence of zidovudine resistance and produces a sustained synergistic antiretroviral effect, (Larder *et.al*, 1995), but HIV strains resistant to both lamivudine and zidovudine may arise (Miller *et al*, 1995). A combination therapy, typically with two nucleoside reverse transcriptase inhibitors and either a non-nucleoside reverse transcriptase inhibitors or an HIV-protease inhibitor, is standard therapy for HIV infection. Treatment with lamivudine-e plus zidovudine has produced better responses than either drug alone in antiretroviral-naive patients, (Katlama *et al*, 1998) and has produced additional responses in antiretroviral-experienced patients, with little additional toxicity (Barlett *et al*, 1998). The addition of lamivudine to existing antiretroviral therapy was reported to slow the progression of disease and improve survival (CAESAR Coordinating Committee, 1997), and treatment with lamivudine, Indinavir, and Nevirapine produced beneficial responses in patients who had previously failed on combined nucleoside analogue therapy (Harris *et al*, 1998). Clinically useful CNS concentrations of lamivudine were achieved in patients with HIV infection given combination therapy with lamivudine and zidovudine or Stavudine (Florida *et al*, 1997).

Lamivudine is also used in prophylactic regimes following occupational exposure to HIV infection and has been tried for reducing vertical transmission from mother to neonate (PETRA Study Team, 2002).

### 2.11.4. Nelfinavir Mesilate

35[2(25',35'), 3a,4aβ, 8aβ]-N-(1, 1-Dimethylethy) decahydro-2-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl-3-isoquinolinecarboxamide monomethanesulphonate.

$C_{32}H_{45}N_3O_4S_1CH_4O_3S$ = 663.9.



Nelfinavir is absorbed from the gastrointestinal tract and peak plasma concentrations occur in 2 to 4 hours. Absorption is enhanced by administration with food. Nelfinavir is extensively bound to plasma proteins (more than 98%). It is distributed into breast milk. Nelfinavir is metabolized by oxidation by cytochrome P450 isoenzymes including CYP3A the major oxidative metabolite has in-vitro antiviral activity equal to that of nelfinavir. The terminal half-life is 3.5 to 5 hours. Nelfinavir is excreted in the faeces mainly as metabolites. Only about 1 to 2% is excreted in the urine (Chinen 2008).

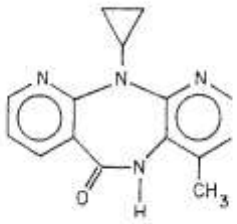
Nelfinavir is a protease inhibitor with antiviral activity against HIV. It is used with nucleoside reverse transcriptase inhibitors for combination therapy of HIV infection.

Nelfinavir is given by mouth as the mesilate, but doses are expressed in terms of the base. Nelfinavir mesilate 292 mg is approximately equivalent to 250 mg of Nelfinavir. Nelfinavir is available as tablets and oral powder. The oral power should not be taken with acidic foods or drinks as this may result in a bitter taste. Nelfinavir is given in an adult dose of 1.25 g twice daily or 0.75 g three times daily with food. Children aged 3 to 13 years may be given 50 to 55 mg/kg twice daily or 25 to 30 mg/kg three times daily.

**2.11.5. Nevirapine**

11-Cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e]-[1,4] diazepin-6-one.

$C_{15}H_{14}N_4O$ = 266.3.




Nevirapine is readily absorbed following oral administration and absorption is not affected by food. Bioavailability is greater than 90%. Peak plasma concentrations occur 4 h after a single dose. Nevirapine is about 60 % bound to plasma proteins. Concentrations in the CSF are

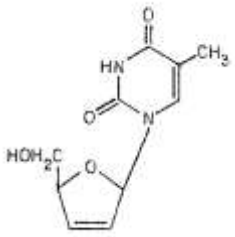
about 45 % of those in plasma. Nevirapine crosses the placenta and is distributed into breast milk. It is extensively metabolized by hepatic microsomal enzymes, principally by cytochrome P450 isoenzymes of the CYP3A family. Auto-induction of these enzymes results in a 1.5- to 2-fold increase in apparent oral clearance after 2 to 4 weeks' administration of usual doses, and a decrease in terminal half-life from 45 hours to 25 to 30 hours over the same period. Nevirapine is mainly excreted in the urine as glucuronide conjugates of the hydroxylated metabolites (Graham 1992).

Nevirapine is a non-nucleoside reverse transcriptase inhibitor with activity against HIV-1. It is used in the treatment of HIV infection. Viral resistance emerges rapidly when Nevirapine is used alone, and it is used in combination with other antiretrovirals.

**2.11.6. Stavudine**

1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine.

$C_{10}H_{12}N_2O_4$ = 224.2.




Stavudine is absorbed rapidly following oral administration producing peak plasma concentrations within 1 h and with a reported bioavailability of about 86%. Administration with food delays but does

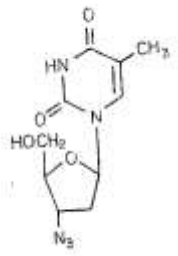
not reduce absorption. Stavudine crosses the blood-brain barrier producing a CSF to plasma ratio of about 0.4 after 4 h. Binding to plasma proteins is negligible. Stavudine is metabolized intracellularly to the active antiviral triphosphate. The elimination half-life is reported to be about 1 to 1.5 hours following single or multiple doses. The intracellular half-life of Stavudine triphosphate has been estimated to be 3.5 hours in vitro. About 40% of a dose is excreted in the urine by active tubular secretion and glomerular filtration (Hurst 1999).

Stavudine is a nucleoside reverse transcriptase inhibitor related to thymidine with activity against retroviruses including HIV. It is used in the treatment of HIV infection, usually in combination with other antiretrovirals. However, use with zidovudine is not recommended. Usual adult doses of Stavudine are 40 mg every 12 hours by mouth for patients weighing 60 kg or more or 30 mg every 12 hours for patients weighing less than 60 kg (Grant 2006).

### 2.11.7. Zidovudine

Azidodeoxythymidine; Azidothymidine; AZT: 3'-Azido-3'-deoxythymidine.

$C_{10}H_{13}N_5O_4$ = 267.2.



Ph. Eur. 5.0 (zidovudine). A white to brownish powder, zidovudine shows polymorphism. Soluble in water; soluble in dehydrated alcohol. Protect from light.

Zidovudine is rapidly absorbed from the gastrointestinal tract and undergoes first-pass hepatic metabolism with a bioavailability of about 60 to 70 %. Peak plasma concentrations occur after about 1 hour. Absorption is delayed by administration with food, but bioavailability is probably unaffected. Zidovudine crosses the blood-brain barrier producing CSF to plasma ratios of about 0.5. It crosses the placenta and is distributed into breast milk. It has been detected in semen. Plasma protein binding is reported to be 34 to 38 %. The plasma half-time is about 1 h (Barry 1994).

Zidovudine is metabolized intracellularly to the anti-viral triphosphate. It is also metabolized in the liver, mainly to the inactive glucuronide, and is excreted in the urine as unchanged drug and metabolite.

Zidovudine is a nucleoside reverse transcriptase inhibitor structurally related to thymidine. It has activity against retroviruses including HIV and is used in the management of HIV infection. Zidovudine is given in combination with other antiretroviral to symptomatic and selected a symptomatic patient. It is used alone to prevent vertical transmission from mother to infant (Burger 1994).

Zidovudine is given by mouth to adults in doses of 500 to 600 mg daily in divided doses. Higher doses maybe required for neurological disease.

Zidovudine may be given by intravenous infusion of a solution containing 2 to 4 mg/ml over 1 hour for short-term management of patients unable to take it by mouth. The adult dose is 1 to 2 mg/kg every 4 h (equivalent to an oral dose of 1 .5 to 3 mg/kg every 4 hours).

For the prevention of maternal-fetal HIV transmission, zidovudine may be given orally after the fourteenth week of pregnancy until the beginning of labor in a dose of 100 mg five times daily. During labour and delivery, zidovudine is given by intravenous infusion in a dose of 2 mg/kg over 1 h, then 1 mg/kf per h until the umbilical cord is clamped. When a caesarean section is planned the intravenous infusion is started 4 h before the operation. The new born infant is given 2 mg/kg orally every 6 hours starting within 12 h after birth and continuing for 6 weeks.

The use of antiretroviral drugs in HIV infection has changed following studies that indicated that combination therapy could improve response. Monotherapy with zidovudine reduced the incidence of opportunistic infections and mortality in patients with AIDS dementia. (Hochester 1999)

Therapy with a combination of zidovudine and other antiretroviral drugs might improve efficacy, minimize toxicity, and delay drug resistance. Results from the Delta study (Delta coordinating committee, 1996) and the US AIDS clinical trial group 175 (ACTG 175) study showed combination therapy to be more effective than monotherapy in antiretroviral-native patients and have led to profound changes in

clinical practice. Both studies showed substantial reductions in mortality at 30 months in antiretroviral-naive patients treated with zidovudine plus either didanosine or zalcitabine compared with those receiving zidovudine alone. Triple therapy with zidovudine combined with another nucleoside reverse transcriptase inhibitor and either an HIV-protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (HAART regimens) have been found to reduce viral loads more effectively than monotherapy or two-drug combination therapy and such regimens are currently regarded as standard (Connor 1994).

2.12 AIMS AND OBJECTIVES:

The main aim of this project is to reduce the "pill-burden" of the HIV/AIDS patient, by formulating as once daily dosage spansule capsules. The means of controlling the release of the ARV drugs from the spansule capsule will be using polymethacrylate polymers as the release controlling agents.

SCOPE OF THE STUDY:

- Seven of the commonly employed ARV drugs namely (didanosine, indinavir, lamivudine, nelfinavir, nevirapine, stavudine and zidovudine) and their clinically approved combinations, will be formulated into controlled release spansule dosage forms.
- Five grades of polymethacrylate polymers manufactured by Rohm Pharma Polymers will be used, namely: Eudragit L 100 which is pH dependant for drug delivery in duodenum and jejunum;

Eudragit S 100 which is pH dependent for drug delivery in ileum; Eudragit RL 100 which is insoluble high permeability, to coat granules for immediate sustained-release; Eudragit NE 100 which is insoluble, low permeability, for intermediate sustained release and Eudragit RS for long term sustained release in colon region.

# CHAPTER THREE

# 3. MATERIALS AND METHODS

## 3.1 MATERIALS

### 3.1.1. CHEMICALS

**a. Anti-Retroviral Drugs.**

Pure powders of seven anti-retroviral drugs listed below were obtained from Cipla Limited, Mumbai Central, Mumbai 400 – 008. India.

(i) Reverse Transcriptase Inhibitors (NRTI):-

- Zidovudine (AZT)
- Didanosine (DDL)
- Stavudine (D4T)
- Lamivudine (3TC)

(ii) Protease Inhibitors:-

- Indinavir
- Nelfinavir

(iii) Non-nucleoside reverse transcriptase Inhibitors:-

- Nevirapine.

Seven proprietary anti-retroviral formulations manufactured by Cipla Limited, Mumbai Central, Mumbai 400 – 008. India, were also used in the study. They are:-

- Zidovir-100$^{R}$ (Zidovudine)
- Divir-100 $^{R}$ (Didanosine)

- Stavir-30 R (Stavudine)
- Nevimune R (Nevirapine)
- Lamivir R (Lamivudine)
- Indivir R (Indinavir)
- Nelvir R (Nelfinavir)

**b. Coating Polymers:** The following polymers, all obtained from Rohm Pharma GmbH & Co. KG Darmstadt. Germany, were used in this study:-

- Polymer Type NE 30 D {Poly (ethyl acrylate-co-methyl methacrylate)}
- Polymer Type NE 30 L {Poly(methacrylic acid-co-methyl methacrylate)}
- Polymer Type S { Poly (methacrylic acid-co-methyl methacrylate)}
- Polymer Type FS {Poly (methacrylate-co-methylmethacrylate-co-methacrylic acid)}
- Polymer Type RL {Poly (ethylacrylate-co-methylmethacrylate-co-trimetlhylammonioethyl methacrylate chloride)}
- Polymer Type RS {Poly (ethylacrylate-co-methylmethacrylate-co-trimetlhylammonioethyl methacrylate chloride)}

**c. Plasticizers**:-

- Triethyl citrate (USP):- Reilly Chemicals, S.A., Bruxelles.
- Polyethylene glycol (USP): – Dow Chemical Company, USA.
- Glyceryl triacetate (USP):- BASF Chemicals, Germany.

**d. Pigments**:-

- Titanium dioxide (USP), Sunset yellow; Blue No2:- Degussa, Germany.

**e. Excipients**:-

- Talc (USP):- Merck GmbH. Frankfurt, Germany.
- Magnesium Stearate: - Merck GmbH. Frankfurt, Germany.
- Microcrystalline Cellulose: - Penwest, Germany.
- Maize Starch: - Roquette GmbH. Frankfur, Germany.

**f. Antifoam Agent**:-

- Simethicone Emulsion: - Dow Corning, USA.

**g. Emulsifiers**:-

- Sodium Carboxymethly Cellulose (USP):- FMC Corp. USA.
- Povidone:- Merck, GmbH, Darmstad, Germany.

**h. pH control Buffers**:-

- Disodium hydrogen orthophosphate anhydrous BDH Laboratory, Poole, England
- Potassium dihydrogen phosphate crystals. Fine chemical Manufacture Division, New Jersey. USA

## 3.2 METHODS

### 3.2.1 Determination of Absorption Peaks

As a tool of identification for quality and quantity control, absorption peaks were determined, using the Helios UV – visible spectrophotometer, (model v 4.60, Switzerland), connected with a H.P. DeskJet 895 cxi printer. Using the Intelliscan mode and a bandwidth of 2 nm, samples of zidovudine, didanosine, stavudine, lamivudine (nucleoside analogs) indinavir, nelfinavir (protease inhibitors) and nevirapine (NRTI) were dissolved in water, ethanol, dilute HCL, and phosphate buffers. These were scanned at wave length range of 198 nm to 1000 nm for absorption peaks. Plots are shown in the Appendix. Peaks of maximum absorption were selected for use in plotting the Beer's plots.

### 3.2.2 Solubility Studies of the antiretroviral drugs:

Preliminary solubility analysis was conducted for all the seven ARV drugs in order to determine saturation concentrations for each drug.

The saturation limits for all the seven antiretroviral drugs were determined as described in the USP under solubility test (USP, 1988). One gram of each powder was weighed and dissolved or suspended in water, ethanol or isopropyl alcohol. This was allowed to stabilize for about one h. A filter paper (labtech, no. 1) was weighed using a Metler analytical balance (Switzerland) and wetted in distilled water. The suspension was then filtered using the wetted paper. The paper was

dried in the oven at 60 ° C and weighed again to find out the amount of solute left. The balance of the solute in water gave the weight in the saturated solution. Based on the resulting values, various drug concentrations were used for the studies.

### 3.2.4 Preparation of Standard Curves

To enable determination of quantities of the drugs during the dissolution studies, calibration curves were made as follows:

For each of the seven ARV drugs, stock solutions and dilutions were prepared in water, ethanol, isopropyl alcohol, dilute HCL and phosphate buffers. The absorbance values were measured spectrophotometrically (Spectrophotometer, Spectrum lab, model 7525, manufactured by B.Bran Scientific and instrument company, England), at predetermined peaks. Beer-Lambard's plots were made as shown in Appendix. Readings were in triplicate and the average plotted.

### 3.2.4 Formulation of Granules by Wet Granulation

The following basic working formular was adopted for each of the active ingredients: -

| Composition | Quantity | Conc. ranges |
|---|---|---|
| Active ingredient (24 hrs daily dose of ARV): | 75% | * |
| Microcrystalline cellulose (Diluent): | 15% | * |
| Carboxymethylcellulose or PVP (Binder): | 5% | * |
| Maize Starch as (granulating agent/disintegrant): | 5% | * |

* the quantities in grams are given for each ARV in their individual working formula tables 3.1 to 3.11.

Microcrystalline cellulose was placed in a mortar; an equal quantity of the active ingredient was added and triturated. Starch was then added with trituration followed by more of the active ingredient, using the doubling up technique (Cooper 1978) until all the decreasing quantities were incorporated. Carboxymethylcellulose was then added and mixed. The powdered mixture was then spread out on the mortar and distilled water sprayed, sparingly on the mixed powder in a controlled manner using a pressure sprayer. The end point was when the powder has absorbed just enough moisture to produce a moist slightly sticky mass.

Approximately 10 to 12 mls of distilled water was used. The moist mass was then passed through laboratory test sieve of 1.00 mm aperture.

The sieved mass was placed in a Gallenkamp BS oven (England), and dried at a temperature set at 60 $^{0}$ C for one hour. The dried granules were dry screened using sieves of 1.00 mm aperture. Granules with sizes of 1.00 mm were selected (with the aid of a micro meter screw gauge-digitech, India) for coating using the different types of polymers. Granules of smaller sizes (< 1.00 mm) were kept for use as the immediate release portion of the dosage form.

The larger size granules were then divided into two equal parts, one part was further divided into four equal portions. These were kept aside, to be coated later with different polymers. This process was done for all the seven ARV drugs and their various combinations in triplicate and all measurements were taken and the average reported.

## 3.3 WORKING FORMULA AND QUANTITIES

### 3.3.1 ARV Monotherapy

**Table 3.1: Working Formula for Spansule Containing 400 mg Didanosine:**

| **Ingredients** | **Granule Core Quantities** |
|---|---|
| Didanosine | 400 mg |
| Microcrystalline cellulose (Diluent) | 25 mg |
| Carboxymethyl cellulose (binder) | 15 mg |
| Starch (granulating agent) | 15 mg |

| **Polymer Type Coating** | **Qty. of ARV** | **Target Release Site** |
|---|---|---|
| **0. L (**Enteric Coated Loading dose) | 200 mg | Duodenum |
| **1. S** | 50 mg | Jejunum/Ileum |
| 2. RL | 50 mg | Small Intestine |
| 3. RS | 50 mg | Large Intestine |
| 4. NE | 50 mg | Colon |

**Table 3.2: Working Formula for Spansule Containing Indinavir 2.400 mg:**

| Ingredients | Granule Core Quantities |
|---|---|
| Indinavir | 2,400 mg |
| Talc | 20 mg |
| Microcrystalline cellulose (diluent) | 360 mg |
| Carboxymethyl cellulose (binder) | 120 mg |
| Starch (granulating agent) | 120 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| 0. Un-Coated Loading dose | 2,400 mg | Stomach |
| 1. S | 400 mg | Jejunum/Ileum |
| 2. RS | 400 mg | Large Intestine |
| 3. NE | 400 mg | Colon |
| 4. NE: RS | 400 mg | Intermediate |

Talc was used as a lubricant to prevent the sticking together of Indinavir granules.

