Design and Evaluation of Oral Controlled Release Tablets of Zidovudine

THESIS

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PUNNA RAO RAVI

Under the supervision of

PROF. RANENDRA N. SAHA

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BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Evaluation of Oral Controlled Release Tablets of Zidovudine**" submitted by **Punna Rao Ravi**, ID. No. 2002PHXF402 for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

Date: 29/07/08

(Prof. RANENDRA N. SAHA)

Professor of Pharmacy
Dean, Faculty Division III &
Educational Development Division
B.I.T.S., Pilani

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List of Abbreviations and Symbols

$A^{1\%}_{1\ cm}$	Specific absorbance value
$[\alpha]_D^{25^\circ C}$	Specific optical rotation value at 25 °C and at sodium D line (589 nm)
#	Sieve size
%	Percentage
% RSD	Percentage relative standard deviation
% RTD	Percentage remaining to be degraded
λ_{max}	Wavelength of maximum absorbance
<	Less than
=	Equal to
≈	Approximately equal to
^0C	Degree centigrade
^0C/min	Degree centigrade per minute
3TC	Lamivudine
ACN	Acetonitrile
AIDS	Acquired immunodeficiency syndrome
AT	Accelerated temperature (40 ± 2 °C/75 ± 5 % RH)
AU/V	Absorbance unit per volt
$AUC_{(0-\infty)}$	Total area under curve
AUFS	Absorbance unit full scale
$AUMC_{(0-\infty)}$	Total area under the first moment curve
BP	British Pharmacopoeia
Cells/mm^3	Cells per millimeter cube
cm^{-1}	Centimeter inverse
C_{max}	Maximum concentration
Conc.	Concentration
Copies/ml	Copies per millilitre
cPs	Centipoises
CR	Controlled release
CRT	Controlled room temperature (25 ± 2 °C/60 ± 5% RH)
CSF	Cerebrospinal fluid
D4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DSC	Differential scannimg calorimetry
EC	Ethylcellulose
ELISA	Enzyme linked immunosorbent assay
Endo	Endothermic peak due to melting

ER/XR	Extended release
et al.	Co-workers
f_2	Similarity factor
FDA	Food and drug administration
F_r	Relative bioavailability
FT	Refrigerated temperature (5 ± 2 °C)
FTIR	Fourier transform infra red
g	Gram
g/l	Gram per litre
GIT	Gastro intestinal tract
h	Hour
h^{-1}	Hour inverse
H15K	HPMC K15M or Hydroxypropyl methylcellulose 15000 cPs
H1K	Hydroxypropyl methylcellulose 1000 cPs
H1L	HPMC K100M or Hydroxypropyl methylcellulose 100000 cPs
H4K	HPMC K4M or Hydroxypropyl methylcellulose 4000 cPs
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type-1
HIV-2	Human immunodeficiency virus type-2
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
HQC	Higher quality control sample
ICH	International conference on harmonization
IP	Indian Pharmacopoeia
IR	Immediate release
IUPAC	International Union of Pure and Applied chemistry
J/g	Joule per gram
K	Release rate constant for Ritger-Peppas' empirical equation
K_0	Zero order release rate constant
K_1	First order release rate constant
K_{deg}	Degradation rate constant
kg	Kilogram
kg/cm^2	Kilogram per square centimeter
K_H	Release rate constant representative of square root kinetics
l	Litre
l/h/kg	Litre per hour per kilogram
l/kg	Litre per kilogram
l/mol/cm	Litre per mol per centimeter
l/mol/kg	Litre per mol per kilogram
LOD	Limit of detection
Log $P_{o/w}$	Log of equilibrium partition coefficient

LQC	Lower quality control
M	Molar
MDT	Mean dissolution time
mg	Milligram
$mg/m^2/day$	Milligram per meter square per day
mg/ml	Milligram per millilitre
MgS	Magnesium Stearate
min	Minute
ml/min	Millilitre per mniute
MQC	Medium quality control
MRT	Mean residence time
MSSR	Mean sum of square residuals
M_t/M_∞	Fraction of drug released at time t
mW/mg	Milliwatt per milligram
n	Diffusional exponent indicative of release mechanism in Ritger-Peppas' empirical equation
Na CMC	Sodium carboxymethyl cellulose
NDDS	Novel Drug delivery system
ng/ml	Nanogram per millilitre
nm	Nanometer
NNRTIs	Nonnucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
P	Probability
pH	Negative log to the base 10 of hydrogen ion concentration
PI	Protease inhibitors
$P_{o/w}$	Equilibrium partition coefficient
QC	Quality control
R^2	Regression coefficient
R_f	Retention factor
RH	Relative humidity
RP-HPLC	Reverse phase-High performance liquid chromatography
rpm	Revolutions per minute
RT	Retention time
S	Slope of the least square regression line
SD	Standard deviation
T	Talc
$t_{1/2}$	Half-life
$t_{50\%}$	Time taken for 50% of the drug remaining to be degraded or time taken for 50% of drug release from formulations
$t_{90\%}$	Time taken for 90% of the drug remaining to be degraded
TDW	Triple distilled water
T_g	Glass transition temperature
TLC	Thin layer chromatography

T_{max}	Time taken to reach maximum concentration
UN	United Nations
USP	United States Pharmacopoeia
UV	Ultra Violet
v/v	Volume by volume
Vis	Visible
w/w	Weight by weight
WHO	World Health Organisation
ZDV	3'-Azido-3'-deoxythymidine/Zidovudine
µg/ml	Micro gram per millilitre
µl	Microlitre
µl/ml	Microlitre per millilitre
µm	Micrometer
σ	Standard deviation of *y* intercept of regression equation

Abstract

The objective of this work was to design and evaluate oral controlled release matrix tablets of zidovudine. To achieve this broad objective, analytical methods for accurate and precise estimation of drug in variety of samples were developed. Extensive preformulation studies were carried out for establishing pharmaceutically relevant physicochemical properties of the drug. Oral controlled release matrix tablets were prepared and their in vitro and in vivo performance was evaluated.

Matrix embedded controlled release tablets were prepared using various polymers either alone or in combination using wet granulation process. The effect of various formulation factors like polymer type, polymer proportion, polymer viscosity and compression force; and effect of dissolution factors like pH of dissolution media and agitation speed on the in vitro drug release were assessed. Designed tablets were characterized for drug content, weight variation, friability, thickness and hardness. Further, in vivo studies of selected controlled release tablets were carried out in rabbits to establish the bioavailability relative to immediate release tablets.

Results indicated that all the developed and validated methods were accurate and precise and facilitated estimation of drug in variety of samples. Preformulation studies indicated that the drug has pH independent solubility and moderate equilibrium partition coefficient. Drug was compatible with all the excipients used.

The designed tablets possessed good physical characteristics with acceptable weight variation and good content uniformity. The designed formulations were stable for at least two years when stored at controlled room temperature conditions. Drug release was affected by polymer type, polymer proportion, polymer viscosity and compression force. Change in agitation speed and pH of dissolution media had an effect of drug release depending on polymer used in the matrix. The release mechanism in almost all the designed tablets was anomalous or non-Fickian release. In vivo studies of selected controlled release tablets in rabbits demonstrated significant increase in bioavailability of zidovudine relative to the immediate release tablets.

The designed controlled release matrix tablets of zidovudine have potential to overcome the disadvantages such as adverse side effects, high dose and frequency of administration, poor patient's compliance and adherence and high cost of multi-dose anti-HIV therapy using conventional formulations. The tablet manufacturing method was relatively simple and can be easily adopted in industries on a commercial scale.

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Chapter 1

Introduction, Literature Survey and Objectives

1.1. HIV and AIDS

Acquired immunodeficiency syndrome (AIDS) and cancer are the two leading causes of death all over the world. AIDS is caused by a family of human immunodeficiency viruses and is predicted to spread further world over unless necessary steps are taken to curtail it. Human immunodeficiency viruses are lentiviruses, a family of mammalian retroviruses evolved to establish chronic persistent infection with gradual onset of clinical symptoms. Unlike herpes viruses, replication is constant following infection, and although some infected cells may harbor non-replicating but infectious virus for years, there is generally no true period of viral latency following infection (Greene and Peterlin, 2002). Humans and chimpanzees are the only known hosts for these viruses. There are two major families of human immunodeficiency virus (HIV), namely human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2). Most of the epidemic involves HIV-1. HIV-2 is a close relative whose distribution is concentrated in western Africa. HIV-1 is genetically diverse, with at least five distinct subfamilies or clades. Both types of HIV infection depletes the helper T-lymphocytes, resulting in continued destruction of the immune system, leading to the occurrence of opportunistic infections and malignancies. A person infected with HIV is defined by Centers for Disease Control and Prevention (CDC) as having positive antibodies against HIV (positive HIV test), with 200 or more helper T-lymphocytes, and the absence of an Acquired Immunodeficiency Syndrome (AIDS) defining illness. By definition then, an HIV infected person with AIDS has fewer than 200 cells/mm^3 CD4 cells or the presence of AIDS defining illness. Ref).

AIDS is considered to be an epidemic and according to estimates from the UNAIDS/WHO AIDS Epidemic Update, December 2007, 33.2 million (30.6–36.1 million) adults and 2.5 million (2.2–2.6 million) children (under the age of 15 years) were living with HIV at the end of 2007. In India alone, around 2.5 million people were living with HIV according to the estimates in 2006. The total number of AIDS deaths in 2007 in the world were estimated to be 2.1 million (1.9–2.4 million) out of which 330000 (310000–380000) were children under the age of 15 years. The annual number of AIDS deaths can be expected to increase for many years to come, unless more effective and patient compliant anti-retroviral medications are available at affordable prices (UNAIDS/WHO AIDS epidemic update: December 2007).

During the last decade, though attempts were being made to eradicate HIV, it was found that eradication of HIV is highly unlikely, and effective antiretroviral therapy is required on a long-term basis to maintain viral suppression and reduce disease progression.

During this decade, effective therapies aimed at continued suppression of HIV replication. Researchers are mainly working on designing vaccine for HIV and designing drugs to destroy or suppress HIV. Targeting of HIV resting reservoirs in brain, lymphatic systems will be critical to prolong survival and renewing hopes for a cure. Thus goals of antiretroviral therapy include, reducing the symptoms of HIV infection and delay disease progression to AIDS, reducing viral load to undetectable levels or lowest level possible for sufficiently longer duration, maintenance of durability of viral suppression, eliminating resting reservoirs of HIV, reducing viral resistance and drug failure, designing effective therapeutic regimens that minimizes the drug adherence problem, reducing total pill burden and interference with quality life.

1.1.1. Virus structure

HIV is a typical retrovirus with a small RNA genome of 9300 base pairs. Two copies of the genome are contained in a nucleocapsid core surrounded by a lipid bilayer, or envelope that is derived from the host cell plasma membrane. Several small genes encode regulatory proteins that enhance virion production or combat host defenses. These include *tat*, *rev*, *nef*, and *vpr* (Greene and Peterlin, 2002). Three enzymes important to the virus's life cycle, reverse transcriptase, integrase and protease, are also within the nucleocapsid.

1.1.2. HIV transmission and progression

HIV is transmitted principally in three ways: by sexual contact, by blood (through transfusion, blood products, or contaminated needles), or by passage from mother to child. Characteristically, an HIV infection can progress for eight to ten years before the clinical syndrome (AIDS) occurs. The long latent period of the virus has contributed to many of the problems relating to diagnosis and control. On the other hand, not all cases exhibit the long latent period, and abrupt progression to AIDS occurs. Many factors, including genetics, determine the speed at which the disease will progress in a given individual. The Centers for Disease Control and Prevention (CDC) has identified the stages of a typical HIV infection, categories A, B, and C.

(a) Category A

It is considered to be the first stage and in this stage it is difficult to determine whether an individual is infected without performing a blood test. While at least half of infected individuals will develop a mononucleosis-like illness (headache, muscle ache, sore

throat, fever, and swollen lymph nodes) within three weeks of exposure, some category A individuals are asymptomatic. Moreover, the symptoms themselves can be the result of many different infections. Most of these signs and symptoms subside, but swollen lymph glands and malaise can persist for years through category A HIV.

(b) Category B

In this stage indications of immune system failure begin. Persistent infections such as yeast infections, shingles, diarrhoea, and certain cancerous conditions of the cervix are apparent.

(c) Category C

HIV (clinical AIDS) occurs once CD4 numbers have fallen substantially (200 cells/mm^3) from the normal level (800-1200 cells/mm^3). Category C is synonymous with AIDS. In this stage the opportunistic infections associated with AIDS appear. An important question relevant to treatment is whether HIV disease is a consequence purely of CD4+ lymphocyte depletion or other factors. Most natural history data suggest the former, although both the amount of virus measurable in the patient's circulation and the CD4 cell count are independent predictors of disease progression (Mellors et al., 1996). Regardless, successful therapy is based on inhibition of HIV replication; treatments designed specifically to boost the host immune system without exerting a direct antiviral effect have had no reliable clinical benefit.