**Table 3.3: Working Formula for Spansule Containing Lamivudine 300mg:**

| Ingredients | Granule Core Quantities |
|---|---|
| Lamivudine | 300 mg |
| Microcrystalline cellulose (diluent) | 25 mg |
| Carboxymethyl cellulose (binder) | 15 mg |
| Starch (granulating agent) | 15 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| 0. Un-Coated Loading dose | 150 mg | Stomach |
| 1. S | 37.5 mg | Jejunum/Ileum |
| 2. RS | 37.5 mg | Large Intestine |
| 3. NE | 37.5 mg | Colon |
| 4. RS: NE: RL | 37.5 mg | Intermediate |

**Table 3.4: Working Formula for Spansule Containing 2,500 mg Nelfinavir:**

| **Ingredients** | **Granule Core Quantities** |
|---|---|
| Nelfinavir | 2,500 mg |
| Microcrystalline cellulose (diluent) | 375 mg |
| Carboxymethyl cellulose (binder) | 125 mg |
| Starch (granulating agent) | 125 mg |

| **Polymer Type Coating** | **Qty. of ARV** | **Target Release Site** |
|---|---|---|
| 0. Un-Coated Loading dose | 1,250 mg | Stomach |
| 1**. S** | 312.5 mg | Jejunum/Ileum |
| 2. RL | 312.5 mg | Large Intestine |
| 3. NE | 312.5 mg | Colon |
| 4**.** RS: NE: RL | 312.5 mg | Intermediate |

**Table 3.5: Working Formula for Spansule Containing 400 mg Nevirapine:**

| **Ingredients** | **Granule Core Quantities** |
|---|---|
| Nevirapine | 400 mg |
| Microcrystalline cellulose (diluent) | 60 mg |
| Carboxymethyl cellulose (binder) | 20 mg |
| Starch (granulating agent) | 20 mg |

| **Polymer Type Coating** | **Qty. of ARV** | **Target Release Site** |
|---|---|---|
| **0. Un-**Coated Loading dose | 200 mg | Stomach |
| 1**. S** | 50 mg | Jejunum/Ileum |
| 2. RS | 50 mg | Large Intestine |
| 3. NE | 50 mg | Colon |
| 4**.** RS: RL | 50 mg | Intermediate |

**Table 3.6: Working Formula for Spansule Containing 80 mg Stavudine:**

| **Ingredients** | **Granule Core Quantities** |
|---|---|
| Stavudine | 80 mg |
| Microcrystalline cellulose (diluent) | 12 mg |
| Carboxymethyl cellulose (binder) | 4 mg |
| Starch (granulating agent) | 4 mg |

| **Polymer Type Coating** | **Qty. of ARV** | **Target Release Site** |
|---|---|---|
| **0. Un-**Coated Loading dose | 150 mg | Stomach |
| 1**. S** | 10 mg | Jejunum/Ileum |
| 2. RS | 10 mg | Large Intestine |
| 3. NE | 10 mg | Colon |
| 4**.** RS: RL | 10 mg | Intermediate |

**Table 3.7: Working Formula for Spansule Containing 600 mg Zidovudine:**

| **Ingredients** | **Granule Core Quantities** |
|---|---|
| Zidovudine | 600 mg |
| Microcrystalline cellulose (diluent) | 90 mg |
| Carboxymethyl cellulose (binder) | 30 mg |
| Starch (granulating agent) | 30 mg |

| **Polymer Type Coating** | **Qty. of ARV** | **Target Release Site** |
|---|---|---|
| **0. Un-**Coated Loading dose | 300 mg | Stomach |
| 1**. S** | 75 mg | Jejunum/Ileum |
| 2. RL | 75 mg | Small Intestine |
| 3. RS | 75 mg | Large Intestine |
| 4**.** NE | 75 mg | Colon |

### 3.3.2 Fixed Dose Combinations for Highly Active Anti Retroviral Therapy (HAART):-

**Table 3.8: Working Formula for Spansule Containing Stavudine (40 mg BD) 80 mg + Didanosine (200 mg BD) 400 mg. 2nd line treatment: Ingredients Granule Core Quantities**

| Ingredients | Granule Core Quantities |
|---|---|
| Stavudine 80 mg + Didanosine 400 mg | 480 mg |
| Microcrystalline cellulose (diluent) | 72 mg |
| Carboxymethyl cellulose (binder) | 24 mg |
| Starch (granulating agent) | 24 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| **0. L Enteric-c**oated Loading dose | 240 mg | Duodenum |
| 1**.** S | 60 mg | Jejunum |
| 2. RS | 60 mg | Large Intestine |
| 3. NE | 60 mg | Colon |
| 4**.** RL: NE: RS | 60 mg | Intermediate |

**Table 3.9: Working Formula for Spansule Containing Nevirapine (200 mg BD) 400 mg + Zidovudine (300 mg BD) 600 mg + Didanosine (200 mg BD) 400 mg. 3rd line treatment:**

| Ingredients | Granule Core Quantities |
|---|---|
| Nevirapine + 400m g + Zidovudine 600 mg + Didanosine 400 mg | 1400 mg |
| Microcrystalline cellulose (diluent) | 210 mg |
| Carboxymethyl cellulose (binder) | 70 mg |
| Starch (granulating agent) | 70 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| 0. L Enteric-coated Loading dose | 700 mg | Duodenum |
| 1. S | 175 mg | Jejunum |
| 2. RS | 175mg | Large Intestine |
| 3. NE | 175mg | Colon |
| 4. RL: NE: RS | 175mg | Intermediate |

**Table 3.10: Working Formula for Spansule Containing Stavudine (40 mg BD) 80 mg + Lamivudine (150 mg BD) 300 mg + Nevirapine (200 mg BD) 400 mg. 1st line treatment:**

| Ingredients | Granule Core Quantities |
|---|---|
| Stavudine 80 mg + Lamivudine 300 mg + Nevirapine 400 mg | 780 mg |
| Microcrystalline cellulose (diluent) | 117 mg |
| Carboxymethyl cellulose (binder) | 39 mg |
| Starch (granulating agent) | 39 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| **0. Unc**oated Loading dose | 390 mg | Stomach |
| 1**.** S | 97.5 mg | Jejunum |
| 2. RS | 97.5 mg | Large Intestine |
| 3. NE | 97.5 mg | Colon |
| 4**.** RL: NE: RS | 97.5 mg | Intermediate |

**Table 3.11: Working Formula for Spansule Containing Zidovudine (200 mg BD) 400 mg + Lamivudine (150 mg BD) 300 mg + Nevirapine (200 mg BD) 400 mg. 1st line treatment:**

| Ingredients | Granule Core Quantities |
|---|---|
| Zidovudine 400 mg + Lamivudine 300 mg + Nevirapine 400 mg | 1100 mg |
| Microcrystalline cellulose (diluent) | 165 mg |
| Carboxymethyl cellulose (binder) | 55 mg |
| Starch (granulating agent) | 55 mg |

| Polymer Type Coating | Qty. of ARV | Target Release Site |
|---|---|---|
| 0. Uncoated Loading dose | 550 mg | Stomach |
| 1. RL | 137.5 mg | Jejunum |
| 2. RS | 137.5 mg | Large Intestine |
| 3. NE | 137.5 mg | Colon |
| 4. RL: NE: RS | 137.5 mg | Intermediate |

### 3.6 Smoothening Of Granules In Coating Pan:

The selected granules were moistened with polymethacrylate dispersions and rounded by dusting with finely powdered ingredients. The size of the final granules gave a core / shell structure of 0.9 to 1.2mm in size. This was determined using a micro meter screw gauge (digitech; India).

### 3.7 Estimation Of Surface Area Of Granule And Polymer Quantities:

The average particle size selected for this work was 1.0mm. The surface area was calculated using the equation:

$$S = \pi \text{ x } . d^2 = mm^2 \qquad (1)$$

Were S is surface area ($mm^2$)

d is diameter (mm)

$\pi = 3.141$

$S = 3.141 \times 1^2$

$= 3.14\ mm^2$

**Polymer Quantity Requirement:**

Amount of coating polymer required is determined using the formular below:-

$$A = S/W \qquad (2)$$

Were A = coating in %

S = surface area in $mm^2$

W = weight of granules in mg

The surface area of the granules was then divided by its weight w (mg), the required coating quantity in % was then obtained, i.e. the polymer quantity required per $cm^2$

Both quantities are linked by the factor 100 which leads to the result in percentage.

Results obtained from investigating various coating thicknesses revealed that polymer applications of about 5% to 10% are required for gastro resistance (Lehmann, 2000). This range becomes the target value. The quantity of polymer required was cross-checked with values stated in the graph produced by (Lehmann, 2000), which relates polymer requirement as a function of particle size (appendix III).

### 3.6 Division of Granules into Initial (Loading) Dose and Follow-Up (Maintainace) Portions:

Table 3.12: Division of Granules into Initial Dose and Maintainace Portions:

| S/No. | Name of Drug And Strength | Dosage Regimen for Adults | Total Daily Daily Dose | Proposed Spansule Formulation Uncoated | Coated/Portion |
|---|---|---|---|---|---|
| 1. | Zidovudine Tabs.(300 mg) | 300 mg b.i.d | 600 mg | 300 mg | 300 mg (75 mg/fraction) |
| **2.** | Lamivudine Tabs.(150 mg) | 150 mg b.i.d | 300 mg | 150 mg | 150 mg (37.5 mg/fraction) |
| 3. | Didanosine Buffered Tabs 100 mg | 100 mg 2 b.i.d (enteric coated) | 400 mg (enteric coated) | 200 mg (enteric coated) | 200 mg (50 mg/fraction) |
| 4. | Stavudine 4 0 mg | 40 mg b.i.d | 80 mg | 40 mg | 40 mg (10 mg/fraction) |
| 5. | Nevirapine 200 mg | 200 mg b.i.d | 400 mg | 200 mg | 200 mg (50 mg/fraction) |
| 6. | Indinavir 400 mg | 400 mg 2 t.i.d | 2,400 mg | 800 mg | 1,600 mg (400 mg/fraction) |
| 7. | Nelfinavir 250 mg | 250 mg 5 b.i.d or 3 t.i.d | 2,500 mg | 750 mg | 1,750 mg (437.5 mg/fraction |

### 3.7.1 Determination of Effective Coating Thickness

The quantities of effective coating polymers used in this study were obtained using the following method:

Since a certain thickness has to be achieved in film coating, the amount of coating material required is related to the surface area of the substance. This amount is expressed in mg of dry polymer substance per $cm^2$ of surface area to be coated.

The surface area of pharmaceutical dosage forms was calculated according to the formular given in equation (1) by Lehmann, 2000.

When the surface area of a substrate S ($mm^2$) is divided by its weight W (mg), the required coating quantity in % is obtained. This represents the Polymer consumption in Kg of dry polymer per 100 kg of substrate for a coating of dry polymer substrate per $cm^2$. When lower or higher coating weights are required, multiplication is done by this additional amount A (mg polymer per $cm^2$) as follows:

$$\text{Coating weight (\%)} = S\ (mm^2)\ .\ A\ mg/\ cm^2\ /\ W\ (mg) \qquad (3)$$

The method of Lehman (2000) outlined above was used. For a dosage form, slightly convex, 7.0 mm in diameter, 3.6mm in thickness, 140mg in weight, quantity of polymer was obtained as follows:

Polymer quantity is obtained by the formular:

$$P = S / W \quad (4)$$

Where P = Coating quantity in %

S = Surface area ($cm^2$)

W = Weight in mg

When additional coating weights are required, the formular becomes:

$$P = S . A / W \quad (5)$$

Where A = Additional amount of coating weight.

### 3.7.2 Preparation of Polymer Solutions

EUDRAGT L100 is available as a solid powder. Redispersion was obtained by addition of sodium hydroxide solution (prepared by dissolving 4 g of sodium hydroxide in 96 g of water). The polymer was added to a beaker containing the stated quantity of water in portions, using a stirrer with adjustable speed. The plasticizer (triethyl citrate 21.78 g) was then added into the beaker and homogenized for 10 min. This was then sieved with a filter paper (0.2 – 0.4 mm).

### 3.7.3 Coating operation method

The suspensions were continuously sprayed unto the rounded granules, which were prewarmed (using Heat Gun. HG – 600B, 2000 watt, manufactured by Woermann, Germany) to about 37˚C, by means of Woermann spray gun (top spray method) using Air Compressor (with pressure control gauge, ABAC model). The coating fluid was sprayed in counter current manner, from above until the desired coating thickness was achieved.

A continuous supply of warm air and sufficient drying time was found to be critical in obtaining good quality coats.

### 3.7.4 Disintegration test

The procedure described in BP (2003) Ph.Eur (2002) USP (2009) for disintegration test was employed. The apparatus was suspended in a one-litre beaker, containing the specified test fluid. The beaker contained enough fluid for the mesh bottom of the basket to be at least 15 mm

below liquid level at its highest point and at least 25 mm away from the vessel bottom at its lowest point. The open ends of the glass tubes were adjusted to always be above liquid surface level. The temperature of the fluid was maintained at $37^0$C with the aid of a thermostat. The test starting liquid was 0.1 M hydrochloric acid, to simulate gastric fluid. The apparatus was kept running for 2 h without disks. Thereafter, the rigid rack was removed and the tablets were inspected for signs of disintegration, (exhibited by fragments of coating or cracks that might lead to release of the active).

Subsequently, the test fluid was replaced with phosphate buffer solution pH 6.8 as simulated intestinal fluid and a loading disk was placed on top of each tablet in its tube. The basket-rack assembly was then moved up and down for 60 minutes, whereupon the condition of the tablets was evaluated. Simulated gastric fluid: 0.1M hydrochloric acid pH 1.0 simulated intestinal fluid: phosphate buffer pH 6.8. At each stage pH was monitored using BBR full range indicator paper, pH 1 – 14: manufactured by Chem.-0-craft Chemical Company, India and pH Meter. Labtech Digital, India.

### 3.8 Processing Granules with Polymethacrylate Polymers.

Spherical or round granules of 1.00 mm – 1.2 mm diameter sizes prepared earlier, were used for the coatings. The granules to be coated (batch size of 1 kg), were put inside a Manesty Pan coating machine, operated at a speed of 25 r.p.m attached to ABAC model air compressor

set, which has a capacity of 125 g/minute. The coating Polymer solution was put into the Woerman spay tank, attached to a spray gun unit. The spray nozzle was set at 1.5 mm distance from the granules. The coating solution was then sprayed onto the granules at a pressure of 2.2 bars. Continuous spraying of the granules was done for about 20 min, at a temperature of 39 C. Continuous spraying of the granules was done in a counter motion to the direction of the pan motion. Spayed granules were first dried at 60 C using HG 600B heat gun for 10 min in the pan and further dried at 40 C for 2 h in the oven.

For safety reasons, ventilation masks with class 1 air filters and plastic eye goggles were worn through out the procedure. The laboratory was kept well ventilated to prevent build up of fumes.

### 3.9 Formulation of Various Types of Polymer Dispersions:

Table 3.13: Composition of Various Types of Polymer Dispersions.

| | Quantities in the Different Polymer Dispersions (gm) | | | | | |
|---|---|---|---|---|---|---|
| **Ingredients** | **1** | **2** | **3** | **4** | **5** | **6** |
| **Polymers:** | | | | | | |
| **Polymer L 30 D55** | 1.670 | --- | --- | --- | --- | --- |
| **Polymer L 100** | **---** | 1.755 | --- | --- | --- | --- |
| **Polymer L 100/S100** | --- | --- | 0.600 | --- | --- | --- |
| **Polymer S 100** | **---** | **---** | **---** | 1.440 | --- | --- |
| **Polymer RL 30 D/ RS 30 D** | --- | --- | --- | 3.334 | --- | |
| **Polymer NE 30 D** | --- | --- | --- | --- | --- | 3.333 |
| **Solvents** | | | | | | |
| **NaOH 4%** | --- | 0.585 | --- | --- | --- | --- |
| **KOH** | --- | --- | --- | --- | --- | 0.734 |
| **Water** | 3.335 | 3.510 | 0.500 | 4.306 | 3.366 | 3.665 |
| **Pigment Suspensions:** | | | | | | |
| **Triethyl Citrate** | 0.050 | 0.175 | 0.060 | 0.720 | 0.175 | 0.060 |
| **Talc** | 0.830 | --- | 0.300 | --- | --- | 1.000 |
| **Glycerol Monostearate** | -- | 0.035 | --- | --- | --- | --- |
| **Titanium dioxide** | 0.500 | --- | --- | --- | --- | --- |
| **Na CMC** | 0.035 | --- | --- | --- | --- | --- |
| **Isopropyl Alcohol** | --- | --- | 8.540 | --- | --- | --- |
| **PEG 6000** | 0.080 | --- | --- | --- | --- | --- |
| **Water** | 3.500 | 3.940 | --- | 2.757 | --- | --- |

### 3.10 Coating of granules

Coatings of the granules were carried out as described in section 3.8.

Agglomeration occurred in the formulation of Indinavir granule. As a result, the spraying was interrupted until the granules were dry and able to flow freely. Subsequently, the processing was continued at a reduced spray rate. To improve the flow of granules, small quantities of talc were added.

### 3.11 Weighing and Encapsulation

The uncoated granules were weighed as specified for each preparation and filled into empty capsule shells, selected as follows:-

Size 000 for 1,000 mg; size 00 for 600 mg and size 0 for 500 mg. In selecting the proper size of capsule, one dose was weighed and by trial, the proper size was ascertained. The smallest capsule that held the dose was selected because it will be the most convenient for swallowing.

The filling of capsule was done in such a way that no air spaces were visible within the capsule. After the size has been selected, the required numbers of capsules were removed from the container; their removal singly during filling process could result in the contamination of the remaining capsule.

In filling the capsules, accuracy and cleanliness were strictly adhered to by using the most sensitive mettle balance (0.1 mg sensitivity). Each capsule was weighed separately because the capsule was designed to

provide an accurate dose of medication as calculated and shown in the individual formulas.

The coated granules (quantity differs with each ARV); to be encapsulated were placed on a paper and pressed down with a spatula until the depth is approximately one third the length of the capsule body. The empty capsule base was held between the thumb and the index finger and was repeatedly pressed vertically until filled. The cap was fitted over the base and the filled capsules weighed using an empty capsule of the same size as the tare.

The attraction of gelatin for moisture required observance of care, in handling the capsule. Traces of moisture on the capsule caused sticky surfaces to which drug material adhered to. The best method of protection was found to be wearing of rubber gloves.