1.1.3. Diagnosis and commencement of treatment

Established HIV infection is diagnosed by finding antibodies to HIV in the plasma using various serological testing methods such as ELISA (Enzyme Linked Immuno Sorbent Assays), Orasure western blot, SUDS (Single Used Diagnostic System), Orasure HIV-1. Generally according to the United States Food and Drug Administration and World Health Organization guidelines, the antiretroviral therapy is commenced with these observations, such as when patients experience severe symptoms of HIV infection or have been diagnosed with AIDS or when the viral load in the blood sample is found to be 50,000 copies/ml or more or when the CD4 cell count is less than 200-350 cells/mm^3.

1.2. Anti-HIV Chemotherapy

Since the early 1990s, the number of available drug therapies for HIV-1 infection has increased from 1 to 14. With this explosion in pharmaceutical armamentarium, potent anti-HIV-1 combination therapies became possible. In 1994, there was debate over whether drug therapy conferred long-term benefit to HIV-1 infected patients. By 1996, researchers were suggesting that with prolonged combination drug therapy, eradication of HIV-1 might be possible (Perelson et al., 1997). While the initial hypothesis regarding eradication of HIV-1 by early initiation of therapy using a single drug has proved to be wrong (Finzi et al., 1997), many patients can now achieve indefinite suppression of HIV-1 replication using combination therapy. Treating viruses is always difficult because viruses use the translational machinery of the host cell. Most drugs that target the virus also damage the host. Drugs that can inhibit enzymes specific to the virus are, therefore, less likely to cause side effects in the host. Most common anti-HIV drugs block key steps in viral reproduction and uptake. Several anti-HIV drugs have been synthesized and proven effective over the years based on the understanding of the viral life cycle. Most of the anti-HIV drugs available till date, act on three important enzymes which are involved in the viral life cycle. The various anti-HIV chemotherapeutic treatments that are being currently followed all over the world are discussed below.

1.2.1. Treatments based on understanding the viral life cycle

HIV-1 has three viral enzymes, reverse transcriptase, protease, and integrase; each of these enzymes is essential in the virus life cycle (Watkins et al., 1995). To date, anti-HIV-1 drugs have targeted reverse transcriptase and the viral protease. There are three classes of anti-HIV-1 drugs which act on either of reverse transcriptase or viral protease (Stephen et al., 1991).

1. Nucleoside reverse transcriptase inhibitors (NRTIs).
2. Nonnucleoside reverse transcriptase inhibitors (NNRTIs).
3. Protease inhibitors (PIs).

1.2.1.1. Nucleoside reverse transcriptase inhibitors (NRTIs)

NRTIs were the first anti-HIV-1 drugs developed. They include zidovudine (ZDV), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), tenofovir and emtricitabine. All compounds in this class are prodrugs which need to be converted intracellularly in the cytoplasm to their active form before exerting their antiviral

activity. The active forms of these drugs are substrates for reverse transcriptase enzyme, and they result in termination of DNA chain elongation of the retrovirus. Each of these drugs is an analog of a cellular nucleoside. As nucleoside analogs, the drugs are incorporated into the proviral DNA by the viral enzyme reverse transcriptase (Furman et al., 1986). However, nucleoside analog incorporation results in chain termination, since the analogs lack the 3' - OH group and cannot be linked to additional nucleosides. In this way, nucleoside analogs terminate reverse transcription. Without reverse transcription, the virus life cycle is also terminated.

1.2.1.2. Nonnucleoside reverse transcriptase inhibitors (NNRTIs)

NNRTIs have been demonstrated to be potent and selective inhibitors of HIV-1 replication (De Clercq, 1999). These drugs noncompetitively inhibit the viral enzyme reverse transcriptase. Presumably, NNRTIs bind to HIV-1 reverse transcriptase at a site away from the catalytic domain. This drug enzyme interaction produces a conformational change which results in the inactivation of HIV-1 reverse transcriptase. These drugs have great selectivity for HIV-1 reverse transcriptase and are not active against HIV-2 or other animal retroviruses. As a class of drugs, they are highly active in vivo, for short periods, but are limited by the relatively rapid emergence of resistant HIV-1 strains. NNRTIs do not require enzymatic modification to become active. NNRTIs also share a common toxicity, an eryathematous skin eruption, which is usually self-limited. Unlike NRTIs, these do not require activation by cellular phosphorylation. They include nevirapine, delavirdine and efavirenz.

1.2.1.3. Protease inhibitors (PIs)

HIV makes many of its structural and enzymatic proteins as part of a large polyprotein (Watkins et al., 1995). The viral protease cleaves the polyprotein into the active smaller units. Proteolytic cleavage of the polyproteins is essential to virus infectivity. The HIV-1 protease activity occurs after virus budding. In the absence of proteolytic activity, the HIV-1 virus fails to mature and is not infectious. HIV-1 protease inhibitors act by blocking the viral protease (Kaul, 1999). Hence, virions that are released in the presence of a protease inhibitor remain immature and cannot infect other cells. In this way the virus life cycle is terminated. They include saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir (always used in combination with ritonavir), atazanavir and fosamprenavir.

Apart form the above three major classifications, a newer class of anti-HIV drugs have been in the literature, which are classified as 'Peptide fusion inhibitors'. These inhibit HIV from entering the target cells by hindering the gp41 protein on the virus. Enfuvirtide is the first representative of this new group of antiretrovirals - fusion inhibitors, which is approved for clinical use. Due to its attachment to the HR1 domain of HIV glycoprotein gp41, enfuvirtide blocks the fusion between the virus and the target cell. Enfuvirtide is chemically a synthetic peptide consisting of 36 amino acids and is not stable in GIT and must be administered only subcutaneously. Enfuvirtide has been registered for use as salvage therapy of patients with multi-resistance, for whom it is not possible to constitute an effective combination of other available antiretrovirals (Machala, 2004; Jamjian et al., 2004).

1.2.2. Combination therapy

Mono-therapy is no longer recommended because incomplete viral suppression can encourage development of resistance. The magnitude and durability of viral suppression is lower with dual antiretroviral combinations compared with combinations containing three or more agents (Nadler, 1996). The current strategy for the treatment of HIV infection is called highly active antiretroviral therapy (HAART) and is based on cocktails of drugs that are currently approved by the Food and Drug Administration (FDA). These drugs include compounds that target the viral entry step and the enzymes reverse transcriptase or protease. For example, generally two NRTIs are combined with antiretrovirals from PI or NNRTI class. Still, HAART is often recommended in the first few weeks after exposure to bring the initial viral load down and not as a long term treatment strategy because of high toxic effects. The following anti-HIV drug combination therapies have been reported in the literature by various research groups and were found to be effective than compared to monotherapy.

(a) NRTI combination therapy

While ZDV, ddI and ddC showed some survival advantage over no therapy, the gains were not great. Consequently, in the early 1990s, investigators began studying combination therapies with two NRTIs. Several other trials involving NRTIs confirmed that combination therapy was superior to mono therapy. By the mid 1990s combination therapy with two NRTIs had become the standard of care for HIV-infected patients (Nadler, 1996). However, even these therapies were not potent or long lasting.

(b) NNRTI combination therapy

Nevirapine was shown to be effective in combination with ZDV and ddI (D'Aquila et al., 1996; Montaner et al., 1998). This three-drug regimen resulted in HIV plasma RNA below 20 copies per ml in 52% of patients, compared to 12% on ZDV/ddI (Montaner et al., 1998). Accordingly, the rate of disease in the three-drug arm was 12% compared to 25% in patients taking ZDV/ddI (Montaner et al., 1998). Similar virologic benefit has been seen in patients on nevirapine/d4T and 3TC (Kaspar et al., 1998). However, a recent trial has reported potent inhibition of HIV-1 with delavirdine/indinavir and ZDV (Daly et al., 1998). Efivarenz is the newest NNRTI. This compound has been shown to be very effective in combination with ZDV and 3TC in DMP 266-005 (Hicks et al., 1998). Finally, a regimen without a NRTI, efavirenz plus indinavir, has shown good HIV-1 viral load suppression (Riddler et al., 1998).

(c) Protease inhibitor combination therapy

The most recently approved protease inhibitor, amprenavir, has worked well in combination therapy. A four-drug regimen, consisting of amprenavir/ABC/ZDV/3TC, produced good viral suppression in both chronically and acutely infected patients (Kost et al., 1998). An interesting combination of only two drugs, amprenavir and ABC, has also demonstrated good short-term antiviral activity (Murphy et al., 1998). This two-drug regimen has a practical advantage in that both drugs are administered twice daily. Long-term data are still needed for amprenavir trials. Overall, each of the approved protease inhibitors has shown potent anti-HIV-1 activity when used in combination with other drugs.

(d) Two-Class combination therapy

Soon after clinical trials established combination NRTI (single class) therapy as the standard of care for HIV-1 infection, two new classes of HIV-1 therapeutics, NNRTIs and PIs, emerged. Clinical trials with the NNRTIs and the PIs began in 1995 - 1996. Over the next few years, multiple clinical studies proved that the addition of an NNRTI or PI to a regimen of two NRTIs was more effective than two NRTIs alone. As of early 1999, over 50 trials have been performed with different drug combinations, which employ two or more drug classes. Currently, two-class combination therapy is the most recommended anti-HIV therapy all over the world.

1.3. Problems Associated with Conventional Therapy

The evolution of HIV variants that are resistant to the more commonly used medications have become a major problem. In one study as many as thirty percent of HIV patients harbored resistant viruses. The virus mutates rapidly, and variants that are able to survive in the presence of drug, particularly when circulating levels of the drug are lower, rapidly take over the population. Patient adherence to drug regimens is critical to reducing the emergence of resistant viruses, even the timing of medication can be important. Unfortunately, given the side effects of current treatments, adherence to the dosage regimen is difficult. Majority of the currently marketed anti-HIV agents are formulated as solid dosage forms, viz., tablets and capsules for oral use; or liquid dosage forms, viz., solutions, suspensions for oral and parenteral use. While the oral dosage forms offer convenience, delivery of drugs via this route suffers from significant first pass effect, variation of absorption and degradation in the gastrointestinal tract due to enzymes and extreme pH conditions. For example, ZDV the first antiretroviral developed, although rapidly absorbed from the intestine, loses considerable potency by the hepatic first pass metabolism (40%) and then rapid elimination from the body with a biological half life of only 1 h. As a shortfall for all the conventional oral dosage forms, the duration of action is limited since the absorption of the drug depends on the resident time of the drug in the gastrointestinal tract (Yong and Patrick, 1999).

1.3.1. Improper bioavailability

Many of these compounds exhibit poor or low bioavailability due to various other factors, namely, physicochemical properties such as dissolution rate and solubility, or biological properties such as permeability (ddI exhibits low intestinal permeability) and metabolism. Studies have shown that solution stability and acid lability have become a significant concern in the dosage form development of dideoxynucleosides (Federici et al., 1998). Thus the causes for poor and variable absorption are vast, but they can primarily be related to physicochemical properties such as dissolution rate and solubility. For example, the oral bioavailability of NNRTIs is limited due to their low aqueous solubility. Thus the bioavailability variability of antiretrovirals may be a significant factor in the failure of some of the drug regimens. Also, after an anti-HIV drug is absorbed and enters the blood circulation, metabolism/elimination and transport barriers will substantially decrease the effective amount of drug reaching the target action site. In order to succeed in an effective therapy for AIDS, it is crucial to maintain the systemic drug concentration consistently

above their target antiretroviral concentration throughout the course of the treatment and to enhance localization and intracellular delivery of the drug. However, because of the short biological half life of number of these drugs, conventional routes are inherently limited in that they can not maintain a constant plasma level with the target therapeutic range for a prolonged duration (Xiaoling and William, 1999). Due to virustatic nature of the drugs, they must be administered for the life of the patient.

1.3.2. Toxic or adverse effects

All these therapeutic moieties exhibit dose-dependent toxic side effects such as hepatotoxicity, hyperglycaemia, hyperlipidemia, lactic acidosis, lipodystropy, osteonecrosis, osteoporosis, osteopenia, skin rashes, resulting from excessive systemic concentration and they often require dosage reduction or even cessation of treatment, since conditions like lactic acidosis may even be fatal. Thus despite their undisputed effectiveness, several complicated clinical issues are associated with the prolong use of these agents (Fischl et al., 1987; Kahn et al., 1992).

Protease inhibitors can cause nausea and diarrhoea, and some of the nucleoside reverse transcriptase inhibitors can cause red or white blood cell levels to drop. Painful nerve damage and inflammation of the pancreas can also result (Kaul et al., 1999). Unfortunately, HAART has several long-term side effects including kidney, liver, and pancreatic problems; and changes in fat metabolism, which result in elevated cholesterol and triglyceride levels and an increased risk for strokes and heart attacks. In addition, some viruses have evolved resistance to HAART.

1.3.3. Patient compliance

Adherence of the patient to the treatment is critical, as loss of antiviral efficacy has been correlated with poor pill taking resulting in loss of viral suppression. The high pill burden [Fosamprenavir - 1400 mg twice daily, nelfinavir - 1250 mg twice daily (10 tablets daily), amprenavir - 1200 mg three times daily (16 tablets daily)] and coordination with the meals make these agents laborious to take (Bruce, 1999).

1.4. Novel Approaches in the treatment of HIV and AIDS

Researchers are being challenged to find new treatment strategies as the currently used drug therapies begin to fail in some patient population, reports on termination of therapy due to drug side effects are mounting, and new drug resistant strains of HIV are

emerging. These strategies include a "multi-drug cocktail" therapy that attacks at several stages of HIV life cycle; a therapeutic vaccine which can boost the immune response against the virus; the development of a preventive vaccine based on a weakened strain of HIV, and the successful maintenance of HIV-inhibitory concentrations at target sites with minimal side effects. To avoid hepatic first pass metabolism and intestinal degradation, efforts are being made to alter the mode and route of delivery of the drug (Haresh and Chien, 1993). Delivery of nucleoside analogues through percutaneous, rectal, buccal, nasal, intrathecal routes and as coated dosage form by oral route, are being studied. Percutaneous absorption has been one of the most reported routes for non-oral administration of anti-HIV agents (Dae and Chien, 1995). Also efforts have been made to design drug delivery systems for anti-HIV agents to reduce the dosing frequency, to enhance the bioavailability, to improve the CNS penetration and inhibit the CNS efflux and to deliver them to the target cells selectively with minimal side effects. Amongst the recent approaches controlled and targeted delivery are the noted ones.

1.5. Need for Controlled Drug Delivery of Anti-HIV drugs

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

1.6. Need for the Controlled Release Drug Delivery Systems of Zidovudine

Zidovudine (ZDV), the first effective antiretroviral agent, was synthesized by Horwitz in 1964 as a false nucleoside with disappointing anticancer activity. It is an analog of thymidine. ZDV (3'-azido-3'-deoxythymidine) has potent in vitro activity against a broad spectrum of retroviruses including HIV-1, HIV-2, and human T-cell lymphotrophic viruses (HTLV) I and II (McLeod and Hammer, 1992). Over the past several years, the wide use of

ZDV in patients with the AIDS has been associated with decreased morbidity, slower progression of the disease and longer survival.

However, treatment of AIDS using conventional formulations of zidovudine (ZDV) is found to have many drawbacks such as poor bioavailability, adverse side effects due to accumulation of drug in multi-dose therapy, poor patient compliance (due to its shorter half-life) and high cost.

Hematological side effects are more likely to occur with higher doses and in patients with more advanced disease. Lactic acidosis, in the absence of hypoxemia, and severe hepatomegaly with steatosis, including fatal cases, has been reported with the use of antiretroviral nucleoside analogs, including ZDV (Fischl et al., 1987). The most frequent side/adverse effects of ZDV are granulocytopenia and anemia. These have been shown to be inversely related to the CD4+ lymphocyte count, hemoglobin concentration, and granulocyte count at the time of therapy initiation and directly related to dosage and duration of therapy. Significant anemia most commonly occurs after 4 to 6 weeks of therapy. The incidence of adverse reactions from ZDV therapy appears to increase as the disease progresses (McLeod and Hammer, 1992). So, controlled release once daily formulation of ZDV can overcome some of these problems.

1.7. Oral Controlled Release Drug Delivery Systems

1.7.1. Conventional oral drug delivery systems and their limitations

From many decades treatment of an acute or chronic disease has been mostly accomplished by delivery of drugs to patients using conventional dosage forms like tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables. Even today these conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescription and the over-the-counter (OTC) drug market place. Oral drug delivery has been known for decades as the most widely utilized and convenient route of drug administration compared to all other routes. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration, cost, as well as the traditional belief that by oral administration the drug is as well absorbed as the food stuffs that are ingested daily (Chien, 1992). Pharmaceutical products designed for oral delivery and currently available on the prescription and over the counter markets are mostly designed for immediate release of drug for rapid absorption. Conventional multidose therapies for long duration of action are not without problem. The problems are, continuous peak and

trough profiles of the drug blood levels with brief optimum therapeutic drug blood level, frequent administration of the dosage forms, adverse/side effects etc (Ainaoui and Vergnaud, 2000). The fluctuating drug levels in blood and tissues lead to a variable influence in the disease treatment (Conard et al., 1982) and are related to an excessive use of the drug that lead to unnecessary wastage of the drug. As the drug concentrations remain higher for a long duration there are increased chances of incidence of toxic or side effects that necessitate discontinuation of the treatment. If the drug concentrations come below minimum effective concentrations for a prolonged period of time or therapy is discontinued, these might result in emergence of drug resistant strains in case of antibiotic treatment. This is another major threat in the treatment of chronic diseases like tuberculosis, malaria, diabetes, hypertension and AIDS. Increased frequency of administration is another drawback in case of conventional medications (Singh and Agarwal, 2002). Less the frequency of administration, more will be the patient compliance and treatment adherence. Apart from these drawbacks, conventional systems also possess many other problems.

1.7.2. Oral controlled release drug delivery systems and their advantages

An ideal drug delivery system should be able to deliver an adequate amount of drug, preferably for an extended period of time, for its optimum therapeutic activity. Most drugs are inherently not long-lasting in the body, and require multiple daily dosing to achieve the desired blood concentration to produce therapeutic activity. To overcome such problems, controlled release and sustained release delivery systems are receiving considerable attention from pharmaceutical industries world-wide. Controlled release drug delivery systems not only prolong the duration of action, but also result in predictable and reproducible drug-release kinetics (Chien, 1982).

The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery systems of existing drugs molecules to maximize their effectiveness in terms of their therapeutic action and patent protection (Parakh et al., 2003). The revolution in oral drug delivery systems has been made possible by the development of two areas of knowledge, pharmacokinetics and pharmaceutical technology. Controlled release drug delivery systems offer important advantage over traditional dosage forms in diseases requiring prolonged duration of therapy. The development of an improved pharmaceutical dosage form such as an oral controlled release preparation should be based upon the pharmacokinetic and the pharmacodynamic properties of the drug. In addition the issues such as minimization of adverse drug reactions,

reduction in the repeated fluctuation of serum and tissue concentration, prevention of the development of the resistant organisms (in the case of antimicrobial therapy), patient compliance factors and treatment cost must be considered (Hoffman et al., 1998).

The controlled release drug delivery systems market was worth over approximately US (United States) $ 17 billion globally in 2007. Drug delivery industry specialists estimated that oral controlled release drug delivery systems contributed the majority of sales around 90% and demonstrated +2% year-on-year growth. By 2015, the drug delivery industry specialist forecasts that controlled release drug delivery systems in development from 22 key companies in USA could generate additional sales of over US $ 11.7 billion. These products utilize a range of delivery platforms including diffusion, degradation and activated controlled-release systems (Cheryl, 2007).

Oral controlled release drug delivery systems of various drugs approved by US or European Union FDA and in developmental or approval filing stage by various companies from the year 2006 are given in Table 1.1 and Table 1.2 respectively. The current global market scenario of various controlled release drug delivery systems is given Figure 1.1. Oral controlled release drug delivery systems contribute approximately 52% of the total controlled release drug delivery systems market globally. The market for oral controlled release drug delivery systems is expected to grow at 9% or more every year. The main reasons for the success of oral controlled release drug delivery systems are attributed to the ease of their manufacturing process and reproducibility of desirable biopharmaceutical properties. The driving forces behind the booming market can be divided into two main groups, patient related factors and market driven factors.

1.7.2.1. Patient related factors - Advantages

Avoiding peak and trough profiles of multidose therapy by maintaining steady and therapeutically effective levels of the drug over a prolonged time period is beneficial for the patient compliance. This results in the effective therapeutic efficacy and better management of the results, with reduction of adverse side effects and improvement in tolerability. Drug plasma levels are maintained at a constant level or within narrow window with no alternate peak and trough profiles and with area under the curve (AUC) of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release dosage form. The enhanced patient compliance is also due to reduction in dosing frequency and reduction in dosage units to be administered. Reduction in total health care cost could probably be the important factor in poor or developing countries like India.

1.7.2.2. Market driven factors - Advantages

Drug delivery is a valuable drug life cycle management tool. The most important force driving the growth and viability of the pharmaceutical industry, the regular introduction of new chemical entities (NCEs), is currently weak. Expenses accrued from drug development are hitting the roof and true innovation is at an all time low. Moreover, FDA's more cautious review process and demand for a greater number of complex clinical trials are increasing the total time period required to market a new molecule. In addition, compliance with 21 CFR Part 11 and the new Health Insurance Portability and Accountability Act (HIPAA) is affecting the clinical trial process. In 1996, FDA approved 53 NCEs; this figure dropped to 27 in 2000, 22 in 2003, 18 in 2006 and 17 in 2007 (Jain, 2004; Business Insights, 2006; Bethan, 2008). In the next five years, at least 20 blockbuster products with combined sales of nearly US $ 40 billion will lose patent protection. Hence the emergence of repatentability, achieved by the introduction of controlled release formulations of existing immediate release products, as an attractive financial option for pharmaceutical companies, in addition to seeking new therapeutic indications for these new products.

The potential for oral controlled release drug delivery technology to extend the period of exclusivity for patent holders has significant financial rewards. Pfizer's Procardia XL® (controlled delivery system of Nifedipine) provides a prime example of a reformulated product generating large revenues and reviving an eroding sales base. Delivery technologies can also mean expanded market for existing drugs, based upon new indications of use and/or the reduction of adverse side effects. Given the staggering costs of drug development, any opportunity to extend or expand the applications of existing intellectual properties represents a significant opportunity (Bill, 2000; Shah, 2004).

Oral controlled release drug delivery systems offer great promise for the pharmaceutical industry like increased therapeutic efficacy, patent extension, product differentiation and delivery of biotechnology products. Many of the complex systems currently in use, whilst effective, are highly complex in design, adversely impacting development, scale-up and manufacturing, and thus severely limiting their application. The future of drug delivery lies in technologies that address these practical issues of formulation, scale-up and production, whilst providing even greater therapeutic control.

1.7.3. Types of oral controlled release drug delivery systems

Oral controlled release delivery system is a drug-containing dosage form that releases the drug continuously in a predetermined pattern for a fixed period of time. Various devices have been studied and built up in order to control the release of the drug through out the GIT through which the pH varies from 1 to 8 (Theeuwes, 1983; Chien, 1982; Jerzewski and Chien, 1991; Chien 1992). They can be divided into five categories as (Jantzen and Robinson, 1996):

1. Dissolution controlled drug delivery systems.
2. Diffusional controlled drug delivery systems.
3. Bioerodable and/or combination of diffusion and dissolution controlled drug delivery systems.
4. Osmotically controlled drug delivery systems.
5. Ion exchange controlled drug delivery systems.

1.7.3.1. Matrix based controlled release drug delivery systems

Matrix controlled release tablets are relatively simple systems that are more forgiving of variations in ingredients, production methods and end-use conditions than coated controlled release tablets and other systems. This results in more uniform release profiles with a high resistance to drug dumping. Matrix systems are relatively easy to formulate and produce on a commercial scale. The performance of many products is already well documented, providing a body of data to refer to and rely upon. This helps speed development work and shortens approval times as well. Tablets are manufactured with existing, conventional equipment and processing methods. This is true for almost any size tablet; thereby it involves direct compression, dry granulation, or wet granulation. Matrix systems are economical. Beyond the possibility of lower development costs and the use of conventional production methods, the ingredients normally used are cost effective.

Matrix-based delivery technologies have steadily matured from delivery drugs by first-order or square-root-of-time release kinetics to much more complex and customized release patterns. In order to achieve linear or zero-order release, various strategies that seek to manipulate tablet geometry, polymer variables and formulation aspects have been applied. Various drug, polymer and formulation-related factors, which influence the in-situ formation of a polymeric gel layer/drug depleting zone and its characteristics as a function of time, determine the drug release from the matrix systems (Verma et al., 2004). In the matrix systems, the drug is homogenously dispersed in either a hydrophobic or hydrophilic polymer matrix. A hydrophilic matrix, controlled release system is a dynamic one involving

polymer wetting, polymer hydration, gel formation, swelling and polymer dissolution. The mechanisms by which drug release is controlled in matrix tablets are dependent on many variables. The main principle is that the water soluble polymer, present through out the tablet, hydrates on outer tablet surface to form a gel layer. Throughout the life of the ingested tablet, the rate of drug release is determined by diffusion (if soluble) through the gel and by the rate of tablet erosion. The release rate from the matrix systems remains unaffected by thin spots, pinholes, and other similar defects, which can be a serious problem with reservoir systems (Soppimath et al., 2001). These advantages, along with the low fabrication cost, outweigh the less desirable feature of declining release rates with time, which is a characteristic of matrix systems.