**3.12. Dissolution and Drug Release Tests**

The DGN-multipurpose drug test device was used for the dissolution tests, model DGN – A Type. Manufactured in China and composed of a 12 chambers disintegration unit and a 6 chambers dissolution unit with time, temperature and speed controls

Test conditions were as specified for dissolution test in the (USP 2000; BP 2007). Temperature was set at 37 ± $1^0$ C. Stirring speed was set at 100 r.p.m. volume of fluid used was 1000 ml. Release tests were first conducted in 0.1N HCL within 2 h and then continued in re-buffer solutions by addition of the stated quantities of disodium hydrogen

orthophosphate and dihydrogen orthophosphate at 2 h intervals, as shown in table 3.13. The pH was monitored using BBR full range indicator paper pH 1 – 14, manufactured by Chem. – 0 – Craft Chemical Company. This method simulates the natural course of events in the body, such as absorption of active ingredients, addition of fresh digestive fluid as gradual increase in pH (Munzel, 2003) and provides a good amount of test fluids for analysis of the drug released.

Samples of 10 ml were taken at the stated intervals, filtered through filtered paper and analyzed spectrophotometrically. Digital pH meter manufactured by Labtech was used in monitoring the pH of all sections of simulated g.i.t fluids. For each spectrum reading, the wave length was adjusted to lambda max. Using the appropriate Beer's plot, ARV drug concentrations were determined. Triplicate dissolution runs were carried out and the average recorded.

**3.13 Test fluids for in-vitro dissolution tests:** The fluids used as dissolution media are as displayed in Table 3.16.

**Table 3.14 Composition of pH in Test Fluids**

| | Test Fluid Composition Qty | pH | Simulated Region | Duration of Test |
|---|---|---|---|---|
| **I** | Disodium hydrogen Orthophosphate 28.8g + Dihydrogen Orthophosphate 11.45g + Distilled water to 1,000ml | 6.8 | Reference Test Fluid | --- |
| **II** | HCL 3.65g + Distilled water to 1,000ml | 1.0 | Stomach | 2 hours |
| **III** | Disodium Hydrogen Orthophosphate 20.3g + Dihydrogen Orthophosphate 8.08g | 4.8 | Duodenum | 2 hours |
| **IV** | Disodium Hydrogen Orthophosphate 23.74g + Dihydrogen Orthophosphate 9.408g | 5.6 | Small Intestine | 2 hours |
| **V** | Disodium Hydrogen Orthophosphate 30.9g + Dihydrogen Orthophosphate 12.26g | 7.3 | Large Intestine | 2 hours |
| **VI** | Disodium Hydrogen Orthophosphate 33.9g + Dihydrogen Orthophosphate 13.44g | 8.0 | Colon | 2 hours |

# CHAPTER FOUR
# RESULTS

All readings under results were taken in multiples and mean recorded, Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated from the mean, according to the following formulas (Ansel and Stoklosa 2006):

SD = √ sum of (deviations) $^2$ / number of deviations minus one

$$= \sqrt{\sum d^2 / n-1} \quad (1)$$

$$RSD = SD / mean \times 100 (\%) \quad (2)$$

Were d is deviations from mean and n is number of readings.

The USP (2003) states the relative standard deviation for oral solids should be less than 6 %.

## 4.1 Peaks of maximum absorption of the Antiretroviral Drugs

| DRUG | Lambda maximum (λ nm) | Absorbance | RSD % |
|---|---|---|---|
| Didanosine | 468.0 | 4.865 | 0.02 |
| Indinavir | 440.0 | 5.110 | 0.01 |
| Lamivudine | 562.0 | 4.772 | 0.02 |
| Nelfinavir | 582.0 | 5.472 | 0.04 |
| Nevirapine | 494.0 | 4.893 | 0.01 |
| Stavudine | 386.0 | 4.927 | 0.01 |
| Zidovudine | 408.0 | 5.631 | 0.02 |

The lambda maximums represent the wave length of maximum absorption for each of the antiretroviral drugs and are shown in figures 1 to 7 of appendix IV. This wave length serves as the selected spectrometer setting for each drug.

## 4.2 Solubility of the Antiretroviral Drugs

The solubility of the substances refers to the approximate solubility at room temperature of 20 $^0$C - 25 $^0$C.

Table 4.2: Solubility of the antiretroviral drugs:

| **ANTIRETROVIRAL** | **SOLUBILITY (mg/ml)** | | |
|---|---|---|---|
| **DRUG** | **Water** | **Ethanol** | **Isopropyl Alcohol** |
| Zidovudine (NRTI) | 27 | 67 | 75 |
| Lamivudine (NRTI) | 90 | 70 | 80 |
| Didanosine (NRTI) | 122 | 80 | 67 |
| Stavudine (NRTI) | 87 | 110 | 165 |
| Nevirapine (NNRTI) | 22 | 60 | 76 |
| Indinavir (PI) | 217 | 54 | 75 |
| Nelfinavir (PI) | 215 | 360 | 340 |

Solubility of the ARV drugs in water is important for this study because distilled water is the dissolution medium recommended for release studies in BP, EP and USP. Figure 4.1 gives the disintegration time of various coating thickness of polymer type L for enteric coating. At 2

mg/cm$^2$, there was no disintegration for 120 min in 0.1 M HCL. Disintegration occurred within 30 minutes in phosphate buffer of pH 6.8.

**4.3 Investigating Different Coating Thicknesses.**

Table 4.3: Effect of Various Coating Thicknesses on weight:

| **Coating wt/Surface area (mg/cm$^2$)** | **Coated Tablet wt. (mg)** | **Polymer Coating wt. (mg)** | **RSD (%)** |
|---|---|---|---|
| 0 | 140.0 | 0 | 0.0 |
| 1 | 141.5 | 1.5 | 1.0 |
| 2 | 143.0 | 3.0 | 1.1 |
| 3 | 144.6 | 4.6 | 2.0 |
| 4 | 146.3 | 6.3 | 2.3 |
| 5 | 147.8 | 7.8 | 2.5 |
| 6 | 149.4 | 9.4 | 2.5 |
| 7 | 150.9 | 10.9 | 2.5 |
| 8 | 152.5 | 12.5 | 2.6 |
| 9 | 154.0 | 14.0 | 3.1 |
| 10 | 155.5 | 15.5 | 3.3 |
| 15 | 163.4 | 23.4 | 3.3 |
| 20 | 171.1 | 31.1 | 3.9 |

The above table shows that the weight of granules increased proportionally with increase in quantity of coating polymer.

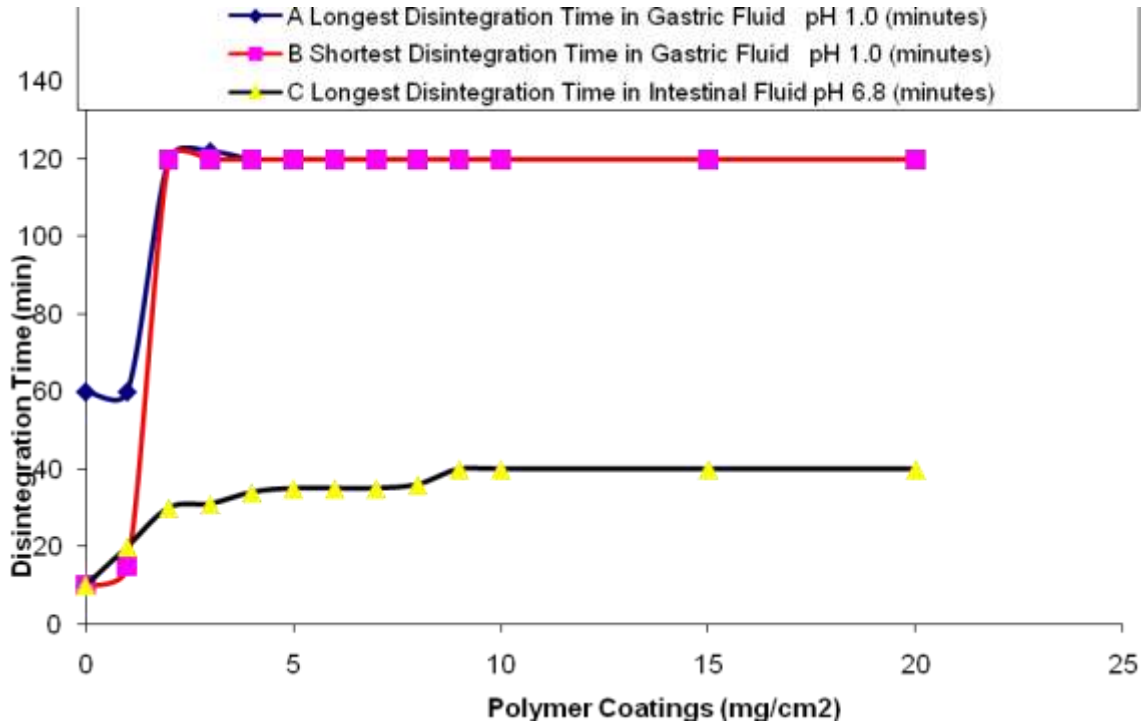



Figure 4.1: Plot of Polymer Coating thickness against disintegration time in gastric and intestinal fluids.

As presented in Fig. 4.1, in spansule formulation using polymer Type L did not disintegrate at a coating thickness of 2 mg/cm² for longer than 120 min. This is the pharmacopeial standard for enteric coating. At this thickness, disintegration occurred within 30 min, at intestinal pH 6.8. This means that drug release commence at this point. To ensure full enteric coating and give additional margin of error, 5 mg/cm² was selected for use in this study. Use of this range is supported by the work of Lehmann (2001).

## 4.3 DRUG RELEASE PROFILES OF FORMULATIONS CONTAINING SINGLE ANTI-RETROVIRAL

### 4.3.1. DIDANOSINE

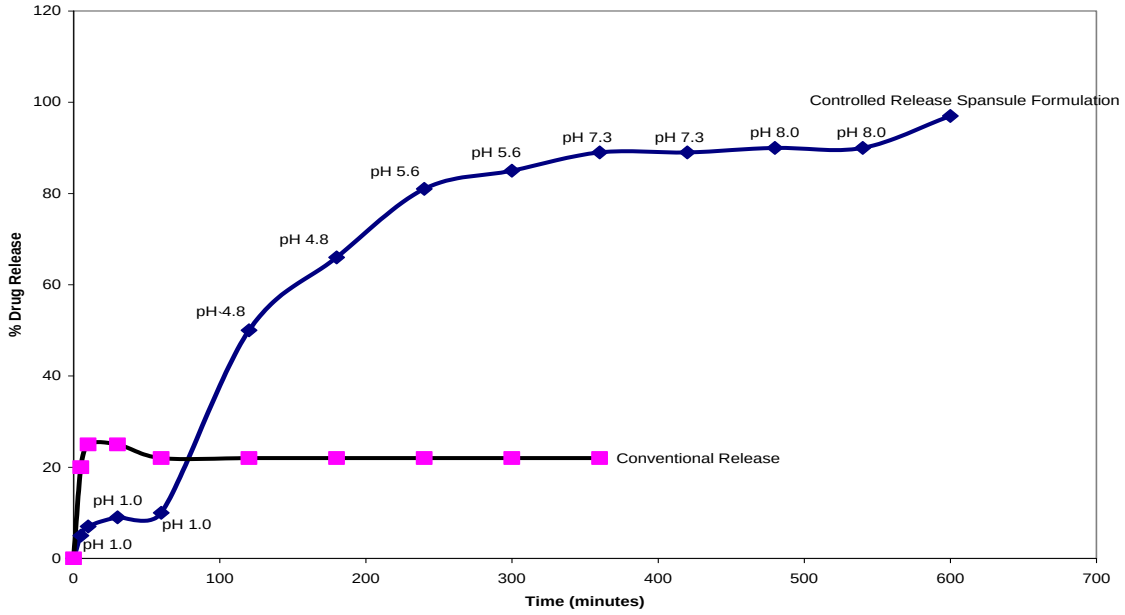



Figure 4.2: Comparative release profile of didanosine from conventional and spansule dosage formulation.

Drug release profiles presented in fig.4.2 showed that conventional tablet of didanosine gave a maximum drug release of 25 % within 5 min, reducing and stabilizing to 22 % from 50 to 360 minutes. Damle (2002) reported that Didanosine is rapidly hydrolyzed in acid media with a release range of 20% to 28%. Sanchez (2002) confirmed this result. In contrast, there was steady and gradual increase in the release of Didanosine from the spansule formulation, reaching 80% after 4 h. Thereafter, further drug release was only slightly increased, peaking at 97% in 10 h.

### 4.3.2. INDINAVIR

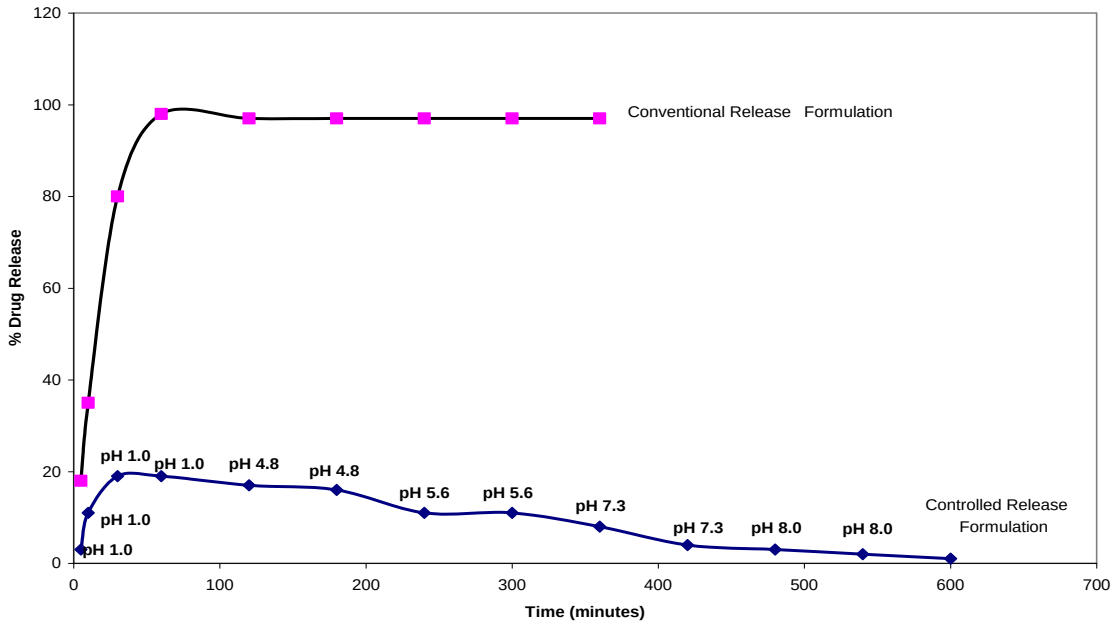



Figure 4.3: Comparative release profile of Indinavir from conventional and spansule dosage formulation.

As shown in Fig. 4.3, conventional Indinavir formulation gave a maximum drug release of 99% within 60 min. This was maintained for over 200 min. In contrast, there was steady and gradual decrease in the release of indinavir from the spansule formulation, after reaching a peak of only 19% at 30 min. The drug release dwindled to 1% by 10 h.

### 4.3.3. LAMIVUDINE

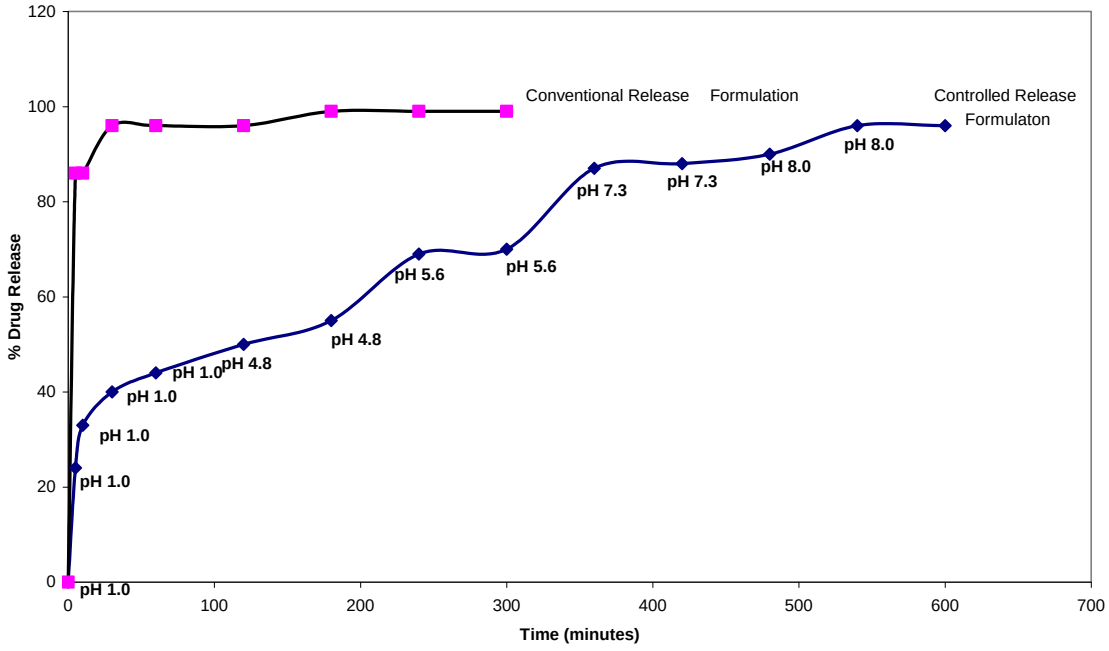



Figure 4.4: Comparative release profile of lamivudine from conventional and spansule dosage formulation.

Fig. 4.4 showed that conventional lamivudine formulation gave a drug release of 96 % within 20 min, stabilizing at this level to a maximum of 99 % at 180 min. In contrast, there was steady and gradual increase in the release of Lamivudine from the spansule formulation, reaching a peak at 10 h.