1.7.3.2. Polymers used in the development of matrix based controlled release formulation

Several hydrophilic and hydrophobic polymers have been reported as carriers/release retardant materials in the development of oral controlled release formulations of the drugs. Selection of the suitable matrix material depends on the dose size, desired release rate and the physicochemical properties of the drug of interest (Verma et al., 2004). Hydrophilic polymers have been paid considerable attention in the formulation of controlled release formulations of many drugs. Hydroxyethyl cellulose (Ranga et al., 1988), Hydroxypropyl cellulose (Nakano et al., 1983), carbopols (Khan and Jhu, 1999), polyvinyl alcohol (Korsemeyer et al., 1983), PHEMA (Polyhydroxyethylmethyl acrylate), methyl methacrylate, vinyl acetate, ethylene oxide, polyethylene oxide (PEO) (Dimitrov and Lambov, 1999), hydroxypropyl methyl cellulose (Lee et al., 1999), methyl cellulose and sodium carboxy methyl cellulose (Ranga et al., 1988) are some of the hydrophilic polymers which have been extensively used in the formulation of controlled release systems. Ethylcellulose (Sajeev and Saha, 2001) is one of the most widely used hydrophobic polymers in the formulation of controlled release systems.

(a) Hydroxypropyl methylcellulose (HPMC)

HPMC, a semi synthetic derivative of cellulose, has its popularity for the formulation of controlled release dosage forms as a swellable and hydrophilic polymer (Velasco et al., 1999; Varghas and Ghaly, 1999; Maggi et al., 1999; Ford et al., 1985). From a commercial point of view, HPMC is the most prominent carrier material in pharmaceutical applications. Its nontoxic property, ease of handling, ease of compression,

ability to accommodate a large percent of drug, negligible influence of the processing variables on drug release rates and relatively simple tablet manufacturing technology make it an excellent carrier material (Alderman, 1984; Skoug et al., 1993; Lee et al., 1999). Various grades of HPMC viz. 1000 cps, 4000 cps, 15000 cps, 100000 cps etc. are available based on the viscocity it produces upon hydration.

HPMC contains methoxyl and hydroxypropyl substituents on its β-*o*-glucopyranosyl ring backbone, which makes it very resistant to changes in pH or ionic content of the dissolution medium. At pH values from 2 to 13, HPMC is relatively stable and the matrix formulations of any drug prepared using HPMC can show pH independent drug release if the drug has pH independent drug solubility (Marcos et al., 1996). Matrix tablets prepared using HPMC on contact with aqueous fluids gets hydrated to form a viscous gel layer through which drug will be released by diffusion and/or by erosion of the matrix (Katzhendler et al., 2000).

Various formulation factors influence the drug release from HPMC particle matrices like polymer viscosity, polymer particle size, drug to polymer ratio, drug solubility, drug particle size, drug loading, compression force, tablet shape, formulation excipients, coatings and processing techniques as well as dissolution medium (Ranga et al., 1988; Velasco et al., 1999; Maichel et al., 2001). The release kinetics of a practically water insoluble drug within two types of HPMC matrices was studied and it was observed to follow non-Fickian release behavior (Eyjolfsson, 1999). However for slightly water soluble drugs, variation of HPMC content and viscosity grade found to produce a wide range of drug release rates (Maggi et al., 1999). The influence of drug to HPMC ratio, drug particle size, polymer particle size and compression force on the release rate has been studied (Velasco et al., 1999). Effects of some formulation variables in the release of promethazine HCl has been investigated (Ford et al., 1985). It was observed that, increased compaction force, increase the apparent density of HPMC based tablets, but the release characteristics were not markedly effected (Ebube and Jones, 2003).

(b) Ethylcellulose

Ethylcellulose (EC) is an inert, hydrophobic polymer and is essentially tasteless, odourless, colourless, noncaloric, and physiologically inert. It has been extensively used as a pharmaceutical vehicle in a number of dosage forms. It has been used as a coating material for tablets and granules (Donbrow and Friedman, 1974), as a tablet binder (Chowhan, 1980), in preparing microcapsules and microspheres (Jalsenjak et al., 1976;

Bodmeier and Chien, 1989) and also as film and matrix-forming material for sustained-release dosage forms (Shaik et al., 1987; Porter, 1989). The release of a drug from hydrophobic EC matrix occurs by dissolution and diffusion of the drug through water filled capillaries within the pore network (Neau et al., 1999).

(c) Carbopol

Carbopol polymers are synthetic, high molecular weight acrylic acid polymers cross-linked with polyalkenyl ethers or divinyl glycol. These polymers readily hydrate, absorb water and swell quickly (Khan and Zhu, 1999) up to 1000 times their volume to form a gel when exposed to pH environment above 4 to 6. In addition to their hydrophilic nature and cross-linked structure, their essential insolubility in water make these polymers potential candidates for use in controlled release formulations (Huang and Schwartz, 1995). Among these, Carbopol 934P and 971P are the most widely used pharmaceutical grade polymers for oral use (Durrani et al., 1994). Since their introduction in 1957 a number of controlled release tablet formulations have been patented.

Jelena et al. (2004) have studied the effect of proportion of carbopol on the release of paracetamol from matrix based tablets. They found that the release of drug from carbopol matrices occurs by swelling controlled diffusion process and/or by controlled relaxation of the polymer. The release rate decreased with increase in polymer proportion. The release mechanism was found to change from non-Fickian release to Super Case II release as the proportion of polymer was increased. Marcos et al. (1996) have studied the release profile of atenolol from carbomer matrix tablets. They found that release profiles followed higuchi's square root kinetics model and compression force had no effect on the drug release. Researchers have reported that increasing the amount of carbopol 934P in the matrix tablet formulation resulted in a reduction in the drug release rate and linearization of the release profiles with shift in the release mechanism (Khan and Zhu, 1999).

(d) Sodium carboxy methylcellulose (Na CMC)

Sodium carboxy methylcellulose is a cellulose ether gum. It is anionic in nature. It is a water-soluble polymer. It is the sodium salt of poly carboxy methyl ether of cellulose, which is produced by reacting alkali cellulose with sodium monochloro acetate under rigidly, controlled conditions. It is available in the form of granules, coarse or fine powders and in varied grades of viscosity. It is an odourless, tasteless and non-toxic polymer which is highly soluble in hot as well as cold water but stable towards hard water, alkalies, acids

and certain electrolytes. However, multivalent metal ions and certain complex cations tend to precipitate sodium carboxy methylcellulose from solution. The solution of sodium carboxy methylcellulose has better resistance to microbiological attack as compared to many natural products.

Sodium carboxy methylcellulose (Na CMC) comes in three molecular sizes: low, medium and high. It has a long history of use as a suspending agent in liquid pharmaceutical preparations. It is also used as tablet binder. Recent work has confirmed the usefulness of cellulose gum in sustained-release applications (Shamsuddin et al., 2006; Paolo et al., 2000; Bhupinder et al., 2006; Madhusudan et al., 2001). In general, the reported results showed that the drug release rate decreases with an increase in the molecular size of Na CMC. The drug release mechanism was influenced by both the molecular size of Na CMC and the presence of polymer additives (Martins et al., 2005, 2006). Martins et al. (2006) have investigated the effect of molecular size of Na CMC and some polymers on the sustained release of theophylline from compressed tablets. Drug release was found to be higher from the formulation containing lower molecular size of Na CMC compared to medium and higher molecular sizes. Drug release was mainly dependent upon the rate and extent of water penetration into the tablet matrix and the relative aqueous solubility of both the matrix material and the drug compound embedded in the matrix.

It is generally accepted that diffusion and release rates decrease with an increase in molecular size. Hydrophilic polymers such as Na CMC rely on water absorption to produce gel swelling and matrix relaxation, which subsequently facilitate drug dissolution and diffusion from the matrix. When Na CMC was used in combination with other polymers the release pattern was found to be different compared to formulation which contains only Na CMC. The release pattern was found to be nearly zero-order when Na CMC is used in combination with polymers like HPMC and Carbopol (Korsmeyer and Peppas, 1983; Emami et al., 2004; Bhupinder et al., 2006).

1.7.3.3. Release kinetics and release mechanism of drugs from matrix formulations

The pharmaceutical industry and the registration authorities do focus, nowadays, on drug dissolution studies. The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used. In some cases, these mathematical models are derived from the theoretical analysis of the occurring

process. In most of the cases the theoretical concept does not exist and some empirical equations have proved to be more appropriate.

Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug is a function of the test time. The quantitative interpretation of the values obtained in the dissolution study is facilitated by the use of generic equation that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage form. The following mathematical models are most frequently used to describe the drug dissolution from matrix based controlled release tablet formulations.

(a) Release kinetics models

1) Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation:

$$M_t = M_0 - K_o t \qquad \text{(I)}$$

Where, M_o is the initial amount of drug at zero time, M_t is the amount of drug released at any time t and K_o is the zero order release rate constant.

This relation can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix with low soluble drugs, coated forms, osmotic system, etc. The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action.

2) First order kinetics

The application of this model to drug dissolution studies was first proposed to describe the absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism in a theoretical basis (Gibaldi and Perrier, 1982). The following equation can be used to describe the drug release which is following first order kinetics.

$$LogM_r = LogM_o - K_1 t/2.303 \qquad \text{(II)}$$

Where, M_o is the initial amount of drug at zero time, M_r is the amount of drug remaining to be released at time t and K_1 is the first order release rate constant.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such a way, that the amount of drug released by unit of time diminishes (Costa and Lobo, 2001).

3) Higuchi's square root kinetics

Higuchi has developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semi-solid and/or solid matrices. The simplified form of the Higuchi square root kinetics model is expressed as follows:

$$M_t/M_\infty = K_H t^{1/2} \qquad \text{(III)}$$

Where, M_t/M_∞ is the fraction of drug released at any time t and K_H is the release rate constant representative of the square root kinetics.

This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems (Costa et al., 1996) and matrix tablets with water soluble drugs (Desai et al., 1996a,b; Schwartz et al., 1968a,b; Costa and Lobo, 2001)

4) Ritger-Peppas' empirical model:

Ritger and Peppas have developed a simple, semi-empirical model, relating the drug release exponentially to the elapsed time. The equation describing the release kinetics is expressed as follows:

$$M_t/M_\infty = Kt^n \qquad \text{(IV)}$$

Where, M_t/M_∞ is the fraction of drug released at any time t and K is a constant incorporating structural and geometric characteristics of the device and n is the release exponent characteristic of the release mechanism. The magnitude of the exponent n allows the general indication of Fickian diffusion, case II transport, or anomalous transport as the release mechanism. For tablets, a n value between 0.43 and 0.5 indicates Fickian diffusion-controlled drug release, and a n value 0.89 indicates a swelling controlled drug release (near zero-order release or case II transport). Values of n between 0.5 and 0.89 can be regarded as an indicator of the superimposition of both phenomenon, commonly called anomalous transport (Ritger and Peppas, 1987a,b; Siepmann and Peppas, 2001; Korsmeyer

et al., 1983). Fickian diffusional release occurs by molecular diffusion of the drug because of the chemical potential gradient. Case II relaxational release is the drug transport mechanism associated with stresses and state transition in hydrophilic glassy polymers. These two mechanisms controlling the drug release are considered additive and can be resolved to get the contribution of either in ultimate drug release.

A thorough understanding of drug release mechanism and kinetics gives a fairly good idea about the variables that can be manipulated in achieving a desired release profile. An extensive review by Siepmann and Peppas on the mathematical modeling of drug release from HPMC matrices deserves a special mention (Siepmann and Peppas, 2001). Narasimhan and Peppas (Narasimhan and Peppas, 1996, 1997) developed mathematical models describing polymer dissolution based on the theory of macromolecular disentanglement and chain repetition. They showed that the dissolution could be either disentanglement or diffusion controlled and also can be a combination of both depending on the polymer weight and the thickness of the diffusion boundary layer.

(b) Release profiles comparison

Several methods were proposed to compare the drug release profiles and they were classified into statistical methods, model-dependent methods and model-independent methods (Shah and Polli, 1996; Ju and Liaw, 1997; Polli et al., 1997; Fassihi and Pillay 1998). However, mean dissolution time (MDT) and similarity factor (f_2), two model-independent methods are the most widely used methods for comparing the drug release profiles (Costa and Lobo, 2001). The MDT values can be calculated using the following expression:

$$MDT = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad \text{(V)}$$

Where, j is the sample number, n is the number of dissolution sample times, $\hat{t}_j$ is the time at midpoint between t_j and t_{j-1} (easily calculated with the expression $(t_j + t_{j-1})/2$) and ΔM_j is the additional amount of drug released between t_j and t_{j-1}.

The similarity factor f_2, is a logarithmic transformation of the sum-squared error of differences between the two release profiles over all the time points.

It can be calculated using the following expression as follows:

$$f_2 = 50 \times \log\left\{\left[1 + 1/n \sum_{j=1}^{n} \left|R_j - T_j\right|^2\right]^{-0.5} \times 100\right\} \qquad \text{(VI)}$$

Where, n is the sample number, R_j and T_j are the percent drug release of the two drug release profiles being compared at each time point j. The similarity factor fits the result between 0 and 100. It is 100 when the two drug release profiles are identical and tends to 0 as the dissimilarity increases.

1.8. Objective of the Present Research Endeavor

ZDV is a potent antiviral agent used in the treatment of AIDS. Conventional formulations of ZDV are administered multiple times a day depending on the dose (300 mg twice daily or 200 mg thrice daily) due to its short half-life (Anthony and Clifford, 2001; Betty, 2000; Laskin et al., 1989). Treatment of AIDS using conventional formulations of ZDV is found to have many drawbacks such as adverse side effects due to accumulation of drug in multi-dose therapy (Chitnis et al., 2002; Chariot et al., 1999), poor patient compliance (Re et al., 2003) and high cost. So, controlled release once daily formulations of ZDV can overcome some of these problems.

Reports were found on the extended release of ZDV from its matrix tablets prepared using combination of hydrophilic (Eudragit) and hydrophobic (ethylcellulose) polymers (Kuksal et al., 2006). The release of ZDV was reportedly extended from 4 to 12 hr. In vitro release of ZDV from ceramic capsules prepared using tricalcium phosphate and alumino-calcium-phosphorous oxide for sustained action had been investigated by Benghuzzi and his co-workers (Benghuzzi et al., 1990). Long-term sustained delivery of ZDV in vivo by means of hydroxyapatite and tricalcium-phosphate ceramic implants was studied by Benghuzzi (Benghuzzi, 2000). It was found that ceramic drug delivery systems can be effectively used in both sustaining and reducing the fluctuations of ZDV concentration levels in blood and tissues.

The present research work was aimed at design and development of oral controlled release tablet formulations of ZDV in order to improve the bioavailability, maintain uniform drug levels in blood, and reduce the dose, to improve patient compliance and to reduce the cost of therapy.

The research endeavor was to prepare oral controlled release matrix tablets of ZDV using various rate controlling polymers either alone or in combination for improving

bioavailability and reduce the dose (thereby the side effects and cost). Research work was carried out in the following stages for achieving this broad objective.

- Preformulation studies of ZDV.
- Preparation of controlled release tablets of ZDV using different polymers alone or in combination and to study the physical characteristics and in vitro drug release characteristics. The effect of following parameters on the in vitro drug release characteristics were studied and analyzed in order to optimize the parameters.
    a. Polymer type (Hydrophilic, Hydrophobic, Anionic etc)
    b. Polymer proportion
    c. Polymer viscosity
    d. Compression force
    e. Agitation speed
    f. pH of the dissolution media
- Stability studies of selected formulations.
- In vivo pharmacokinetic and bioavailability studies of selected formulations.

The proposed work required suitable and sensitive analytical methods for analysis and estimation of drug in variety of samples like bulk powders, formulations, in vitro drug release samples, stability samples and biosamples. So it was also planned to develop and validate suitable analytical techniques like ultraviolet (UV) spectrophotometry and high performance liquid chromatography (HPLC) for the estimation of drug in variety of samples.

Table 1.1: Oral controlled release drug delivery systems approved by United States or European Union FDA since the year 2006 till date (Rajeev, 2007)

Product	Drug	Company	Approval Date	Technology
OPANA XR Tablets	Oxymorphone hydrochloride (5/10/20/40 mg)	Endo, USA	June 22, 2006	Matrix Tablets
SERQUEL XR Tablets	Quetiapine fumarate (50/200/300/400 mg)	Astrazeneca, USA	May 17, 2007	Matrix Tablets
ZYFLO CR Tablets	Zileuton 600 mg	Critical, USA	May 30, 2007	Matrix Tablets
SANCTURA XR Tablets	Trospium chloride 60 mg	Indevus, USA	August 3, 2007	Matrix Tablets
CLARINEX-D 12 HOUR Tablets	Desloratadine (2.5 mg) + Pesudoephedrine sulfate (120 mg)	Schering, USA	February 1, 2006	Bilayered Tablets (Desloratadine as IR and pesudoephedrine sulfate as ER)
COREG XR Capsules	Carvedilol phosphate (10/20/40/80 mg)	SB Pharmco, USA	October 20, 2006	Multiparticulates
AMRIA ER Capsules	Cyclobenzaprine hydrochloride (15/30 mg)	ECR, USA	February 1, 2007	Multiparticulates

XR/ER – extended release, IR – immediate release

Table 1.2: Oral controlled release drug delivery systems under developmental or approval filing stage by various companies (Rajeev, 2007)

Product	Drug	Company	Current status
JURNISTA Tablets	Hydromorphone	Johnson & Johnson, USA	Approved recently by European Union/Out licensed in USA
LAMICTAL XR Tablets	Lamotrigine	GlaxoSmithKline, USA	Approved recently
PRISTIQ ER	Desvenlafaxine succinate	Wyeth, USA	Approved recently
REQUIP ER	Ropinirole	GlaxoSmithKline, USA	NDA filed
AVANDAMET XR	Rosiglitazone maleate + Metformin hydrochloride	GlaxoSmithKline, USA	Phase III trials
GEPIRONE ER	Gepirone	GlaxoSmithKline, USA	Phase III trials
GABAPENTIN GR	Gabapentin	Depomed, USA	Phase III trials

XR/ER – extended release, GR – gastro retentive

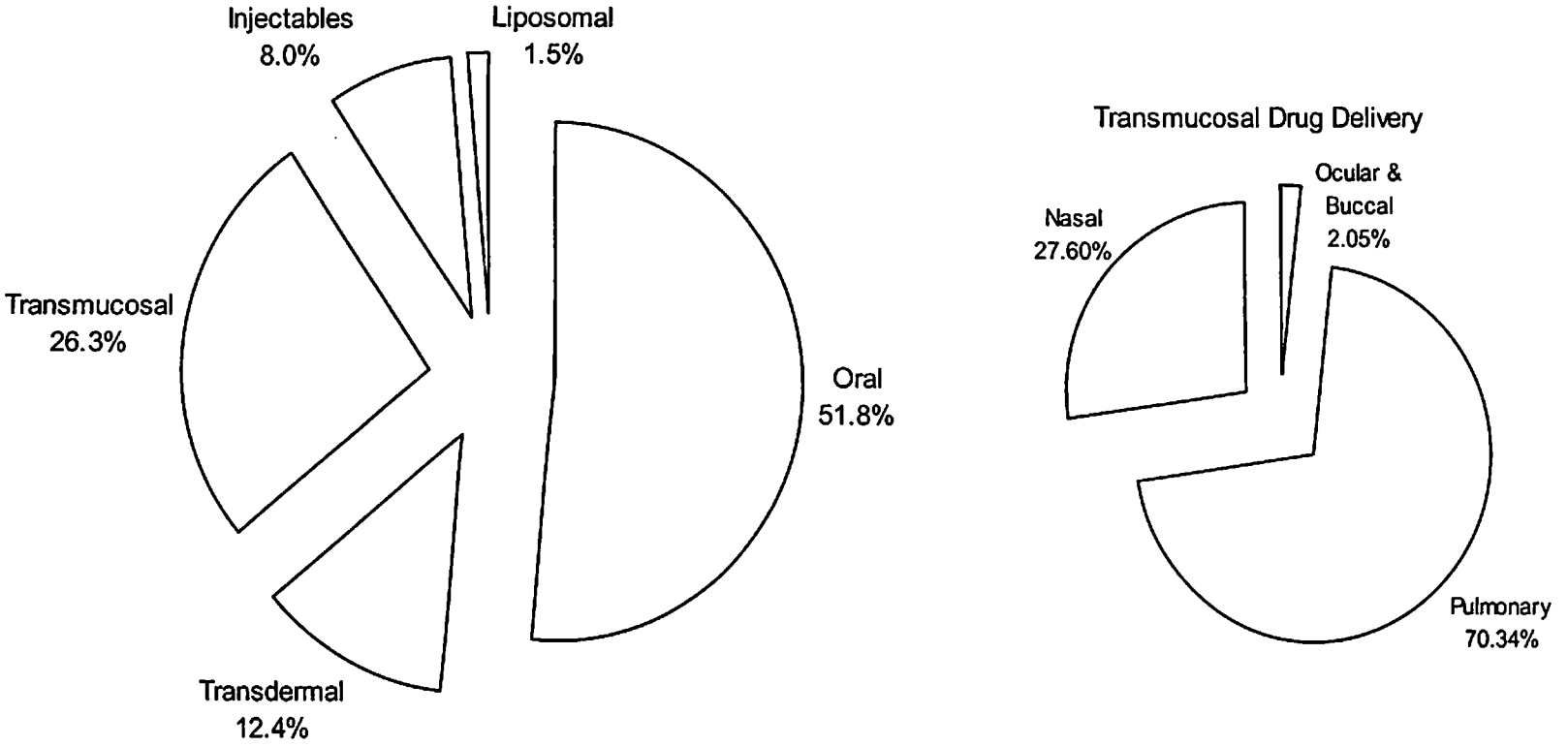

Figure 1.1: Current global market scenario of various controlled release drug delivery systems

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Chapter 2

Drug Profile – Zidovudine

2.1. Zidovudine

The first effective antiretroviral agent, zidovudine (ZDV), was synthesized by Horwitz in 1964 as a false nucleoside with disappointing anticancer activity. The drug was shown by Osterag in 1972 to inhibit the in vitro replication of a murine type D retrovirus (McLeod and Hammer, 1992). Clinical studies of ZDV began that same year, and by 1987 ZDV was approved and marketed for the control of HIV infection based on the results of a small but definitive randomized clinical trial (Fischl et al., 1987).

It is a member of a class of compounds called dideoxynucleosides. It is an analog of thymidine. The molecular structure of ZDV is given in Figure 2.1. It was scientifically proved by a research group in 1985 that ZDV inhibits replication of human immunodeficiency virus HIV (Mitsuya et al., 1985). ZDV (3'-azido-3'-deoxythymidine) has potent in vitro activity against a broad spectrum of retroviruses including HIV-1, HIV-2, and human T-cell lymphotrophic viruses (HTLV) I and II (McLeod and Hammer, 1992). Over the past several years, the wide use of ZDV in patients with the acquired immunodeficiency syndrome has been associated with decreased morbidity, slower progression of the disease and longer survival. It is also active against many animal retroviruses, such as feline leukemia virus, Friend murine leukemia virus, Harvey murine sarcoma virus and simian T-lymphotropic virus type 3 (Furman and Barry, 1988).

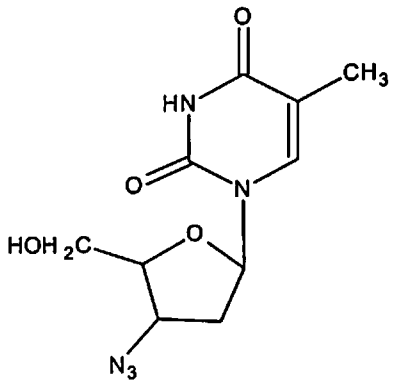

Figure 2.1: Molecular structure of zidovudine

2.2. Description

2.2.1. Nomenclature, molecular formula and molecular weight

The chemical abstracts' name is 1-[(2*R*, 4S, 5S)-4-azido-5-(hydroxymethyl) tetrahydrofuran-2-yl]-5-methyl-pyrimidine-2, 4(1*H*, 3H)-dione (IUPAC). The chemical

name of ZDV is 3'-azido-3'-deoxythymidine. Molecular formula of ZDV is $C_{10}H_{13}N_5O_4$. Molecular weight of ZDV is 267.24.

2.2.2. Appearance, odour and colour

ZDV is a white to beige, odourless, crystalline solid.

2.2.3. Melting range

The melting range of ZDV is 124 - 126 °C (The International Pharmacopoeia, 2005).