### 4.3.4. NELFINAVIR

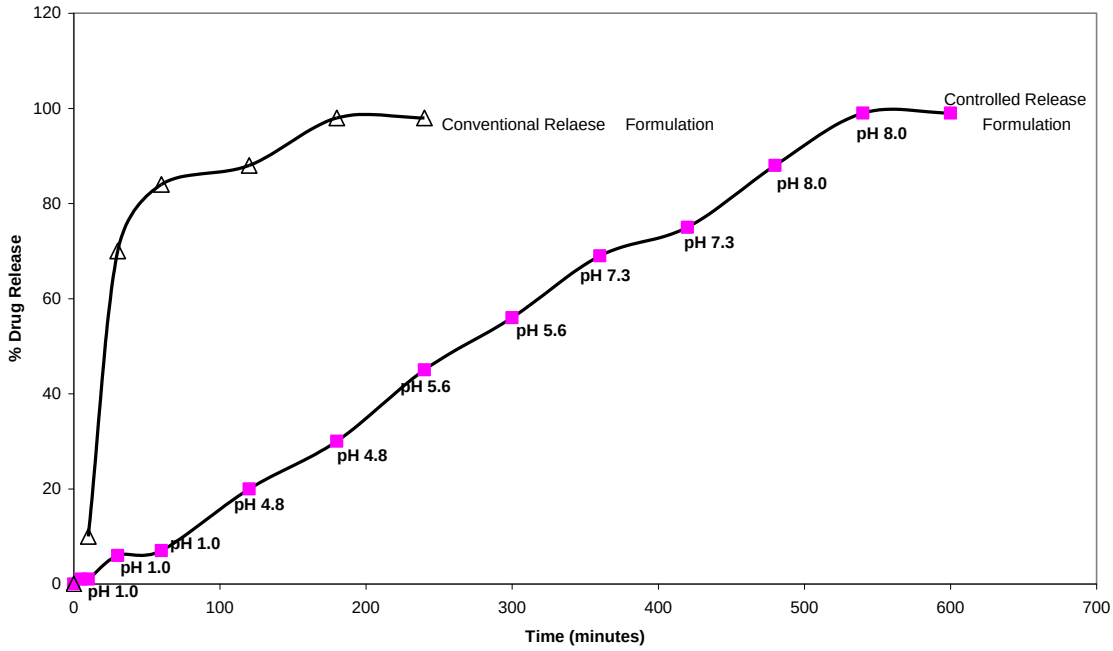



Figure 4.5: Comparative release profile of nelfinavir from conventional and spansule dosage formulation.

As shown in Fig. 4.5, conventional nelfinavir formulation gave a drug release of 84 % within 40 min, which steadily rose to a maximum of 98 % at 180 min. In contrast, there was steady and gradual increase in the release of nelfinavir from the spansule formulation with time, reaching a peak at 500 min. little of drug in the spansule formulation was released at pH 1.0 (less than 10 %), but at pH 4.6, release increased to 30 %. Generally, as the pH of the medium increased, more of the drug was released from the spansule formulation.

### 4.3.5. NEVIRAPINE

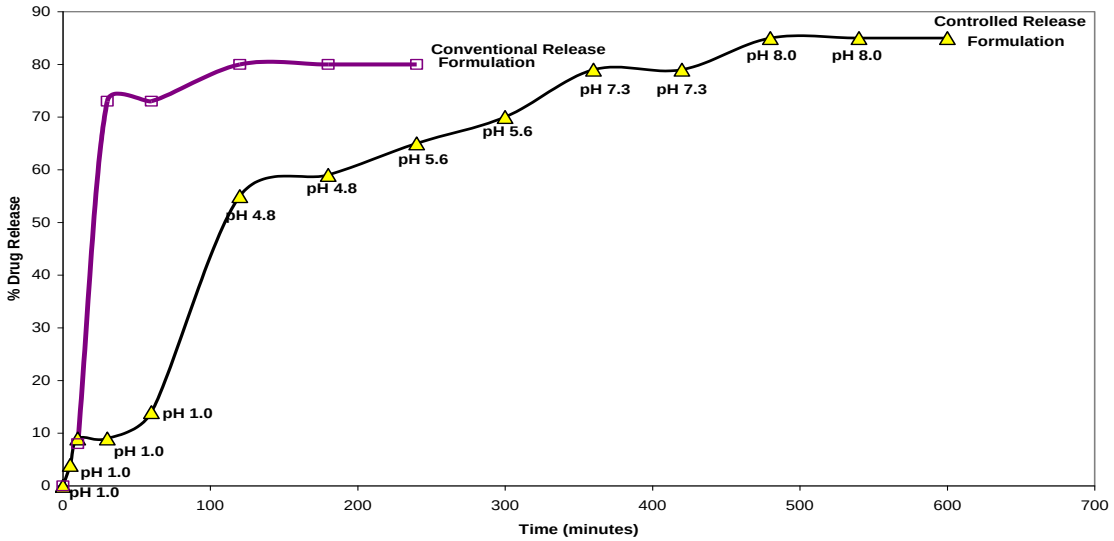



Figure 4.6: Comparative release profile of nevirapine from conventional and spansule dosage formulation.

Fig. 4.6 showed that conventional nevirapine formulation gave a drug release of 73 % within 30 min; this peaked and stabilized at a maximum of 82 % at 400 min. In the case of nevirapine formulation, there was an initial steep rise to 60 % by 140 min, followed by gradual increase in the release and reaching a peak of 87 % at 10 h, evaluation time. Relatively low amount of nevirapine was released in acidic environment of pH 1.0. Higher increase in the amount of drug released occurred as the pH increased from 1.0 to 4.6. At alkaline pH environments (pH 7.3 to 8.0) drug release still took place but not substantially.

## 4.3.6. STAVUDINE

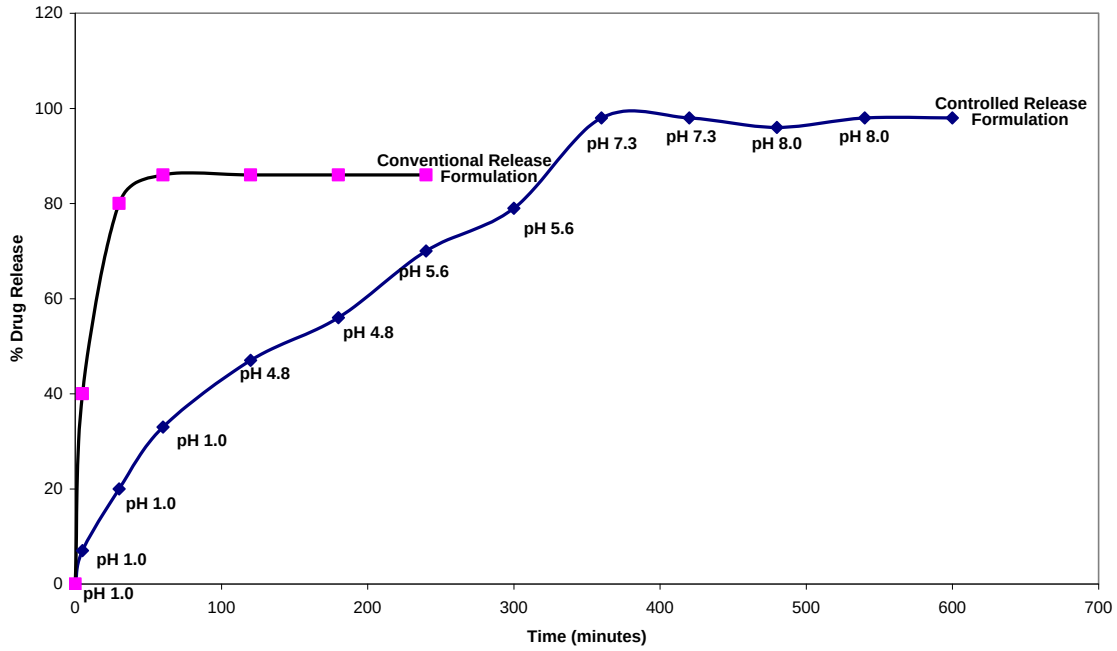



Figure 4.7: Comparative release profile of stavudine from conventional and spansule dosage formulation.

Data presented in fig. 4.7 showed that, conventional formulation of Stavudine gave a maximum % drug release of 85 % within 50 min, stabilizing to 87 % from 50 to 360 min. In contrast, the release profile from the spansule formulation was steady and gradual, reaching a maximum of 97 % after 360 min. This level was maintained for 600 min. While the conventional formulation of stavudine had a peak release of 87 %, the spansule formulation had a 99 % drug release, which was maintained in the small intestine and the colon.

## 4.3.7. ZIDOVUDINE

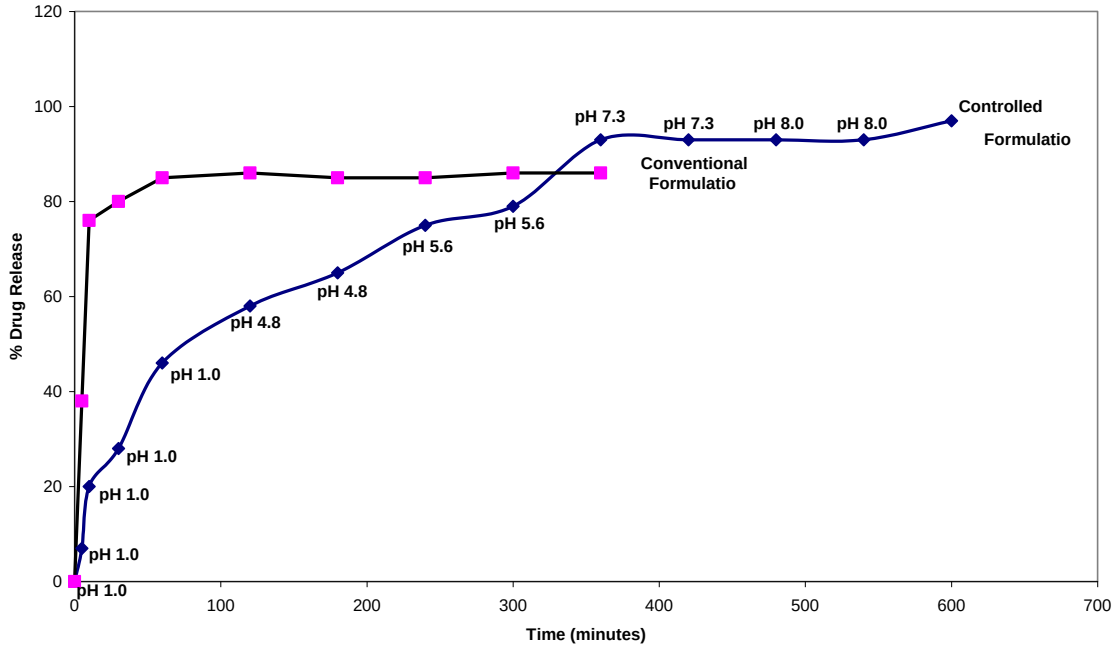



Figure 4.8: Comparative release profile of zidovudine from conventional and spansule dosage formulation.

As shown in Fig. 4.8, conventional zidovudine formulation gave a maximum drug release of 85% within 40 min, stabilizing at this level for a further 360 min. In contrast, there was steady and gradual increase in the release of zidovudine from the spansule formulation, reaching a maximum of 97 % after 360 min. This level was maintained up till 600 min evaluation period.

## Drug Release Profiles of Spansule Controlled Release Formulations Containing Mixtures of Anti-Retroviral Compounds.

The tables are given in appendix II. The RSD (Relative Standard Deviations) ranged from 1 % to 4 %. This fall with in the limits set by USP (2003) of 6 %.

### 4.3.8. STAVUDINE 80 MG + DIDANOSINE 400 MG

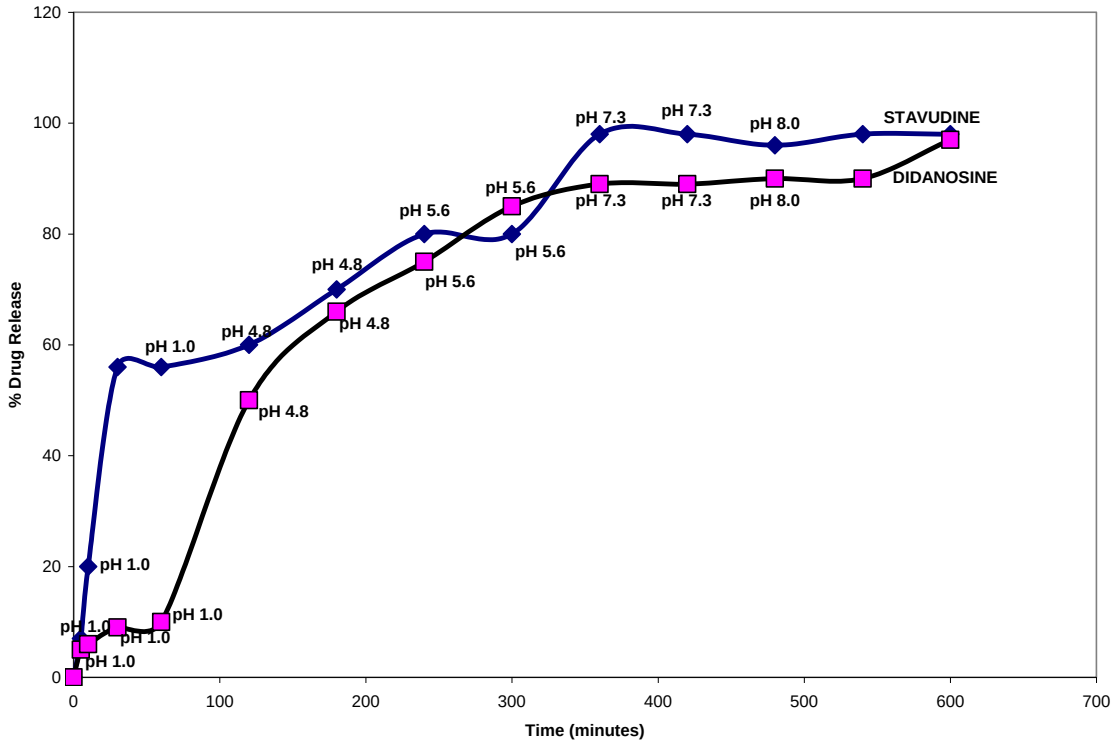



Figure 4.9: Drug Release Profile of Spansule formulation, Containing Stavudine 80mg + Didanosine 400mg

Figure 4.9 drug release profile of spansule formulation containing stavudine (80 mg) and didanosine (400 mg) it showed low release of didanosine during the first 80 min, followed by a sharp increase up to 80 % at 250 min. Thereafter, drug released increased gradually, peaking at

98 % in 10 h. Stavudine release reached 57 % within 45 min, thereafter increasing gradually to a peak of 98 % at 320 min. This was maintained for 10 h.

### 4.3.9. NEVIRAPINE 400 MG + ZIDOVUDINE 600 MG + DIDANOSINE 400 MG

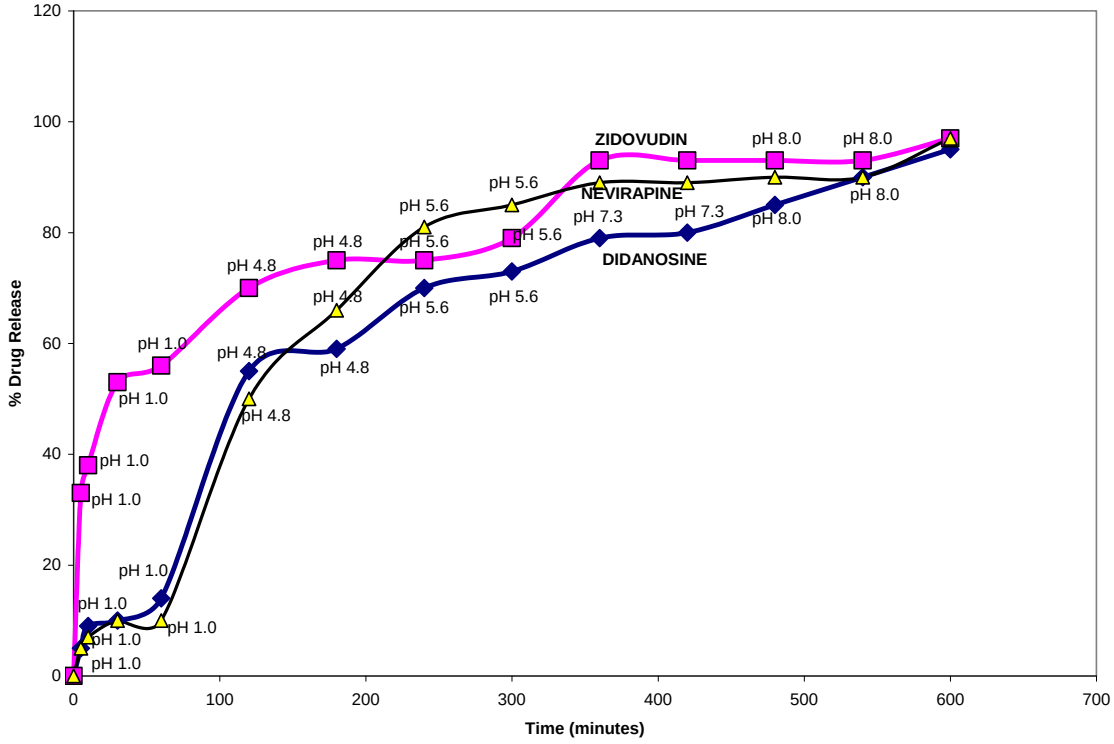



Figure 4.10: Drug Release Profile of Spansule formulation, Containing Nevirapine 400 mg + Zidovudine 600 mg + Didanosine 400 mg

Data presented in Fig. 4.10 showed that there was low release of didanosine and nevirapine during first 100 min, which sharply increased at pH 4.8. Thereafter, a gradual increase peaking at 97 % in 10 h was observed. Zidovudine showed a slightly higher than expected release initially (pH 1.0); thereafter, the released marched those of didanosine

and nevirapine as the pH increased. All three drugs peaked at 98 % by the 10 h evaluation period. At pH 1.0, percentage drug release for didanosine, nevirapine and zidovudine are 15, 10 and 5 % respectively.

### 4.3.10. STAVUDINE 80 MG + LAMIVUDINE 300 MG + NEVIRAPINE 400 MG

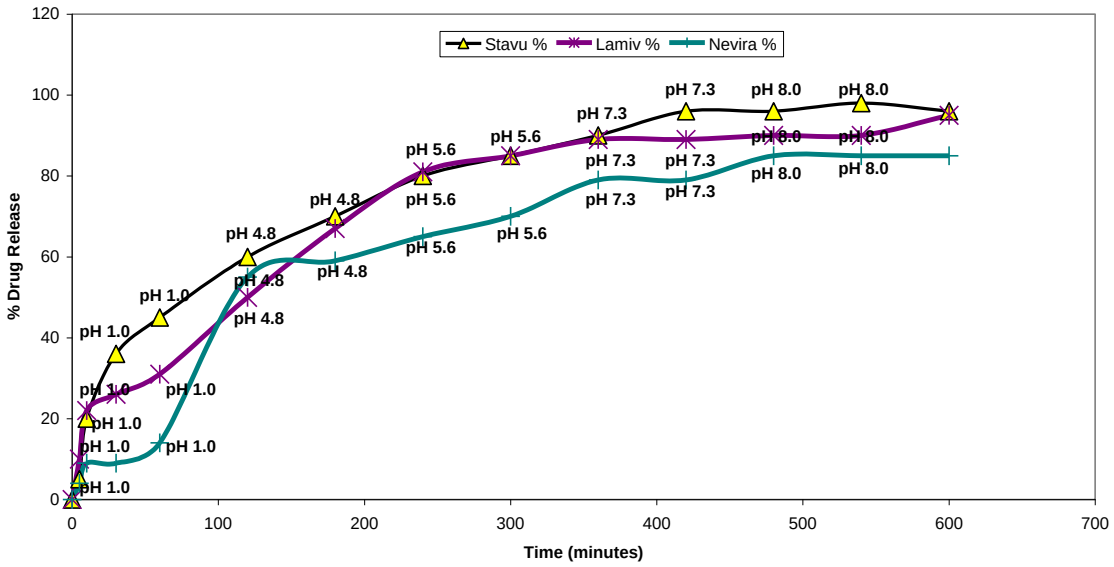



Figure 4.11: Drug Release Profile of Spansule formulation, Containing Stavudine 80 mg + Lamivudine 300 mg + Nevirapine 400 mg

Fig. 4.11 shows that stavudine, lamivudine and nevirapine were released gradually, in a steady step like manner as pH changes from acid to alkaline. Differences during the first 100 min there was 10 % drug release for nevirapine as against 48 % drug release for stavudine at pH

1.0 nevirapine release peaked at 87 % while stavudine and lamivudine peaked at 97 %.