2.2.4. Specific optical rotation

When a 10 mg/ml solution of ZDV in ethanol (95%) is used and calculated with reference to the dried substance, the specific optical rotation value, $[\alpha]_D^{25^\circ C}$ = +60° to + 63° (The International Pharmacopoeia, 2005).

2.2.5. Differential scanning calorimetry

The DSC thermogram of ZDV shows a sharp melting endotherm at 125 °C with a normalized energy of 92.5 J/g (Araujo et al., 2003).

2.2.6. Solubility

ZDV is soluble in ethanol (~750 g/l in 95% ethanol solution). It is sparingly soluble in water (The International Pharmacopoeia, 2005). Solubility is 20.1 mg/ml in water at 25°C. (Remington, 2000)

2.2.7. Dissociation constant

ZDV is a weakly acidic drug. It contains an acidic hydrogen on the thymine moiety (thymine P_{BH}^K = 9.9). The dissociation constant of ZDV is reported to be 9.8 ± 0.2 (Kashuba et al., 1999; Keith, 2000).

2.2.8. UV and Infrared spectral information

The UV absorption spectrum of a 15 µg/ml solution of ZDV in methanol when observed between 210 nm and 300 nm, exhibits one maxima at about 266 nm and the specific absorbance value ($A_{1\,cm}^{1\%}$) is between 360 to 398 (The International Pharmacopoeia, 2005).

The infrared spectrum of ZDV shows a characteristic peak of carbonyl group at 1694 cm^{-1} and azide group at 2012 cm^{-1} (Araujo et al., 2003).

2.3. Pharmacology

2.3.1. Mechanism of action

HIV replication requires the action of RNA-dependent DNA polymerase (reverse transcriptase), an enzyme present in all retroviruses. Once HIV enters the host cell, it loses its protein coat, exposing its genome and reverse transcriptase. The reverse transcriptase directs the synthesis of a complementary-strand DNA copy of the viral RNA and then catalyzes the production of a second, positive-strand DNA copy. The viral genetic information is thus encoded in a double-stranded DNA. This viral DNA is carried to the nucleus, where it is integrated into the DNA of the host. Viral replication occurs through transcription of RNA copies of the DNA provirus by the cellular RNA polymerase (Anthony and Clifford, 2001; Betty, 2000).

ZDV inhibits HIV replication by acting as a reverse transcriptase inhibitor. This action requires intracellular conversion of the drug to ZDV triphosphate (Yarchoan et al., 1990). ZDV is first converted into the monophosphate derivative by cellular thymidine kinase. The monophosphate is further converted into the diphosphate derivative by cellular thymidylate kinase and into the triphosphate derivative by another cellular kinase. Since phosphorylation of ZDV depends on cellular rather than viral enzymes, conversion to the active triphosphate derivative occurs in both virus-infected and uninfected cells.

ZDV triphosphate is a structural analog of thymidine triphosphate, the essential substrate for the formation of proviral DNA by reverse transcriptase. ZDV triphosphate competes with thymidine triphosphate for incorporation into the proviral DNA. After ZDV is incorporated into the proviral DNA chain, DNA synthesis is prematurely terminated because the 3'-azido group of ZDV prevents further addition of nucleosides (Mitsuya et al., 1987). ZDV has a 100 to 300 fold greater affinity for inhibiting HIV reverse transcriptase than it does for inhibiting human DNA polymerase (Furman et al., 1986).

2.3.2. Resistance

Strains of HIV with reduced in vitro sensitivity to ZDV have been isolated from patients who have received the drug for six months or longer (Larder et al., 1989). ZDV resistance appears to be due to multiple mutations in the reverse transcriptase gene affecting

four specific amino acid residues (Larder and Kemp, 1989). Because of the number of mutations that must occur, the period required for the development of resistance is fairly long. Although the development of ZDV resistance often parallels clinical deterioration, the relationship between the two is unclear.

2.3.3. Clinical toxicities

The most serious adverse effect of ZDV is bone marrow suppression resulting in macrocytic anemia or granulocytopenia, or both. Bone marrow toxicity is directly related to the dosage and duration of therapy and occurs most frequently in patients with CD4 T-lymphocyte counts below 200 cells/mm^3 and those with pre-existing anemia or leukopenia (Chitnis et al., 2002).

Significant anemia most commonly occurs after four to six weeks of therapy. Anemia appears to result from impaired erythrocyte maturation; an increase in mean corpuscular volume precedes the development of anemia. Erythropoietin levels, which are inappropriately low in HIV-associated anemia, increase after ZDV treatment. The bone marrow does not respond to the erythropoietin, because of direct toxic effects of ZDV (Spivak et al., 1989). Anemia usually resolves when ZDV is discontinued or the dosage is decreased.

Granulocytopenia occurs most commonly after six to eight weeks of therapy. ZDV should be temporarily discontinued if the granulocyte count falls below 750 cells/mm^3. If the count remains stable or increases, therapy may be reinstated at the original dose or a lower dose. Rarely, thrombocytopenia may occur during ZDV therapy. In patients with HIV-associated thrombocytopenia, platelet counts rise during the first few weeks of ZDV therapy (Pottage et al., 1988). Later, however, the drug's toxic effect may cause the platelet count to decline. Rare instances of pancytopenia with hypoplastic bone marrow have also been described.

Other adverse effects of ZDV include nausea, headache, insomnia and myalgia, which may sometimes resolve despite continued administration (Richman et al., 1987). Temporarily discontinuing therapy or lowering the dose should be considered, however, if a patient's compliance is threatened by these side effects. Drug fever may occur and should be considered, along with opportunistic infections and neoplasms, in the differential diagnosis of fever occurring within the first weeks after initiation of ZDV therapy (Jacobson et al., 1989).

Rarely, patients have developed neurologic toxicity manifested by confusion, stupor, cerebellar dysfunction or seizures. A dark blue discoloration at the base of the finger nails and toe nails has also been observed, more commonly in black patients (Don et al., 1990). Changes in liver function tests, including elevated serum aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase levels, may occur two to three weeks after initiation of treatment.

The carcinogenic and mutagenic potential of ZDV in man has not yet been fully investigated. Rodents maintained on high doses of the drug for prolonged periods had an increased incidence of vaginal neoplasm.

2.3.4. Therapeutic use and clinical activity

The first double-blind, placebo-controlled trial of ZDV in patients with advanced symptomatic HIV infection was conducted in 1986 (Fischl et al., 1987). In this study, 281 patients were randomly assigned to receive either ZDV, 250 mg every four hours, or placebo every four hours. After a mean follow-up of four and one-half months, patients in the ZDV group had a significantly lower mortality rate and frequency of opportunistic infections than the placebo group. Additional beneficial effects noted in the ZDV-treated patients were improved neuropsychiatric status and maintenance of body weight. Significant increases in the CD4 T-lymphocyte counts were also seen in patients receiving ZDV; for patients with AIDS, these changes were transient.

Additional data in 4805 patients who received ZDV confirmed the clinical benefits (Creagh-Kirk et al., 1988). In March 1987, the Food and Drug Administration approved the use of ZDV in HIV-infected patients who either had Pneumocystis carinii pneumonia or CD4 counts below 200 cells/mm^3. Follow-up studies of patients with AIDS who have received ZDV for up to 21 months suggest that the decreased mortality and reduced frequency of opportunistic infections are maintained. Therapy with ZDV has been shown to prolong survival and decrease the incidence of opportunistic infections in patients with advanced HIV disease at the initiation of therapy and to delay disease progression in asymptomatic HIV-infected patients. ZDV combined with lamivudine, didanosine, or zalcitabine is more effective than ZDV alone (Hammer et al., 1996). Greater benefit is achieved when ZDV is combined with two nucleoside analogs (Saag et al., 1998). The current standard of care for treatment-naive patients is to combine ZDV with a potent protease inhibitor and another nucleoside analog (Hammer et al., 1997) or with an NNRTI and another nucleoside analog (Staszewski et al., 1999). ZDV monotherapy reduced the risk

of perinatal transmission of HIV by 67% (Connor et al., 1994), and combining ZDV with other antiretroviral drugs is even more efficacious in this setting. ZDV is also recommended as a component of combination therapy administered to healthcare workers soon after exposure to contaminated blood or body fluids to prevent HIV transmission (Cardo et al., 1997). ZDV in combination with certain antiretroviral agents has been shown to be superior to monotherapy in one or more of the following: delaying death, delaying development of AIDS, increasing CD4 cell counts, and decreasing plasma HIV RNA. Use of ZDV in some combinations is based on surrogate marker data.

Patients treated with ZDV demonstrate a reduction in the serum level of p24 antigen, a proposed marker of progressive infection (Jackson et al., 1988); serum p24 antigen levels increase after discontinuation of ZDV therapy, which reflects the virustatic effect of ZDV (Spear et al., 1988). Reduced antigenemia also occurs in asymptomatic HIV-infected patients treated with ZDV (de Wolf et al., 1988).

Neurologic dysfunction is common in HIV-infected persons. ZDV has been shown to improve cognitive function in patients with symptomatic HIV infection. This improvement is most apparent in patients with more advanced disease (Dournon et al., 1988; Schmitt et al., 1988). A syndrome of acute transient meningoencephalitis, characterized by fever, neck stiffness, headache and confusion, has occurred following abrupt reduction of the ZDV dosage (from 1200 to 600 mg/day) because of severe neutropenia or thrombocytopenia. This response to dosage reduction may indicate that the higher dosage is required for control of HIV-associated neurologic dysfunction (Helbert et al., 1988).

2.3.4.1. Use in children

Both oral and intravenous ZDV has been used in a limited number of children with AIDS who acquired HIV infection either perinatally or through blood transfusion (Pizzo et al., 1988; Blanche et al., 1988). Therapy generally continued for at least six months and resulted in clinical and immunologic improvement. In addition, neuropsychologic status improved in many children who had shown signs of encephalopathy prior to ZDV therapy.

Approximately one-third to one-half of children born to HIV- infected mothers will be infected with the virus. Transmission may occur as early as the second trimester or after birth. Whether ZDV can reduce the perinatal transmission of HIV is not known. However, because of teratogenic concerns about administering a DNA-chain terminator, ZDV is not currently recommended for use in pregnant women.

2.3.4.2. Use following occupational exposure

The safety and efficacy of ZDV chemoprophylaxis following occupational exposure to HIV has not been evaluated. HIV infection develops in three to four of 1000 individuals who have incurred percutaneous exposure to blood from an infected patient. At some institutions, ZDV chemoprophylaxis (200 mg every four hours for four to six weeks) is offered to employees who report, within 24 h, percutaneous or mucous membrane exposure to HIV-positive blood or blood-containing body fluids. There are, however, no data to support this use of ZDV.

2.3.4.3. Use in early infection

As mentioned previously, ZDV was initially approved by the FDA for use in HIV-infected patients who either had R carinii pneumonia or were asymptomatic but had CD4 T-lymphocyte counts below 200 cells/mm^3. The dosage initially recommended was 200 mg orally every four hours. Subsequent studies have investigated the efficacy of other dosage regimens, as well as earlier initiation of therapy.

In a multicenter clinical trial of ZDV, 524 AIDS patients who had recovered from one episode of R carinii pneumonia were randomized to initially receive either 250 mg every four hours (1500 mg/day) or 200 mg every four hours (1200 mg/day) for one month, followed by 100 mg every four hours (600 mg/day). Survival rates and the frequency of opportunistic infections were comparable in both treatment groups, but the incidence of hematologic toxicity was lower in patients who received the lower dosage.

The results of a multicenter, double-blind, placebo-controlled trial of ZDV in 1338 adults with asymptomatic HIV infection were reported (Volberding et al., 1990). Patients in the study had CD4 T-lymphocyte counts below 500 cells/mm^3. They were randomly assigned to receive either 100 mg of ZDV, 300 mg of ZDV or placebo every four hours while awake (five times a day). Both of the ZDV treatment groups had significantly lower rates of progression to AIDS; the mean follow-up was 55 weeks (range: 19 to 107 weeks). Clinical benefit was demonstrated both in those persons with fewer than 200 CD4 cells/mm^3 and in those with 200 to 500 CD4 cells/mm^3 when they entered the study. Changes in immunologic and virologic parameters (i.e., CD4 cell count and serum p24 antigen level) paralleled the observed clinical benefits.

ZDV was much better tolerated in this population than in patients with more advanced disease. Furthermore, the dosage of 500 mg/day was as effective as (and significantly less toxic than) a dosage of 1500 mg/day. The results of this trial showed that

ZDV therapy can delay the onset of AIDS when it is given to asymptomatic persons with CD4 cell counts below 500 cells/mm^3. In another placebo-controlled trial (Fischl et al., 1990), 711 HIV-infected patients with early manifestations of the disease and CD4 T-lymphocyte counts of 200 to 800 cells/mm^3 were assigned to receive either placebo or 200 mg of ZDV orally every four hours (1200 mg/day). ZDV therapy significantly delayed the development of AIDS in patients who had CD4 cell counts between 200 and 500 cells/mm^3. No clear benefit was seen in those with CD4 cell counts above 500 cells/mm^3.