### 4.3.11. ZIDOVUDINE 400 MG + LAMIVUDINE 300 MG + NEVIRAPINE 400 MG

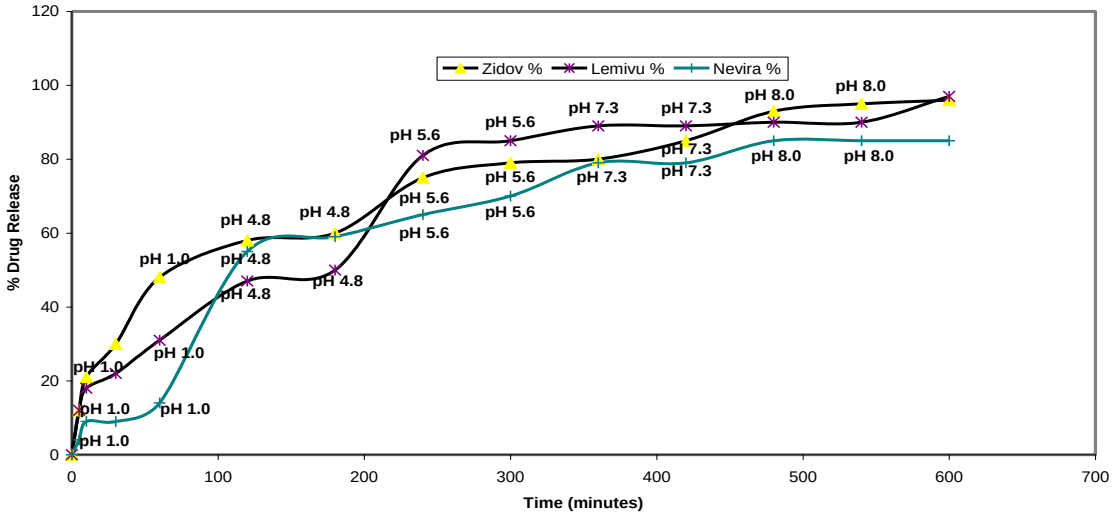



Figure 4.12.: Drug Release Profile of Spansule formulation, Containing Zidovudine 400 mg + Lamivudine 300 mg + Nevirapine 400 mg

Figure 4.12 shows that zidovudine; lamivudine and nevirapine are released gradually from the spansule, in a steady step like manner as pH changes from ac id to alkaline. Amount of release during the first 100 min ranged from 10% drug release for nevirapine to 50 % for zidovudine at pH 1.0

# CHAPTER 5

# DISCUSSION

Solubility studies of the seven ARV drugs shows them to be sufficiently soluble in water to maintain sink conditions during drug release studies. This result is in line with solubility data given by other researchers (Sanchez-Lafuente *et.al*, 2002) and Martindale Pharmacopoeia 35th edition (2007).

Investigation on the effectiveness of various coating thickness of polymer coatings, using Eudragit L for enteric coating showed that a coating of 2mg/cm$^2$ was sufficient to provide the desired property of enteric effect. In order to give a safety margin, 5mg/cm$^2$ coatings was used. This result is supported by extrapolation from the graph of relationship between particle size, surface area and polymer requirement (Appendix). At this coating, enteric effect can be guaranteed with minimal weight gain. Above this, carries the twin disadvantages of weight gain and economic waste of expensive polymers.

## 5.1 DRUG RELEASE PROFILES OF FORMULATIONS CONTAINING SINGLE ANTI-RETROVIRAL (CONVENTIONAL VERSUS CONTROLLED RELEASE)

### a. Didanosine Spansule Formulation

Damle (2002) reported that didanosine is rapidly hydrolyzed in the acid medium. Conventional didanosine formulation contains magnesium and

aluminum antacids to neutralize this acid in order to protect the drug. In this study conventional formulation of didanosine was hydrolyzed at pH 1.0, despite the antacids, as only a maximum of 25% drug release was achieved. The conventional formulations have to be thoroughly chewed or crushed in water. This leads to poor taste in the mouth reducing patient compliance, as well as causing nausea. By comparism, the spansule controlled release formulation contains no antacid that can give rise to drug-drug interaction (e.g. with ketaconazole, HIV-protease inhibitors, dapsone and tetracyclines) and drug-food interactions (e.g. with fruit juice and milk). The polymethacrylate polymers have released didanosine from the spansule dosage forms in a step-wise manner as the pH was adjusted to simulate the digestive tract pH. As shown in this study the relatively low % of didanosine in stomach pH (pH 1.0) in the first 2 h of dissolution is in full compliance with pharmacopeial requirement for enteric coatings.

The figure also shows that Eudragit L 100 coatings encasing the loading dose dissolved at rising pH values to release didanosine in the duodenum pH value of over 4.8.

Drug release data from conventional formulations showed that pH changes from acidic pH of 1.0 to alkaline pH 8.0 did not result in differences to the release profiles. This can be explained by the fact that conventional formulations are designed to release actives within the first one h. This was seen to be the case with all the conventional dosage

forms studied. On the other hand, a substantial quantity of the drug in the spansule formulation was released in pH conditions simulating duodenal, jejunum and colon environment. This release profile, which is characteristic of controlled release dosage forms, clearly highlights the significant advantage of the formulated spansule formulation over the conventional one.

**b. Inidnavir Spansule Formulation**

Indinavir was found to be a difficult drug to handle during formulation, because of its hydroscopic nature. Unlike other formulations, the drug volume was too large to fit into one size 000 capsule. Conventional Indinavir must be stored in its original container which has a desiccant in the cap and another small desiccant sachet in the bottle, without this indinavir is only stable for about 3 days (Murphy 1999).

The drug release profile of conventional indinavir formulation showed a formulation that almost completely released the drug. The spansule formulation performed dismally, because of the method employed in its formulation.

Indinavir has been documented to be hygroscopic (Kanaker, 1987) with possible crystalline changes as a result. The wet granulation method employed by this project is the likely explanation for the low drug release. Remington 21$^{st}$ edition (2005) attributed the problem to the fact that indinavir loses an ethanol molecule from its structure on exposure to moisture and is then converted to the hydrate form.

The wet formulation method of granulation is therefore inappropriate in preparing granules of indinavir. It is therefore advisable to use dry granulation method or use anhydrous wetting agents in the processing of granules of indinavir. Hygroscopic propensity of indinavir should reduce when mixed with methylcellulose and protected by sprayed layers of polymethacrylate polymers. These polymers are known to be impermeable to atmospheric moisture (Lehmann, 2001).

**c. Lamivudine Spansule Formulation**

The drug release profile of the spansule formulation of lamivudine clearly showed that significant amounts of the drug are released at various regions of gastro-intestinal tract. The continuous release of the drug over 10 h indicates that the formulated spansule formulation of lamivudine can serve as a once daily regimen in place of the twice daily dosing of the conventional formulation.

**d. Nelfinavir Spansule Formulation**

Changes in pH from 1.0 to 8.0 resulted in pH sensitive polymers (Eudragit L and S) releasing the loading dose. Eudragit RL, RS and NE controlled the maintenance doses.

The amount of drug needed for spansule formulation was high, although this did not prevent successful formulation into controlled release spansule form, ways of scaling down this amount without compromising the successful drug release may have to be considered.

**e. Nevirapine Spansule Formulation**

Drug release profiles following changes from acidic (1.0) to alkaline (8.0) pH, shows that the pH sensitive polymer coating (Eudragit S) and sustained release coatings (Eudragit RL, RS and NE) were effective in controlling release of the drug. The release profile of the spansule formulation of nevirapine indicates that a significant proportion of the drug is released in the duodenum and the jejunum, but generally protected in the stomach (as evidenced by low release at pH 1.0). The release profile of the spansule formulation of nelfinavir indicates that it can serve as a good replacement to the multiple dose conventional form.

**f. Stavudine Spansule Formulation**

The stavudine spansule formulation drug release profile showed that an appreciable amount of the drug was released at all levels. Loading dose was substantial (about 35 %), and as much as 20 % increase in drug concentration was noticed when drug transited from the ileum to the colon environment. The sustained, continues release of the drug from the spansule conforms to designed characteristics of controlled release formulation, and ensures less frequent administration of the formulation.

**g. Zidovudine Spansule Formulation**

The drug release profile of the spansule formulation of zidovudine fits within the expected release design of spansule formulation. The initial

loading dose of un-modified 300 mg, reached the required C max as rapidly as was allowed by the dissolution of zidovudine. The four different portions of the sustained released formulation coated with Eudragit S, RS, NE and RL were able to release their dosage, gradually and on schedule, at the designated p.H changes of pH 1.0 to 8.0, thereby maintaining the level reached by the loading dose. This result is considered to be satisfactory.

**5.2 Pill Burden reduction**

**Table 5.1:** Results showed that pill-burden reduction was achieved as follows

| **ANTIRETROVIRAL DRUG** | **Conventional Dosage Form per day** | **Spansule Capsule size** | **Spansule Caps per dosing (O.D)** |
|---|---|---|---|
| Didanosine 200mg | 2 | 0 | 1 |
| Indinavir 400mg | 5 | 000 | 3 (Failed) |
| Lamivudine 150mg | 2 | 0 | 1 |
| Nelfinavir 150mg | 10 | 000 | 3 |
| Nevirapine 200mg | 2 | 0 | 1 |
| Stavudine 30mg | 3 | 0 | 1 |
| Zidovudine 300mg | 2 | 00 | 1 |
| **HAART** | | | |
| **1**. Stavudine 40mg BD + Didanosine 200mg BD | 4 | 0 | 1 |
| **2**. Nevirapine 200mg BD + Zidovudine 300mg BD + Didanosine 200mg BD | 6 | 00 | 2 |
| **3**. Stavudine 40mgBD + Lamivudine 150mgBD + Nevirapine 200mg BD | 6 | 00 | 1 |
| **4**. Zidovudine 200mg BD + Lamivudine 150mg BD + Nevirapine 200mg BD | 6 | 00 | 2 |

Hard gelatin capsules and their approximate capacities:

| Caps. no. | 000 | 00 | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|
| Content (mg) | 1.000 | 800 | 500 | 300 | 250 | 200 | 150 | 100 |

## 5.3 SPANSULE FORMULATIONS CONTAINING MIXTURES OF ANTI-RETROVIRAL DRUGS.

### a. Combination of Stavudine and Didanosine (Double drug combination spansule formulation)

The drug-release patterns of the two drugs in the spansule formulation compared favorably with those of the individual drugs, indicating that the two drugs do not interfere with each others release from the spansule formulation.

### b. Triple drugs combination spansule formulation combination of nevirapine, zidovudine and didanosine.

The drug release profiles of the triple combination of nevirapine, zidovudine and didanosine also showed that the proportion of nevirapine and didanosine release do not differ significantly from their corresponding single drug spansule formulations. On the other hand, while percentage release of Zidovudine in the single drug spansule formulation was about 40%, it increased to about 57% in the triple drug combination spansule formulation.

The drug release profiles of the other two triple drug combination spansule formulations:- Stavudine/Lamivudine/Nevirapine and Zidovudine/Lamivudine/Nevirapine, also showed that release profiles of individual components in the formulation were also not significantly modified. This implies that the formulations will not diminish the

bioavailability of individual drugs and therefore, presents a novel and simplified drug delivery system that will improve patient's compliance in ARV therapy.

**5.4 INTERPRETATION OF RELEASE PROFILES.**

Following the determination of drug release profiles from conventional and spansule formulations, the conditions of the *in-vitro* test should allow for the correlation of the measured data with the behavior of the drug in vivo. All drug release tests were conducted in accordance to USP XXI (2007). In practice, they reflected the physicochemical properties of the active ingredients whose release was carefully controlled from the formulations.

For the purpose of this formulation study, which was conducted *in vitro*, the result can be correlated with behavior of the drug in vivo, if the drug release pattern can be shown to reflect the blood plasma-level as the drug is being released from the formulation (Oliver *et al*, 2009).

Ranbaxy, a company that specializes in the manufacture of antiretroviral drugs has, in 2005 published a detailed report of its anti-retroviral portfolio (Ranbaxy, 2005). The studies consisted of comparative, randomized, two-treatment, two-period, two-sequence, crossover fed/fasting bioavailability study of single and fixed combination tablets of lamivudine, zidovudine and nevirapine; lamivudine, nevirapine and stavudine. Another bio-equivalent study was conducted in 2007, it involved determination of mean plasma versus time (24h) in healthy male

subjects, of single dose formulations for lamivudine, zidovudine, stavudine, nevirapine and didanosine, as well as their combinations, (Ranbaxy, 2007).

Data obtained from these studies indicated that controlled drug delivery systems, clearly present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral drug therapy. The use of such systems which began in the early 1990s, has witnessed greater interest in the past 5 years, particularly in the continued drive for simplification of ARV therapy.

Various researchers (Anderson, 1988; Kim, 1995; Sanchez-Lafuente, 2002; Flexner, 2007 and Chinen, 2008) reported efficient controlled drug release, under in vitro conditions. The complexity of the disease, the formulation optimization needed and evaluation studies require, multidisciplinary research, for eventual adoption of CDDS containing ARV drugs in clinical practice.

The drug release patterns obtained for the spansule controlled release formulations in this study followed a model which can be defined as **initial rapid release** of the loading dose, followed by a **slow first order release** of the four maintainace doses**.** Such a formulation is ideally suited for antiretroviral (ARV) drugs. This is because they have a half life that ranges from one hour to four hours, except for nevirapine.

The sustaining release polymer (Eudragit RL, RS and NE) only began releasing ARV drugs, after the loading dose polymers (Eudragit L and S)

have reached their peaks of release and are just beginning to fall. The equation describing the time course of plasma drug concentration with this type of formulation contains two portions, one to describe the rapid first order drug release and the other for slow first order release from the maintenance portion (Brahmankar and Sunil, 2000).

Evidence of successful control of drug release was seen in all the drug release patterns obtained except for indinavir formulation. The four different maintainace dose portions were clearly seen in the release patterns, each un-folding its effect as pH changed from 1.0 to 8.0.

A comparative analysis of the plasma drug level patterns conducted by Ranbaxy (2005) and the drug release patterns obtained by this study showed clear correlations. The correlation can theoretically be explained as follows: As a drug is released from a dosage form, it dissolves and is then absorbed into the blood. This follows Fick's law of dissolution of solids. Our method of achieving control is using drug release from the dosage form as a rate limiting step. As the two patterns were found to be similar, it means there is a correlation. It is not the aim of this project to temper with the absorption, distribution, elimination, metabolism (ADEM) of the antiretroviral (ARV) drugs, but rather, to control the rate and amount of the ARVs that are made available for ADEM. The results obtained have confirmed this achievement in all but one, of the formulations.

**5.5 Effect of pH on Drug Release:**

The drug profiles of all the controlled release capsule dosage forms show that the amount of drug release depended on the length of time the formulation was in contact with designated pH at which the polymer coating becomes perforated to release the drug (e.g. gastric or intestinal pH). This function was performed by coatings of Eudragit L and S. Some of the drug was released independent of pH to provide sustainability in drug level by Eudragit RL, RS and NE; this was seen clearly in the drug release patterns. In effect, release was seen to have been effectively controlled by the different types of polymethacrylate polymer coatings. Dissolution characteristics of the ARV were then activated as the drugs were made available by the dosage form design of pH sensitive and pH independent polymer barriers.

The test duration was ten to twelve hours, with pH changes at interval of two hours. *In-vivo*, it can be expected that natural g.i.t movements will control these timings, which will be at longer intervals. This is expected to be approximately twenty four hours, with slight variations among individuals.

Results of this study also showed that pill burden has been reduced substantially in all cases to once daily dosing. In the case of indinavir and nelfinavir 3 size 000 capsules were required to fit in the daily dose. This is a major disadvantage, because of the dose size.

**5.6 Stability of the ARVs**

The spectrophotometric analysis conducted to determine drug release accounted for 98% to 100% of the drugs in spansule formulation, except for indinavir (19%). This indicates they are stable in formulation and the result is supported by data published in Martindale pharmacopoeia 35th edition (2007) and by Deshmukh *et al.* (2003). The instability of indinavir can be explained by the same sources, which stipulates the drug be protected from moisture, light and a maximum temperature of $25^0$c. The wet granulation and other processing methods used in this study therefore explain the cause of indinavir instability. Recommendations have been made to reformulate this drug.

**5.7 Evaluation of Controlled Release Spansule dosage form:**

Lehmann (1991) listed the criteria to be addressed during formulation of controlled release products. The spansule formulated products in this study were subjected to these criteria as follows:-

- It has been found that controlled release products are subject to either of two modes of failure: Insufficient dose is released, or too much drug is made available too quickly.

In this study, all formulations, with the sole exception of indinavir formulation did not dump and did not fail to release ARV drugs. Actives were evenly released in conformity with drug release design.

Because the mechanism of drug release control is pH changes, all drugs were fully released under experimental g.i.t pH conditions by Eudragit L

and S. Levels were then maintained by sustaining pH independent portions by Eudragit RS, RL, NE.

The principal physiologic variable in the design of this spansule controlled release formulation is pH changes as the formulation makes its way down the g.i.t; this pH variation was taken into account by tests using phosphate buffers. Changes of pH were initiated at two hours interval to mimic the g.i.t variations. Results have shown excellent conformity with the expected properties of polymethacrylate polymers.

Loading dose was released immediately and maintenance dose released gradually, in conformity with drug release design.

Unit to unit variation was found to be minimal because the spansule formulations were tested for drug release three times (from three batches) and results were an average of the three. All showed reproducible drug release.

Stability of formulation with respect to its drug release was found to be stable, with the exception of indinavir, which was unstable because of its hygroscopic nature and the fact that indinavir converts to the hydrate form in the presence of moisture. All other drug formulations were found to be stable.

Obtained release profiles have fully met the design of the spansule micro encapsulation of loading dose release, followed by gradual release of the four portions of maintainace doses. This is expected to unfold as the dosage form makes its way down the g.i.t.

Comparism of release profiles of conventional and controlled release formulation have shown conclusively that spansule controlled release formulations using Polymethacrylate Polymers, have met all expectations. As such it is recommended for adoption to reduce pill burden in HIV/AIDS treatment.

### 5.8 Comparism of results of this study with other works

Betageri *et al.* (2001) improved the oral absorption of didanosine by delivering it over a prolonged period of time as well as prolonging retention on the mucosa. Their formulation contained Polyox WSRN-303, Carbopol 974-NF and Methocel K4M. These researchers concluded that a single polymer could be used for sustained release but for simultaneous optimization, the blending of various polymers may be required. They went on to say these systems remain to be investigated. Results from this study have confirmed that blending of various polymers is necessary of optimal drug release.

Sanchez-Lafuente *et al.* (2002a) formulated controlled release matrix tablets of ARV drugs using Eudragit RSPM and ethylcellulose (Ethocel 100). The experimental values obtained from these optimized formulations highly agreed with the predicted values, thereby validating the model used in preparation of the tablets. This is very similar to what has been obtained in this study.

Deshmukh *et al.* (2003) used HPMCP 5.5, Methocel K4M, WSRN-303 and Polyox to make matrix tablets of didanosine. The formulation was shown

to be resistant to dissolution in 0.1 N HCL but dissolved within 10 min in phosphate buffers pH 7.4. This result is similar to what was obtained in this study.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

The co-relation between mean plasma concentrations with release of drug from the dosage forms (Ranbaxy, 2005) and other works (Anderson, 1988; Betageri *et al.* 2001; Sanchez-Lafuente *et al.* 2002; Deshmukh *et al.* 2003; Chinen *et.al*, 2008) provided conclusive evidence of the viability of the drug-formulation methods employed in this study to reduce the pill burden associated with HIV/AIDS treatment.

The ability of the polymethacrylates to protect the active ingredient from the actions of other formulation ingredients and protect the drug from the physiological environment of the gastrointestinal tract and to deliver with precision to a targeted release site is clearly demonstrated by the drug release profiles exhibited in this study.

The successful formulation of all seven ARV drugs by the polymethacrylate polymers in a spansule design, inspite of their varied physic-chemical properties shows the versatile application of this technique, especially to other classes of drugs with formulation problems similar to those of ARV therapy.

The fear of dose dumping can be eliminated by formulating as coated granules which are expected to become widely dispersed in the gastro intestinal tract as each granule can release the active ingredient at a controlled rate. Because the granules are widely scattered, local high

concentrations can be avoided and enabling a smooth, steady drug release to be maintained.

## 6.2 RECOMMENDATIONS FOR FURTHER STUDIES

1. Indinavir was found to be highly hygroscopic presenting problems during the wet granulation method employed. Recent studies have shown that in the presence of moisture, indinavir is converted to the hydrate form. The maximum drug released by the CDDS was only 19%. This formulation is a failure. Further studies are recommended using dry granulation methods and employing non aqueous solvents such as ethanol or isopropyl alcohol. Direct compression method, using Avicel$^{R}$, Emdex$^{R}$ or Fast-Flo$^{R}$, should be considered.

2. The dose size of indinavir was considered too large for once daily formulation. It is therefore recommended that it is broken down to twice daily dosing.

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# APPENDIX- I

## Beer-Lambert's Plots

Figure 1: Didanosine Beer's Plot

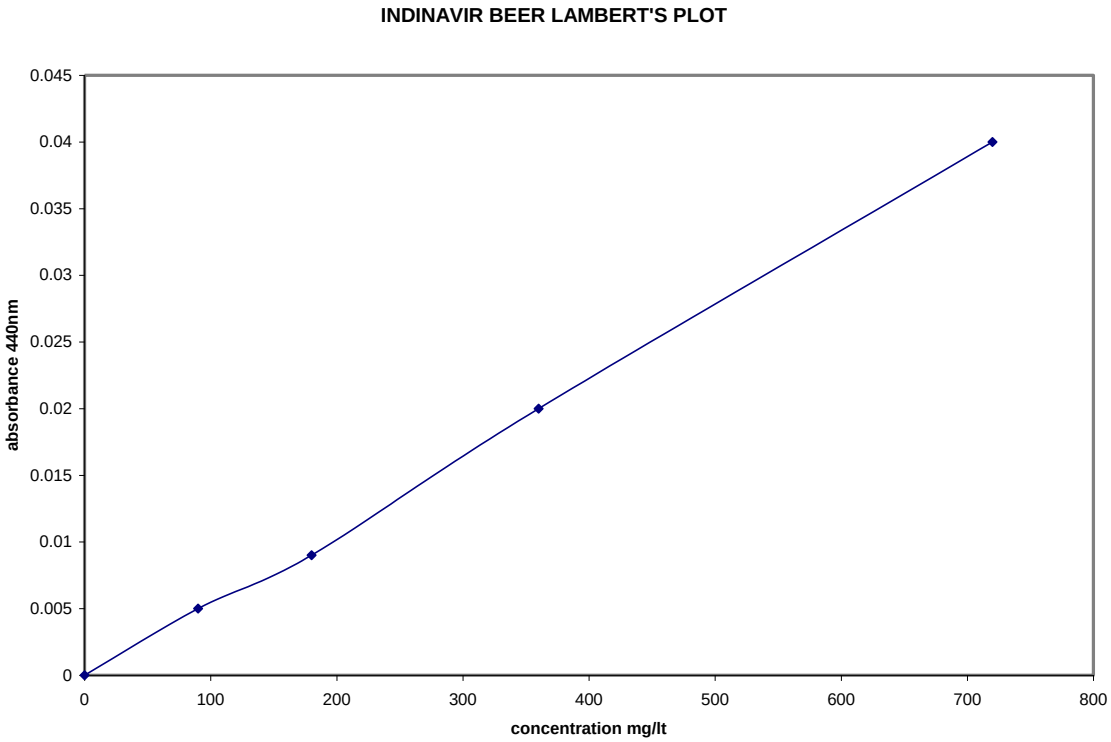


Figure 2: Indinavir Beer's Plot

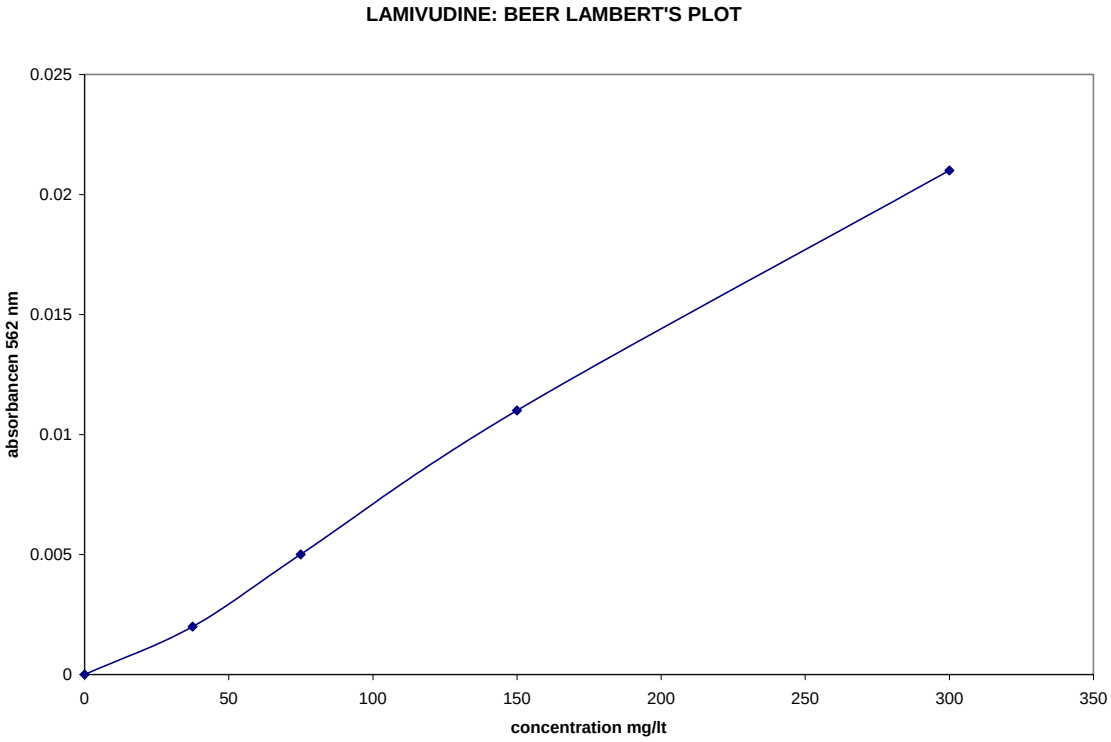


Figure 3: Lamivudine Beer's Plot

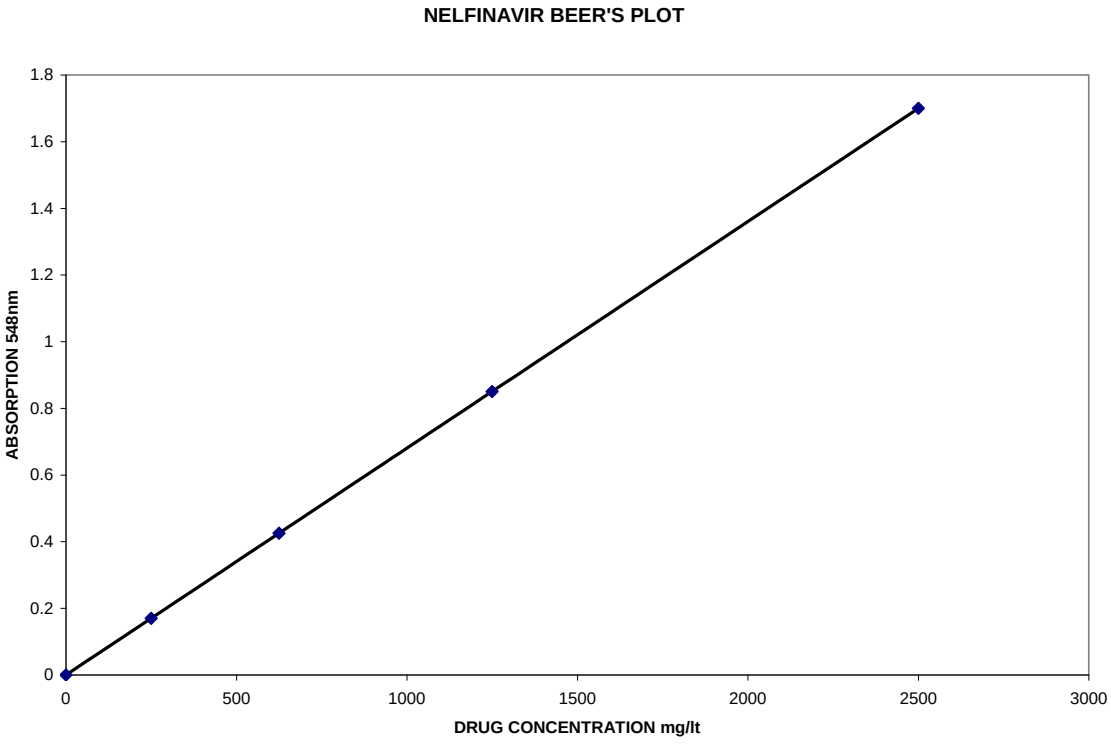


Figure 4: Nelfinavir Beer's Plot

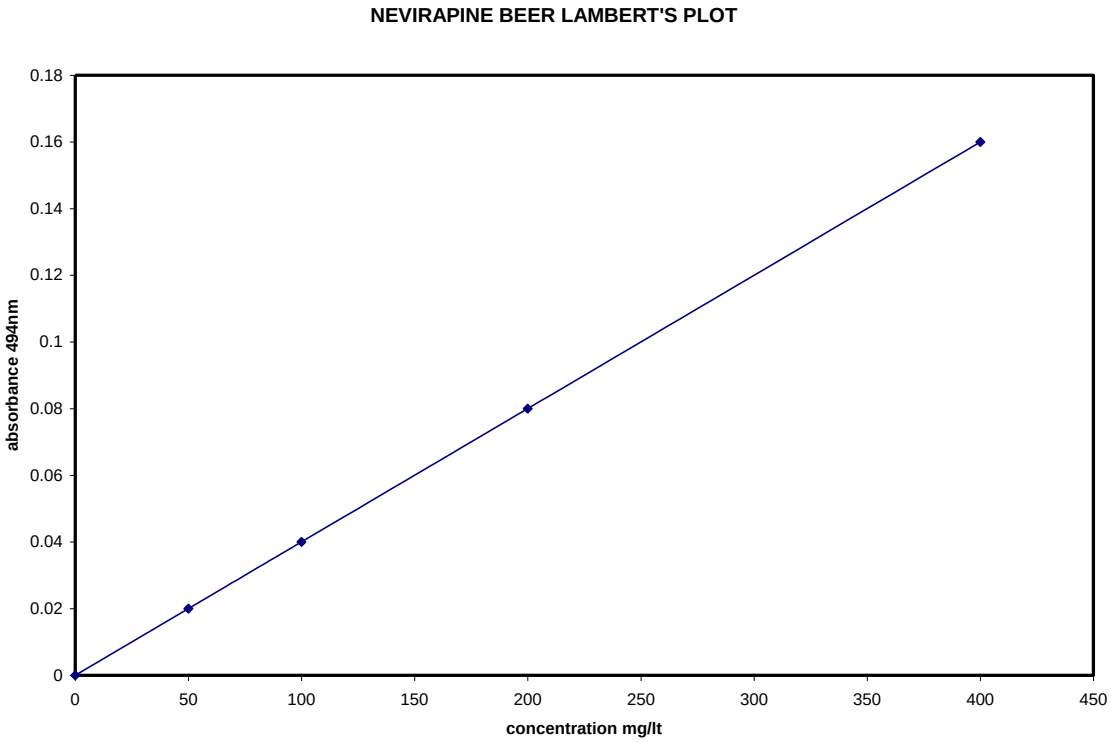


Figure 5: Nevirapine Beer's Plot

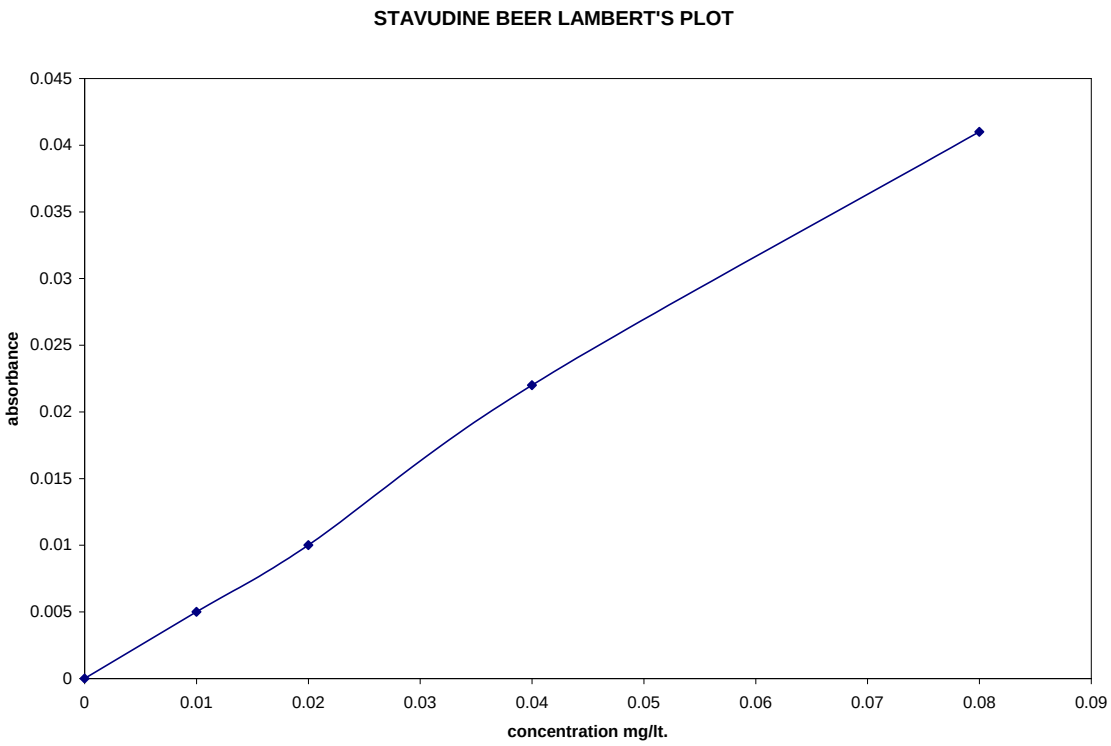


Figure 6: Stavudine Beer's Plot

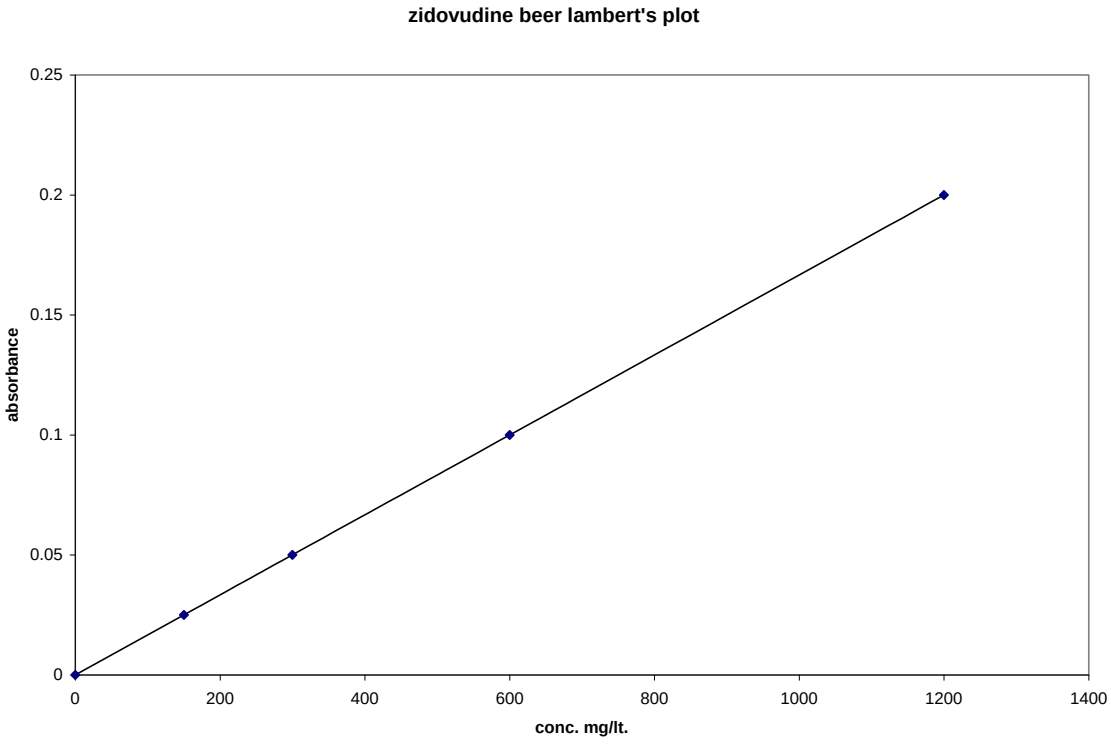


Figure 7: Zidovudine Beer's Plot

# APPENDIX- II

## Drug Release Tables of Spansule Controlled Release Formulations Containing Mixtures of Anti-Retroviral Compounds. (Fixed Dose Combinations of HAART)