2.3.4.4. Dosage

(a) Adults

For adults with symptomatic HIV infection, including AIDS, the recommended starting dose of ZDV is 200 mg every four hours (maximum 1200 mg/day). After one month, the dose may be reduced to 100 mg every four hours (maximum 600 mg/day). It is unknown whether this lower dose improves the neurologic dysfunction associated with HIV infection. For patients unable to take oral medications, ZDV may be administered intravenously (1 to 2 mg/kg, infused over one hour, every four hours) until oral therapy can be given. For persons with asymptomatic HIV infection and CD4 T-lymphocyte counts below 500 cells/mm^3, the recommended dose is 100 mg every four hours.

(b) Pediatrics

For children three months to 12 years of age with HIV infection, the recommended dose is 180 mg/m^2 every six hours (720 mg/m^2/day), not to exceed 200 mg/m^2 every six hours.

(c) Neonatal dosing

In neonatal babies, the dose is 2 mg/kg orally every six hours starting within 12 h after birth and continuing through 6 weeks of age. Neonates unable to receive oral dosing may be administered intravenously at 1.5 mg/kg, infused over 30 minutes, every six hours. Careful monitoring of hematologic indices every two weeks is recommended during the therapy in neonates. Significant anemia [hemoglobin less than 7.5 g/dl and/or granulocytopenia (granulocyte count less than 750 cells/mm^3)] may mandate cessation of therapy until evidence of marrow recovery is observed. For less severe anemia or granulocytopenia, a temporary reduction in the daily dosage may be adequate. In addition,

some patients may benefit from treatment with erythropoietin, although the specific indications for the use of this drug in HIV-infected patients remain to be defined (Fischl et al., 1990).

Various conventional or immediate release formulations of ZDV available in the Indian market are given in Table 2.1.

2.4. Pharmacokinetics

2.4.1. Absorption

ZDV is absorbed rapidly and completely after oral administration, with peak serum concentrations occurring in 0.5 to 1.5 h. ZDV is absorbed through out the gastro-intestinal tract (Kuksal et al., 2006). More than 90 percent of an orally administered dose is absorbed, but because of significant first-pass metabolism in the liver, overall bioavailability averages 63 percent (Blum et al., 1988). The rate of absorption is slowed, with lower peak levels, if ZDV is taken with a high-fat meal (Collins and Unadkat, 1987).

2.4.2. Distribution

ZDV crosses the blood-brain barrier. Distribution of ZDV to cerebrospinal fluid (CSF) averages approximately 68% of the plasma concentration in children (age: three months to twelve years) and 60% of the plasma concentration in adults (Langtry et al., 1989; Yarchoan et al., 1990). It is also reported that ZDV can cross the placental barrier. One case report and a study in three pregnant women found that the ZDV concentration in the infant cord blood was slightly higher than simultaneous maternal serum concentration, and that the amniotic fluid concentration was several times higher than the simultaneous umbilical cord concentration. The concentration of ZDV in the central nervous system (CNS) tissue of a gestational 13-week fetus was below effective antiviral concentrations (Liebes et al., 1990). ZDV is found to concentrate in the semen of HIV-infected patients, with concentration ranging from 1.3 to 20.4 times those found in the serum. The estimated volume of distribution is 1.6 l/kg. The plasma protein binding of ZDV is reported to be around 30 to 38%.

2.4.3. Metabolism

ZDV is metabolized in the liver by glucuronide conjugation to major, inactive metabolite, 3'-azido-3'-deoxy-5'-O-beta-D-glucopyranuronosylthymidine (GZDV). In

children under 1 year of age, the glucuronide conjugation pathway is under developed at birth. However, a study done in infants older than 30 days of age found that the clearance and half-life of ZDV were comparable to those in adults.

The half-life of ZDV in adults with normal renal function is found to be approximately 1.1 h (range 0.5 to 2.9 h). In the renal impaired patients with the renal function value less than 30 ml/min, the half-life is found to be between 1.4 to 2.9 h. In patients suffering from liver cirrhosis the half-life is reported to be approximately 2.4 h. In children in the age group of 2 weeks to 13 years the half-life is reported to be approximately 1 to 1.8 h. In children up to 14 days of age half-life is approximately 3 h and in neonates (mother receiving ZDV) half-life is approximately 13 h.

2.4.4. Elimination

In adults, the mean systemic clearance of ZDV is approximately 1.6 l/h/kg. 90% of ZDV and its major metabolite, 5'-glucuronylzidovudine, are excreted in urine. Approximately 14 to 18% of ZDV that reaches the systemic circulation is excreted by glomerular filtration and active tubular secretion in urine.

In children (age 14 months to 12 years), approximately 30% of ZDV that reaches the systemic circulation is excreted by the kidneys. The pharmacokinetics in children over one year of age is similar to those observed in adults (Balis et al., 1989). Because children younger than one year do not have complete liver enzyme systems, they may not be able to sufficiently metabolize ZDV.

The pharmacokinetic parameters of ZDV obtained following administration of the drug through intravenous route in HIV infected adult patients and pediatric patients are given in Table 2.2 and Table 2.3 respectively. The pharmacokinetic parameters of ZDV obtained following oral administration of the drug in HIV infected adult patients under fasting conditions is given in Table 2.4.

Table 2.1: Various conventional or immediate release formulations of zidovudine available in Indian market

Product	Dose	Brand or Generic Name	Company
Tablets	300 mg	Retrovir® Tablets	GlaxoSmithKline
Capsules	100 mg	Retrovir® Capsules	
Syrup	50 mg per 5 ml	Retrovir® Syrup	
Tablets	300 mg	Zidovir® Tablets	Cipla Pharmaceuticals
Capsules	100 mg	Zidovir® Capsules	
Oral solution	50 mg per 5 ml	Zidovir® Syrup	
Tablets	300 mg	Zidovudine Tablets	Aurobindo Pharma
Oral solution	50 mg per 5 ml	Zidovudine Oral Solution	
Tablets	300 mg	Zidovudine Tablets USP 300 mg	Ranbaxy Laboratories

Table 2.2: Pharmacokinetic parameters of zidovudine obtained following administration of the drug through intravenous route in HIV infected adult patients (GlaxoSmithKline, 2006a)

Parameter	Value[a] (n=22)
Apparent volume of distribution (l/kg)	1.6 ± 0.6
Plasma protein binding (%)	Less than 38
CSF:plasma ratio[b]	0.6 (0.04 to 2.62)
Systemic clearance (l/h/kg)[b]	1.6 (0.8 to 2.7)
Renal clearance (l/h/kg)	0.34 ± 0.05
Elimination half-life (h)[b]	1.1 (0.5 to 2.9)

[a] Data presented as mean ± standard deviation except where noted
[b] Median (range)

Table 2.3: Pharmacokinetic parameters of zidovudine obtained following administration of the drug through intravenous route in HIV infected pediatric patients (GlaxoSmithKline, 2006a)

Parameter	Parameter value obtained in different age groups[a]		
	Birth to 14 days (n = 15)	14 days to 3 months (n = 17)	3 months to 12 years (n = 18)
Apparent volume of distribution (l/kg)	89 ± 19	61 ± 19	65 ± 24
Plasma protein binding (%)	no data	no data	0.26 ± 0.17
CSF:plasma ratio[b]	0.65 ± 0.29	1.14 ± 0.24	1.85 ± 0.47
Systemic clearance (l/h/kg)	3.1 ± 1.2	1.9 ± 0.7	1.5 ± 0.7
Renal clearance (l/h/kg)	89 ± 19	61 ± 19	65 ± 24
Elimination half-life (h)	3.0 ± 0.5	no data	0.26 ± 0.17

[a] Data presented as mean ± standard deviation except where noted
[b] CSF ratio determined at steady-state on constant intravenous infusion

Table 2.4: Pharmacokinetic parameters of zidovudine obtained following oral administration of the drug in HIV infected adult patients in fasting state (GlaxoSmithKline, 2006b)

Parameter	Value[a]
Oral bioavailability (%)	64 ± 10 (n = 5)
Apparent volume of distribution (l/kg)	1.6 ± 0.6 (n = 8)
Plasma protein binding (%)	Less than 38
CSF:plasma ratio[b]	0.6 (0.04 to 2.62) (n = 39)
Systemic clearance (l/h/kg)[b]	1.6 (0.8 to 2.7) (n = 6)
Renal clearance (l/h/kg)	0.34 ± 0.05 (n = 9)
Elimination half-life (h)[b]	1.0 (0.5 to 2.9) (n = 19)

[a] Data presented as mean ± standard deviation except where noted
[b] Median (range)

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Chapter 3

Analytical Method Development

3.1. Introduction

An analytical method which is accurate and precise in the estimation of drug is an important and integral part of the formulation development process of the drug. A simple and suitable analytical method for the estimation of drug in formulations and in different samples of study will be very useful in fastening the formulation development of the drug. If the analytical method is not sensitive and selective, there is a possibility of erroneous results, which will lead to false conclusions. Therefore, proper analytical method development is the first step of formulation development process. Though literature may provide methods for drug analysis, however such methods may not always be found suitable for the specific formulation development purpose. In that case it becomes essential to develop a need based simple, sensitive and cost effective method for the estimation of drug. Different sensitive methods like UV-visible, fluorescence and high performance liquid chromatography can be developed according to the need of the study.

3.2. Analytical Methods for Estimation of Zidovudine

Zidovudine (ZDV) is the first effective antiretroviral agent that was approved and marketed in the year 1987 for the control and treatment of HIV infection. One UV spectrophotometric method (The International Pharmacopoeia, 2005) and few HPLC methods (US Pharmacopoeia, 2007a; British Pharmacopoeia, 2008) for the estimation of ZDV are available in the leading pharmacopoeias. The UV spectrophotometric method involves preparation of several reagents and is very tedious particularly when analysis of large number of samples has to be done. The HPLC methods available in the leading pharmacopoeia involve multiple organic solvents systems like chloroform, methanol, and acetonitrile in large quantity due to large retention times and high flow rates involved in the methods. So it is not economically viable to use such methods when large numbers of samples are to be estimated as the cost of the organic solvents is very high.

A UV spectrophotometric method based on the oxidation of ZDV by a known excess of oxidant N-bromosuccinimide, in buffer medium of pH 1.5, followed by the estimation of unreacted amount of oxidant with metol and sulphanilic acid is reported (Basavaiah and Anil, 2006b). The same research group has also reported two more spectrophotometric methods for the determination of ZDV in pharmaceuticals. The methods use chloramine-T and two dyes, methylene blue and rhodamine-B, as reagents and are based on addition of a known excess of chloramine-T to ZDV in hydrochloric acid medium followed by determination of residual oxidant by reacting with a fixed amount of either

methylene blue and measuring the absorbance at 665 nm (Method A) or rhodamine-B and measuring the absorbance at 555 nm (Method B) (Basavaiah and Anil, 2006a). A validated and selective HPLC method has been reported for the estimation of ZDV during stability studies (Dunge et al., 2005). A reverse phase HPLC method for the simultaneous estimation of ZDV and lamivudine in tablet dosage forms has been reported (Palled et al., 2005). Few HPLC methods for the estimation of ZDV in biological fluids such as plasma, serum, urine and amniotic fluid in humans, rats, pigs and monkeys have been reported (Savaser et al., 2007; Tove et al., 1998; Hedaya and Sawchuk, 1989; Lingling et al., 2007; Rao et al., 2008). Some methods have been reported for the simultaneous estimation of ZDV and other antiretroviral drugs like lamivudine, stavudine, abacavir etc. using HPLC/HPLC with tandem mass spectrometry (Geetha et al., 2006; Yazen et al., 2004; Fan and Stewart, 2002; Summer et al., 2007).

The literature survey revealed that none of the reported UV spectrophotometric methods were suitable for routine analysis of ZDV in formulations and in vitro samples of present research endeavor. Though the reported HPLC methods were sensitive and selective, but most of them suffer from the drawback of tedious sample preparation, use costly solvents in large quantities and in some cases use mass spectrometry in tandem with HPLC.

So, in the present research work, a simple, inexpensive, sensitive and rapid spectrophotometric method was developed for the routine estimation of drug in bulk, formulations and in vitro release samples. Liquid chromatographic methods were developed for estimation of drug in formulation, stability and biosamples. All developed methods were validated according to the standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2007b). Suitable statistical tests were performed to validate the developed methods (Bolton and Bon, 2004). These developed and validated methods were used for the estimation of drug in bulk, formulations, in vitro release samples, stability samples and biosamples.