**Table 1: Drug Release Profile of Spansule formulation, Containing Stavudine 80mg + Didanosine 400mg:**

| TIME minutes | pH | DRUG RELEASE **Stavudine** | | | DRUG RELEASE **Didanosine** | | |
|---|---|---|---|---|---|---|---|
| | | mg | RSD % | % | mg | RSD % | % |
| 0 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 1.0 | 6 | 1 | 7 | 20 | 1 | 5 |
| 10 | 1.0 | 16 | 1 | 20 | 24 | 2 | 6 |
| 30 | 1.0 | 45 | 2 | 56 | 36 | 2 | 9 |
| 60 | 1.0 | 45 | 2 | 56 | 40 | 2 | 10 |
| 120 | 4.8 | 48 | 2 | 60 | 200 | 2 | 50 |
| 180 | 4.8 | 56 | 3 | 70 | 300 | 2 | 75 |
| 240 | 5.6 | 64 | 3 | 80 | 320 | 2 | 80 |
| 300 | 5.6 | 64 | 3 | 80 | 325 | 3 | 81 |
| 360 | 7.3 | 79 | 3 | 98 | 355 | 3 | 89 |
| 420 | 7.3 | 79 | 3 | 98 | 360 | 4 | 90 |
| 480 | 7.3 | 77 | 3 | 96 | 360 | 4 | 90 |
| 540 | 7.3 | 79 | 4 | 98 | 360 | 4 | 90 |
| 600 | 7.3 | 79 | 4 | 98 | 390 | 4 | 97 |

**Table 2: Drug Release Profile of Spansule formulation, Containing Nevirapine 400mg + Zidovudine 600mg + Didanosine 400mg:**

| TIME minutes | pH | DRUG RELEASED **Nevirapine** | | | DRUG RELEASED **Zidovudine** | | | DRUG RELEASED **Didanosine** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mg | RSD % | % | mg | RSD % | % | mg | RSD % | % |
| 0 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 1.0 | 20 | 1 | 5 | 198 | 1 | 33 | 40 | 1 | 10 |
| 10 | 1.0 | 35 | 2 | 9 | 228 | 1 | 38 | 44 | 2 | 11 |
| 30 | 1.0 | 40 | 2 | 10 | 300 | 2 | 53 | 48 | 1 | 12 |
| 60 | 1.0 | 55 | 3 | 14 | 336 | 2 | 56 | 76 | 2 | 19 |
| 120 | 4.8 | 220 | 3 | 55 | 420 | 2 | 70 | 212 | 2 | 53 |
| 180 | 4.8 | 235 | 3 | 59 | 450 | 3 | 75 | 270 | 2 | 67 |
| 240 | 5.6 | 280 | 4 | 70 | 450 | 3 | 75 | 325 | 3 | 80 |
| 300 | 5.6 | 292 | 4 | 73 | 475 | 3 | 79 | 340 | 2 | 85 |
| 360 | 7.3 | 315 | 3 | 79 | 560 | 4 | 93 | 355 | 2 | 89 |
| 420 | 7.3 | 320 | 4 | 80 | 560 | 3 | 93 | 358 | 3 | 89 |
| 480 | 7.3 | 340 | 4 | 85 | 560 | 4 | 93 | 360 | 4 | 90 |
| 540 | 7.3 | 360 | 4 | 90 | 560 | 4 | 93 | 380 | 4 | 95 |
| 600 | 7.3 | 380 | 4 | 95 | 582 | 4 | 97 | 380 | 4 | 95 |

**Table 3: Drug Release Profile of Spansule formulation, Containing Stavudine 80mg + Lamivudine 300mg + Nevirapine 400mg:**

| TIME minutes | pH | DRUG RELEASED **Stavudine** mg | RSD % | % | DRUG RELEASED **Lamivudine** mg | RSD % | % | DRUG RELEASED **Nevirapine** mg | RSD % | % |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 5 | 1.0 | 4 | 1 | 5 | 30 | 1 | 10 | 15 | 2 | 4 |
| 10 | 1.0 | 16 | 1 | 20 | 66 | 1 | 22 | 35 | 2 | 9 |
| 30 | 1.0 | 29 | 2 | 36 | 105 | 2 | 26 | 35 | 2 | 9 |
| 60 | 1.0 | 36 | 2 | 45 | 125 | 2 | 31 | 55 | 3 | 14 |
| 120 | 4.8 | 48 | 2 | 60 | 150 | 3 | 50 | 220 | 3 | 55 |
| 180 | 4.8 | 56 | 3 | 70 | 201 | 3 | 67 | 235 | 3 | 59 |
| 240 | 5.6 | 64 | 3 | 80 | 243 | 3 | 81 | 260 | 3 | 65 |
| 300 | 5.6 | 68 | 2 | 85 | 255 | 3 | 85 | 280 | 3 | 70 |
| 360 | 7.3 | 72 | 3 | 90 | 267 | 3 | 89 | 315 | 4 | 79 |
| 420 | 7.3 | 77 | 3 | 96 | 267 | 4 | 89 | 315 | 4 | 79 |
| 480 | 7.3 | 77 | 3 | 96 | 270 | 3 | 90 | 340 | 4 | 85 |
| 540 | 7.3 | 79 | 3 | 98 | 270 | 4 | 90 | 340 | 4 | 85 |
| 600 | 7.3 | 77 | 4 | 96 | 285 | 4 | 95 | 340 | 4 | 85 |

**Table 4: Drug Release Profile of Spansule formulation, Containing Zidovudine 400mg + Lamivudine 300mg + Nevirapine 400mg:**

| TIME minutes | pH | DRUG RELEASED **Zidovudine** | | | DRUG RELEASED **Lamivudine** | | | DRUG RELEASED **Nevirapine** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mg | RSD % | % | mg | RSD % | % | mg | RSD % | % |
| 0 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 1.0 | 48 | 1 | 12 | 36 | 1 | 12 | 15 | 1 | 4 |
| 10 | 1.0 | 84 | 1 | 21 | 54 | 1 | 18 | 35 | 1 | 9 |
| 30 | 1.0 | 120 | 2 | 30 | 66 | 1 | 22 | 35 | 1 | 9 |
| 60 | 1.0 | 192 | 2 | 48 | 125 | 1 | 31 | 55 | 2 | 14 |
| 120 | 4.8 | 232 | 3 | 58 | 141 | 2 | 47 | 220 | 2 | 55 |
| 180 | 4.8 | 240 | 3 | 60 | 150 | 2 | 50 | 235 | 2 | 59 |
| 240 | 5.6 | 300 | 3 | 75 | 243 | 2 | 81 | 260 | 2 | 65 |
| 300 | 5.6 | 316 | 3 | 79 | 255 | 3 | 85 | 280 | 3 | 70 |
| 360 | 7.3 | 320 | 4 | 80 | 267 | 3 | 89 | 315 | 3 | 79 |
| 420 | 7.3 | 340 | 4 | 85 | 267 | 3 | 89 | 315 | 3 | 79 |
| 480 | 7.3 | 372 | 4 | 93 | 270 | 4 | 90 | 340 | 4 | 85 |
| 540 | 7.3 | 380 | 4 | 95 | 270 | 4 | 90 | 340 | 4 | 85 |
| 600 | 7.3 | 384 | 4 | 96 | 291 | 4 | 97 | 340 | 4 | 85 |

# APPENDIX- III

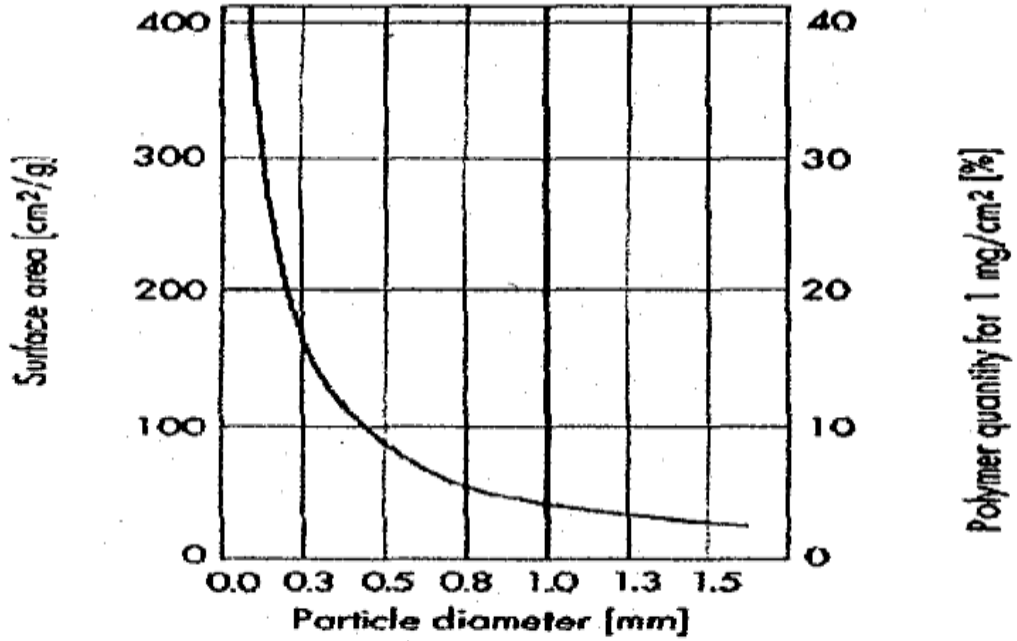


**FIGURE 1: RELATIONSHIP BETWEEN PARTICLE SIZE, SURFACE AREA AND POLYMER REQUIRMENT.**

# APPENDIX- IV

## Calculations involving Coating Thickness and Polymer Solutions

$$1mg/cm^2 \text{ coating weight (\%)} = \frac{156mm^2 . 1.0mg/cm^2}{140mg} = 1.1\%$$

$$2mg/cm^2 \text{ coating weight (\%)} = \frac{156mm^2 . 2mg/cm^2}{140mg} = 2.2\%$$

Using this approach, polymer requirement was calculated as follows:-

Table: Polymer Requirements

| Coating Polymer Requirement ($mg/cm^2$) | Polymer Quantities (%) | Polymer Factor |
|---|---|---|
| 1 | 1.1 | 0.33 |
| 2 | 2.2 | 0.66 |
| 3 | 3.3 | 1 |
| 4 | 4.5 | 1.36 |
| 5 | 5.6 | 1.69 |
| 6 | 6.7 | 2.03 |
| 7 | 7.8 | 2.36 |
| 8 | 8.9 | 2.60 |
| 9 | 10.0 | 3.03 |

| | | |
|---|---|---|
| EUDRAGT L100 | 66g | 29.1% |
| Triethyl citrate | 7g | 3.1% |
| Water | 154 g | 67.8% |
| Coating solution | 227 g | 100.0% |

| | |
|---|---|
| Polymer quantity: | 66 g |
| Plasticizer | 7 g |
| Solids content | 32.2% |

Coating weight 3.3%

Using the Polymer Factor (PF) to convert the formular while maintaining the quantity ratio to the other excipients:-

Polymer Factor (P.F) = required amount of polymer (g) / amount of polymer in sample formulation (g).

The Polymer coating solution used in this study had the following Composition, as recommended by Lehman (2000):

| | | | |
|---|---|---|---|
| 1mg/cm$^2$ coating weight (%): | 1.1%: 3.3% = | 0.33 P.F | |
| EUDRAGT L100 | 66g . 0.33 = | 21.78g | 29.1% |
| Triethyl citrate | 7g . 0.33 = | 2.31g | 3.1% |
| Water | 154 g . . 0.33 = | 50.82g | 67.8% |
| Coating solution | 227 g | =74.91g | 100.0% |
| Polymer quantity: | 21.78 g | | |
| Plasticizer | 2.31 g | | |
| Solids content | 32.2% | | |
| Coating weight | 1.1% | | |

| | | | |
|---|---|---|---|
| 2mg/cm$^2$ coating weight (%): | 2.2%: 3.3% = | 0.66 P.F | |
| EUDRAGT L100. | 66g. 0.66 = | 43.56g | 29.1% |
| Triethyl citrate. | 7g . 0.66 = | 4.62g | 3.1% |
| Water | 154 g. 0.66 = | 101.64g | 67.8% |
| Coating solution | 227 g | =149.82g | 100.0% |
| Polymer quantity: | 43.56 g | | |
| Plasticizer | 4.62 g | | |
| Solids content | 32.2% | | |
| Coating weight | 2.2% | | |

The calculations were done serially to 20mg/cm$^2$, and result presented in the table below:-

Table: Polymer Coating Weights for 2kg of Granules

| Coating Polymer Requirement (mg/cm$^2$) | Qty. Coating Solution Required (g) |
|---|---|
| 1.0 | 74.91 |
| 2.0 | 149.82 |
| 3.0 | 227.00 |
| 4.0 | 308.72 |
| 5.0 | 643.68 |
| 6.0 | 460.81 |
| 7.0 | 648.72 |
| 8.0 | 580.63 |
| 9.0 | 667.81 |

Example:

Let polymer requirement, P = 1 mg polymer per cm$^2$

For a size d = 7mm, h = 3.6mm

Using the formular: $S = \pi . (d . h + 0.5 . d^2)$

$S = 3.14 (7mm.3.6mm + 0.5 . 7^2 mm^2)$

$= 156mm^2$

# APPENDIX- V

Table 1: Disintegration time of different coating thickness

Eudragit L-IOO. (Enteric Coating)

| **Coating wt. / Surface area ($mg/cm^2$)** | **Disintegration Time (minutes) in Gastric Fluid (pH 1.0)** | **Intestinal Fluid (pH 6.8)** |
|---|---|---|
| 0 | 10 – 60 | 10 |
| 1 | 15 – 60 | 20 |
| 2 | > 120 | 30 |
| 3 | > 120 | 31 |
| 4 | > 120 | 34 |
| 5 | > 120 | 35 |
| 6 | > 120 | 35 |
| 7 | > 120 | 35 |
| 8 | >120 | 36 |
| 9 | > 120 | 40 |
| 10 | > 120 | 40 |
| 15 | > 120 | 40 |
| 20 | > 120 | 40 |

# APPENDIX- VI

## ABSORPTION SPECTRA OF THE SEVEN ARV DRUGS

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :DIDANOSN AQ
TIME :11:31:42 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 364.0 | 400.0 | 444.0 | 468.0 | 504.0 | 544.0 | 568.0 | 594.0 | 628.0 | 656.0 |
| ABS | 3.734 | 3.129 | 4.427 | 4.865 | 3.164 | 3.354 | 4.510 | 2.894 | 3.145 | 3.716 |

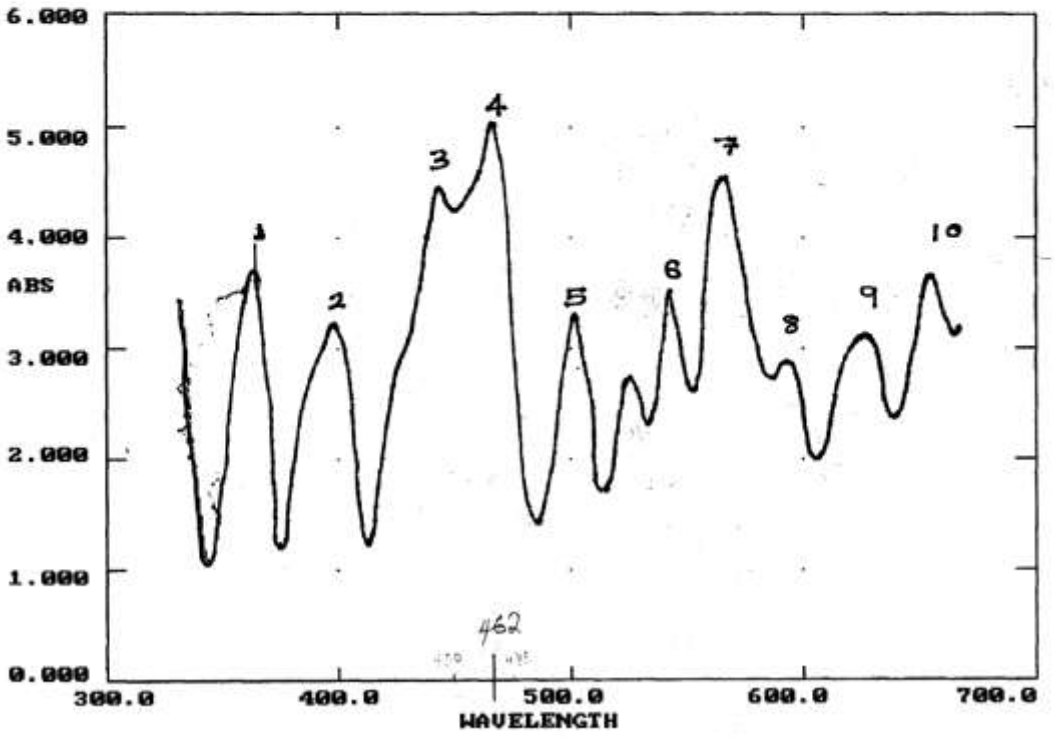


**Figure 1: Absorption Spectra for Didanosine**

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :INDINA AQ
TIME :11:52:50 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 366.0 | 412.0 | 440.0 | 468.0 | 492.0 | 512.0 | 534.0 | 558.0 | 586.0 | 616.0 |
| ABS | 3.861 | 4.380 | 5.110 | 2.480 | 3.849 | 3.252 | 3.583 | 4.019 | 4.135 | 4.575 |

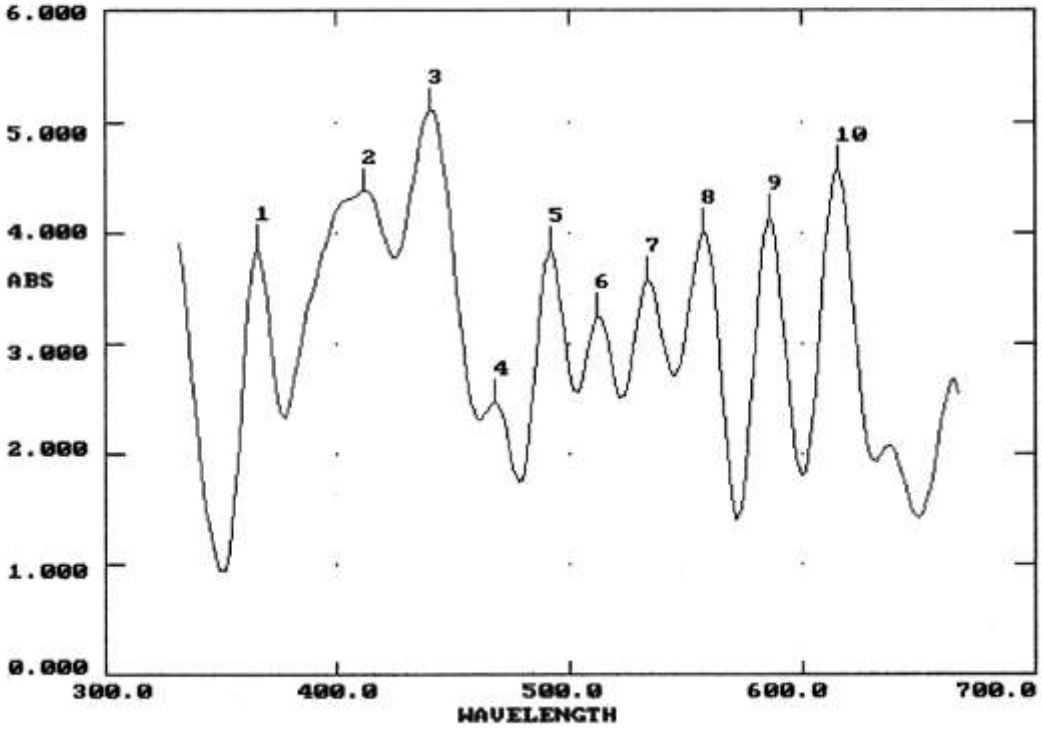


**Figure 2: Absorption Spectra for Indinavir**

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :LAMIVUD AQ
TIME :11:19:24 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 346.0 | 380.0 | 406.0 | 434.0 | 500.0 | 532.0 | 562.0 | 608.0 | 624.0 | |
| ABS | 4.154 | 2.539 | 2.229 | 4.599 | 2.097 | 2.809 | 4.772 | 3.763 | 3.791 | |

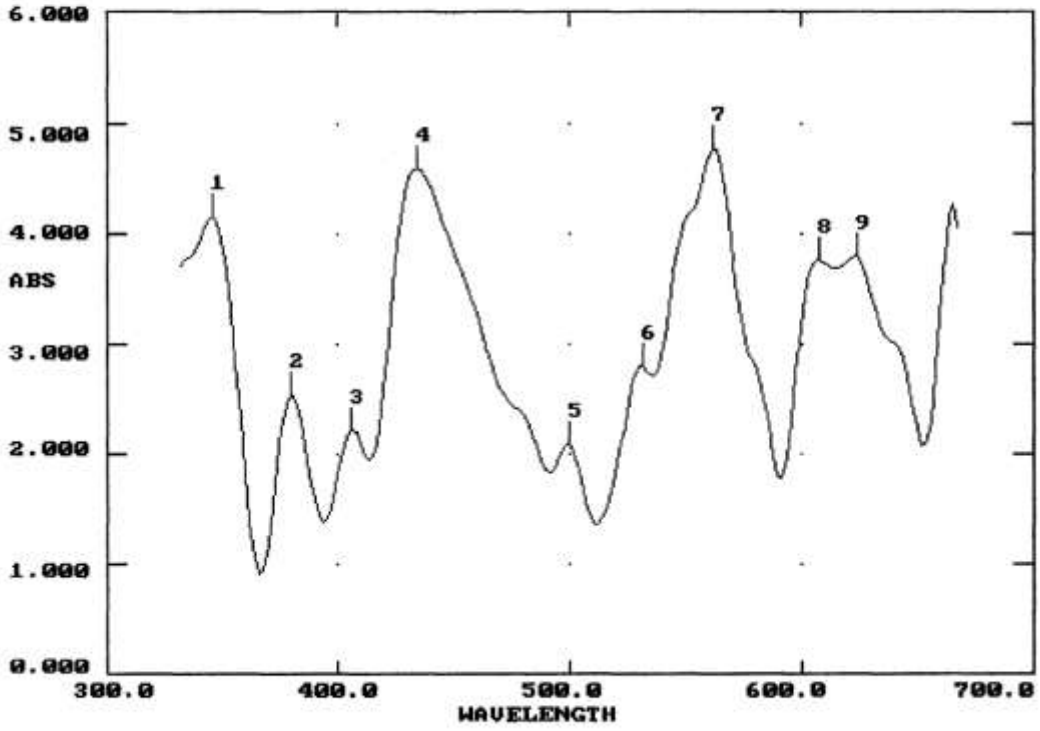


**Figure 3: Absorption Spectra for Lamivudine**

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :NELFINA AQ
TIME :12:09:30 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 336.0 | 376.0 | 406.0 | 448.0 | 464.0 | 488.0 | 514.0 | 548.0 | 582.0 | 614.0 |
| ABS | 5.040 | 3.968 | 4.083 | 4.873 | 4.508 | 3.482 | 4.127 | 4.785 | 5.472 | 4.480 |

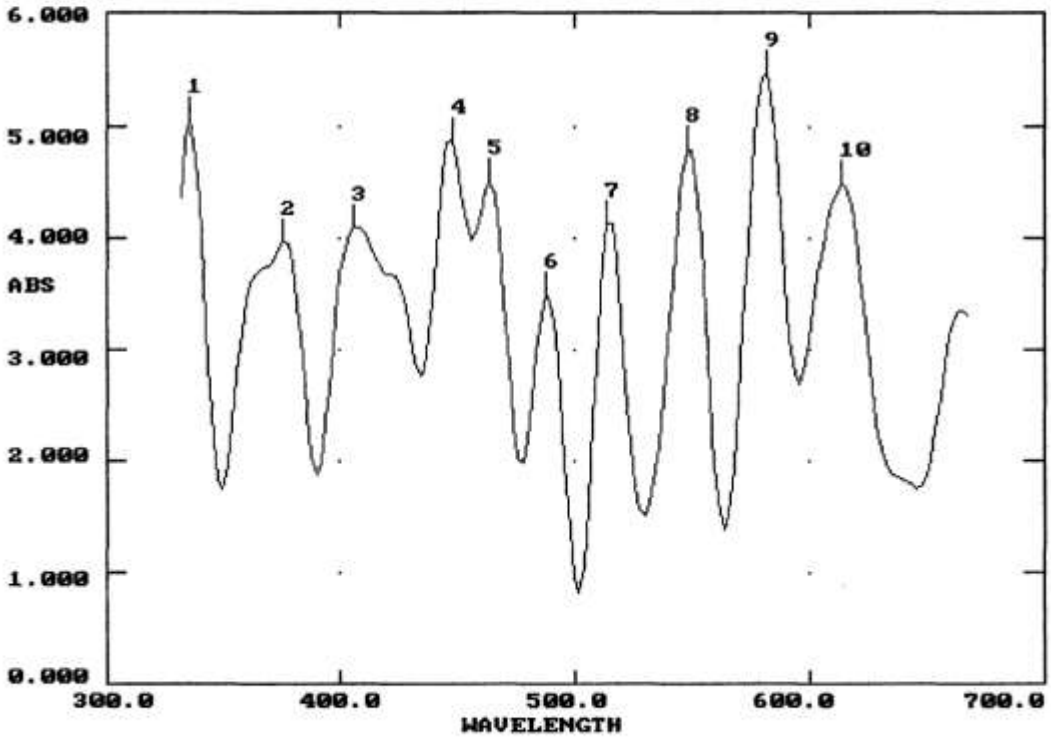


**Figure 4: Absorption Spectra for Nelfinavir**

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :NEVIRA AQ
TIME :12:18:06 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 336.0 | 354.0 | 376.0 | 414.0 | 454.0 | 494.0 | 526.0 | 560.0 | 586.0 | 644.0 |
| ABS | 2.365 | 3.431 | 2.777 | 2.655 | 3.771 | 4.893 | 2.789 | 4.421 | 4.694 | 3.667 |

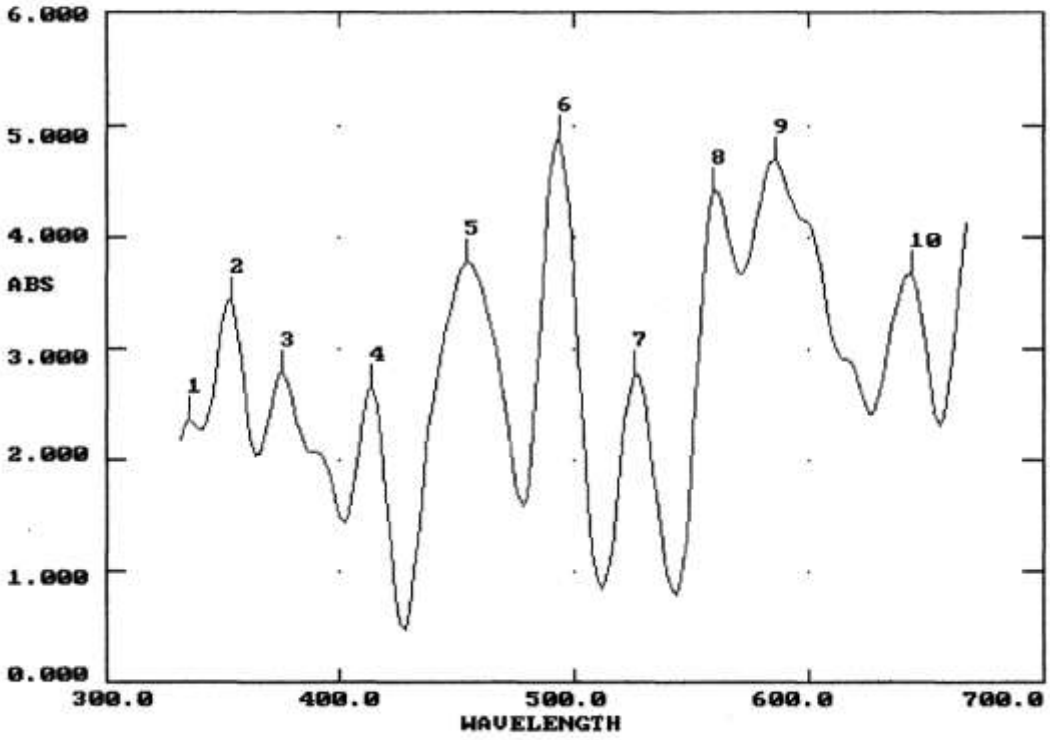


**Figure 5: Absorption Spectra for Nevirapine**

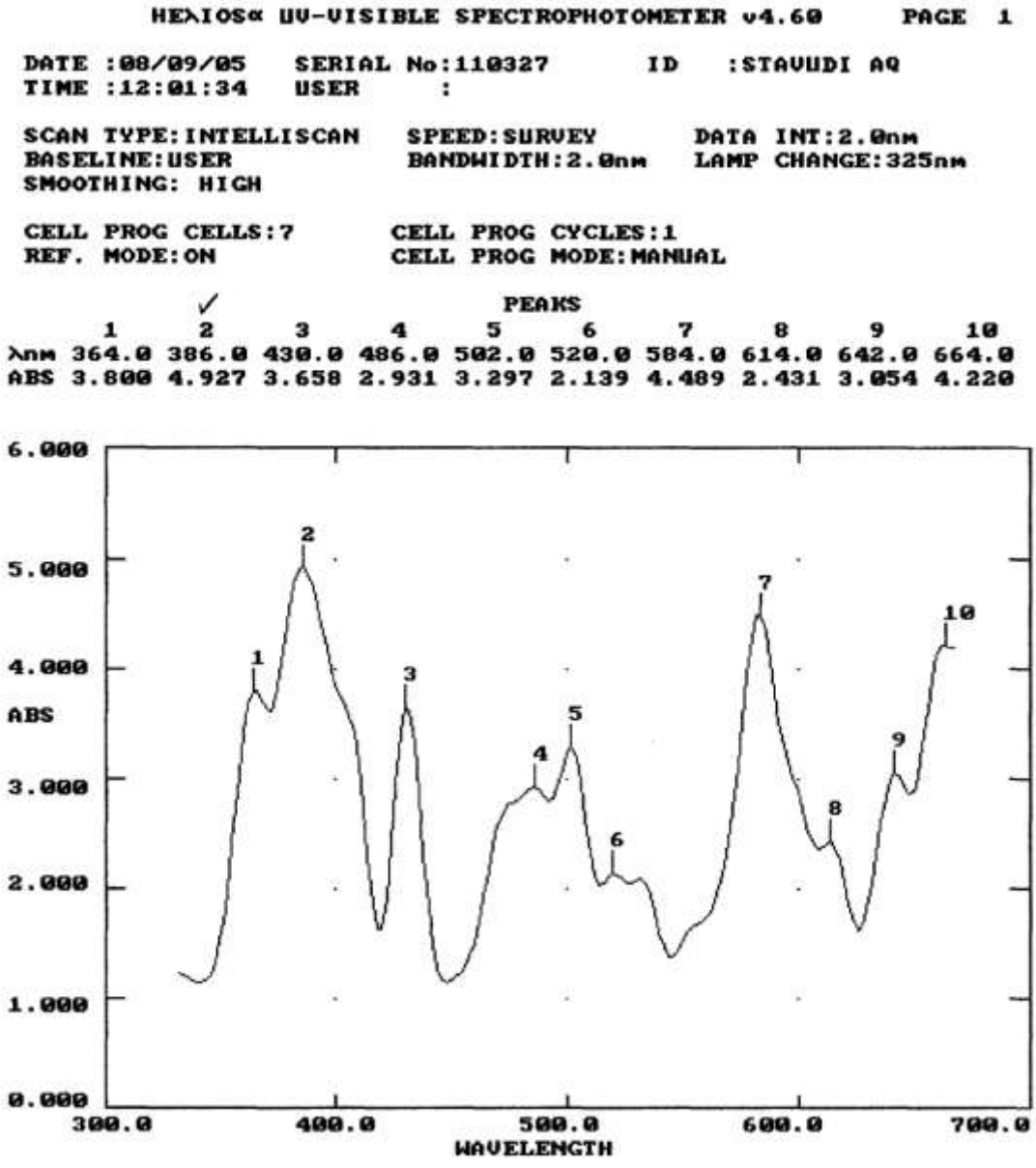


**Figure 6: Absorption Spectra for Stavudine**

HEλIOSα UV-VISIBLE SPECTROPHOTOMETER v4.60 PAGE 1

DATE :08/09/05 SERIAL No:110327 ID :ZIDOVUDN AQ
TIME :11:42:00 USER :

SCAN TYPE:INTELLISCAN SPEED:SURVEY DATA INT:2.0nm
BASELINE:USER BANDWIDTH:2.0nm LAMP CHANGE:325nm
SMOOTHING: HIGH

CELL PROG CELLS:7 CELL PROG CYCLES:1
REF. MODE:ON CELL PROG MODE:MANUAL

PEAKS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| λnm | 342.0 | 378.0 | 408.0 | 470.0 | 508.0 | 534.0 | 552.0 | 600.0 | 652.0 | |
| ABS | 3.680 | 4.534 | 5.631 | 4.442 | 4.080 | 2.404 | 3.519 | 2.974 | 3.867 | |

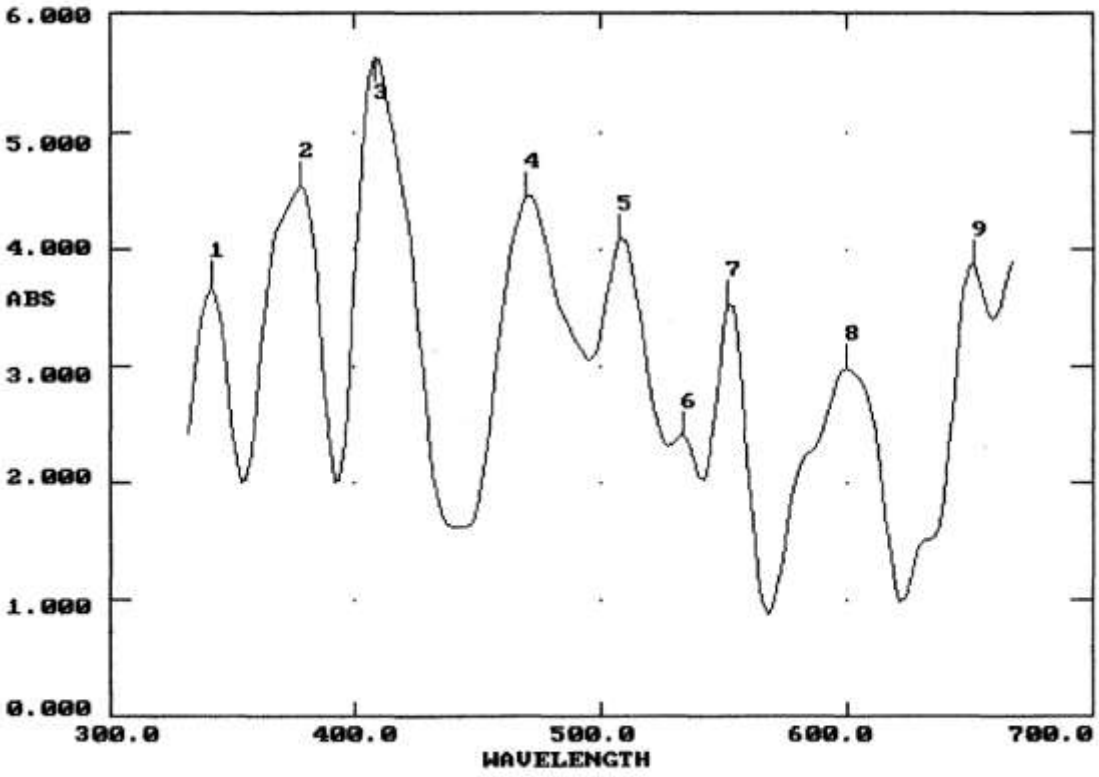


**Figure 7: Absorption Spectra for Zidovudine**