3.3. Materials

Pure ZDV was obtained as gift sample from Strides Arcolab Limited, Bangalore, India. Acetonitrile (ACN), methanol and glacial acetic acid were of HPLC grade and purchased from Spectrochem, Mumbai, India. Potassium dihydrogen orthophosphate and sodium hydroxide were of analytical grade and purchased from Qualigens, India. In-house prepared triple distilled water (TDW) was used for preparation of buffers used in spectrophotometric method. In case of HPLC method, for preparing aqueous phase,

Millipore water (Millipore, USA) was used. The aqueous phase after the preparation was further passed through 0.22 μ Millipore membrane filters (Millipore, USA). The polymers like hydroxypropyl methylcellulose (HPMC) (1000 cPs, 4000 cPs (METHOCEL K4M Premium), 15000 cPs (METHOCEL K15M Premium) and 100000 cPs (METHOCEL K100M Premium)), ethyl cellulose (ETHOCEL™ Standard Premium, 10 cPs) Sodium carboxy methyl cellulose and carbopol (Carbopol 971P NF) were obtained as gift samples from IPCA Laboratories, Mumbai, India.

Formulations containing ZDV: Retrovir® tablets, labeled claim of 300 mg of ZDV per tablet; Retrovir® capsules, labeled claim of 100 mg of ZDV per capsule (GlaxoSmithKline Pharmaceuticals Limited, Mumbai, India) and Zidovir tablets, labeled claim of 300 mg ZDV per tablet (Cipla Pharmaceuticals Limited, Mumbai, India) were obtained from local market.

3.4. Analytical Equipments

A double-beam Jasco (Japan) UV-Vis-NIR spectrophotometer, model V570 connected to a computer loaded with Spectra Manager software was used for spectrophotometric method development. The UV spectrophotometer has an automatic wavelength correction with wavelength accuracy of 0.1 nm. Matched quartz cells of 10 mm path length were used in the spectrophotometric studies.

The liquid chromatography equipment employed was Shimadzu HPLC (Shimadzu, Japan) with solvent delivery system of two pumps (Model LC 10AT VP Shimadzu LC, Shimadzu, Japan), autoinjector (Model SIL HT A Shimadzu autosampler, Shimadzu, Japan), column oven (Model LC 10AT VP Shimadzu LC, Shimadzu, Japan) and UV-Vis detector (Model SPD 10A VP Shimadzu, Shimadzu, Japan). Data collection and integration was accomplished using LC Solutions software.

3.5. Reagents

(a) Preparation of 0.2 M sodium hydroxide

0.8 g of sodium hydroxide was transferred carefully into a 100 ml volumetric flask and 50 ml of TDW was added into the flask to dissolve sodium hydroxide completely. The final volume was made up to 100 ml with TDW (US Pharmacopoeia, 2007c).

(b) Preparation of pH 6.8 phosphate buffer

6.805 g of potassium dihydrogen orthophosphate was transferred carefully into a 1000 ml volumetric flask and 500 ml of TDW was added into the flask to dissolve potassium dihydrogen orthophosphate completely. Then 112 ml of 0.2 M sodium hydroxide was added into the volumetric flask and the final volume was made upto 1000 ml with TDW (US Pharmacopoeia, 2007c).

(c) Glacial acetic acid (0.1% v/v)

1 ml of glacial acetic acid was carefully transferred into a 1000 ml standard flask and the volume was made upto 1000 ml with Millipore water.

3.6. Analytical Method 1: UV Spectrophotometric Method

3.6.1. Experimental

(a) Optimization of media

Different media were investigated to develop a suitable UV spectrophotometric method for the analysis of ZDV in bulk, formulations and dissolution samples. For selection of media the criteria employed were solubility of the drug, ease of sample preparation, sensitivity of the method, cost of solvent and applicability of the method to various purposes. Absorbance values of ZDV in the selected media at respective λ_{max} were determined and apparent molar absorptivity values were calculated.

(b) Calibration curve

Primary stock solution of 100 µg/ml of ZDV was prepared in phosphate buffer (pH 6.8) by dissolving 10 mg of the drug in 100 ml of media. Six different concentrations in the range of 5-30 µg/ml (5, 10, 15, 20, 25 and 30) were used to develop the calibration curve. These six concentrations were prepared by transferring required aliquots of stock solution into six different 10 ml standard flasks and making up the volume to 10 ml with the media. ZDV was estimated at 266 nm. To establish linearity of the proposed method, nine separate series of drug solutions in the above mentioned concentration range were prepared from the stock solution and analyzed. Average absorbance of each concentration was substituted in the regression equation to calculate corresponding predicted concentration. The data was subjected to least square regression analysis. ANOVA test (one-way) at 95% level of

significance was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions.

(c) Analytical method validation

The developed method was validated for various parameters according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2007b; Bolton and Bon, 2004).

Specificity and selectivity of the method was assessed by preparing a drug concentration of 15 µg/ml from pure drug stock and commercial sample stock in selected medium and scanning the two solutions separately. The two spectra were compared for any change in absorbance pattern of ZDV in the presence of excipients. Solutions of the drug with and without different excipients used in the formulation were prepared and analyzed for any change in absorbance spectra of ZDV.

To determine the accuracy of the proposed method, different quality control levels of drug concentration (Lower quality control [LQC] sample – 7 µg/ml, Medium quality control [MQC] sample – 12 µg/ml and High quality control [HQC] sample – 27 µg/ml) were prepared independently from stock solution and analyzed (n = 6). Solutions were analyzed by proposed method and predicted concentrations were calculated using regression equation. Accuracy was assessed as the mean percentage recovery and percentage bias (% Bias = 100×[(Predicted concentration – Nominal concentration)/Nominal concentration]. Further, different concentrations of the pure drug (10, 15, 20 µg/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 5) to check the analytical recovery. The percent analytical recovery (% Analytical recovery) of the added pure drug was calculated as, % Analytical recovery = $[(C_v - C_u)/C_a] \times 100$, where C_v is the total drug concentration measured after standard addition, C_u is the drug concentration in the formulation sample, C_a is the drug concentration added to the formulation sample.

Precision was determined by studying repeatability and intermediate precision. Repeatability was determined by using different quality control levels of drug concentration (same concentration levels as taken in accuracy study), prepared from independent stock solutions and analyzed (n = 6). Inter-day and intra-day variations were found out to determine the intermediate precision of the proposed method. Different quality control levels of drug concentration in triplicates were prepared at two different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study

inter-day variation (n = 18). The percentage relative standard deviation (% RSD) of the predicted concentration from the regression equation was taken as intermediate precision.

The limit of detection (LOD) and limit of quantitation (LOQ) of ZDV by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively, where σ is the standard deviation of y-intercept of regression equation and S is the slope of the regression equation. Robustness of the proposed method was determined by changing the pH of media by ± 0.1 and checking the stability of ZDV in the selected media by observing the spectral changes at room temperature (25 ± 2 °C) for 36 h.

(d) Estimation of drug content of formulations

Two different tablet brands (Retrovir® and Zidovir tablets) and one capsule brand (Retrovir® capsule) of ZDV that were commercially available were taken for estimation of total drug content per unit dosage form (tablet/capsule). In case of tablets, 20 tablets were weighed and pulverized. Amount of powder equivalent to 10 mg of ZDV was taken and suitably dissolved in selected media to prepare a final concentration of 100 µg/ml. The resulting solution was filtered through Whatman filter paper no. 40. Further dilutions were made with selected medium in order to obtain concentrations within the linearity range and the samples were analyzed using the proposed method (n = 5). In case of capsules, content of 20 capsules were emptied and mixed properly. Amount of powder equivalent to 10 mg of ZDV was taken and suitably dissolved in selected media and same steps were repeated as mentioned above to obtain concentration in linearity range and the final samples were analyzed (n = 5).

3.6.2 Results and discussion

(a) Optimization of media

For media optimization various aqueous media like TDW and phosphate buffers (pH 4.5 to 7.4) were investigated. ZDV showed a slight variation in the UV absorption spectra in the various phosphate buffers. Phosphate buffer (pH 6.8) was selected as the media based on the sensitivity, cost, ease of preparation and applicability of the method in analyzing in vitro drug release studies samples. The spectra of different concentrations of ZDV in the phosphate buffer (pH 6.8) are shown in Figure 3.1. The λ_{max} of ZDV in phosphate buffer (pH 6.8) was found to be 266 nm. No change in the UV absorption spectra was observed till

36 h of storage at room temperature (25 ± 2 °C). Apparent molar absorptivity of ZDV was found to be 1.083×10^4 l/mol.cm.

(b) Calibration curve

The linear regression equation obtained was: Absorbance = 0.0399×Concentration (µg/ml) + 0.0116. Different standard concentrations and their absorbance values were shown in the Table 3.1. At all the concentration levels the standard deviation (SD) was low and the percentage relative standard deviation (% RSD) did not exceed 0.97. The predicted concentrations were nearly matching with the nominal concentrations (Table 3.1). In selected medium the linearity range was found to be 5 – 30 µg/ml. The standard error of slope, intercept and estimate were found to be 2.17×10^{-4}, 2.80×10^{-3} and 3.69×10^{-1} respectively. The individual values of slope and intercept were found to be within 95% confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9998), low standard error of estimate (3.69×10^{-1}), MSSR (1.25×10^{-4}) and low F_{cal} value (2.19×10^{-3}) than F_{crit} value (2.15) [$P < 0.05$, F_{crit} (8,45) = 2.15]. Lower values of parameters like MSSR, standard error of slope, intercept and estimate indicated high precision of the proposed method.

(c) Analytical method validation

The overlaid spectra of pure drug solution and solution containing admixture of ZDV with HPMC 15000 cPs in 1:1 ratio in selected media is shown in Figure 3.2. The UV spectrum of ZDV was not changed in the presence of various excipients. There was no difference in absorbance values or spectra of drug solutions prepared from various stock solutions. Absorption spectrum of pure drug sample was matching with the marketed formulation sample. Therefore the proposed method is specific and selective for the drug.

All the three quality control levels (LQC, MQC and HQC) showed an accuracy (% Bias) ranging from -0.16 to 1.03 (Table 3.2). The high mean percentage recovery values (nearly 100) and their low standard deviation (SD) values (SD < 1.01) indicated high accuracy of the proposed method. The validity and reliability of the proposed method was evaluated by recovery studies from standard addition method. The mean percentage recovery (± SD, SD is given in the parenthesis) for 10, 15 and 20 µg/ml concentrations in the standard addition method studies were found to be 100.83 (± 0.94), 99.79 (± 0.74) and 101.32 (± 1.12) respectively. These results revealed accuracy of the proposed method.

In the repeatability study, % RSD values ranged from 0.47 to 1.14 at all the different quality control levels of concentrations (Table 3.2). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 0.67 and inter-day variation not more than 1.03 (Table 3.3). Low % RSD values indicated the excellent precision of the proposed method.

LOD and LOQ were found to be 0.69 µg/ml and 2.11 µg/ml respectively. The mean percentage recovery (± SD) for 2.5 µg/ml in triplicate was found to be 100.07 (± 1.24). Variation of pH of phosphate buffer by ± 0.1 did not have any significant effect on UV absorbance of ZDV. No change was observed in the UV absorbance spectra of ZDV solutions till 36 h when kept at room temperature (25 ± 2 °C), which confirm the robustness of the proposed method.

(d) Estimation of drug content in formulations

The % recovery values for the estimation of ZDV in different commercially marketed pharmaceutical formulations ranged from 99.81 to 100.87% with standard deviation not more than 1.02. Assay values of formulations were found to be same as that mentioned in the labeled claim, suggesting that the interference of excipient matrix was insignificant in the estimation of ZDV by proposed method. The estimated drug content with low values of standard deviation establishes the precision of the proposed method (Table 3.4).

3.7. Analytical Method 2: RP-HPLC Method for Estimation of Zidovudine in Formulations

3.7.1. Experimental

(a) Chromatographic conditions

Separation was performed on a reverse phase LiChroCART® RP-8 (250×4 mm, 5 µm) column. Mobile phase consisted of a mixture of aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40. The aqueous phase was filtered through 0.22 µm membrane filter before use. The organic phase (ACN) was degassed by sonication (Bransonic Cleaning Company, USA). Injection volume was 50 µl. Flow rate was adjusted to 0.6 ml/min. The sensitivity parameters were set at auxiliary range = 1.0 AU/V (Absorbance unit/volt) and recorder range = 1.0 AUFS (Absorbance unit full scale). The

wavelength of the UV detector was set at 266 nm. The column oven temperature was maintained constant at 25 °C.

(b) Selection of mobile phase

Different aqueous media [water, phosphate buffer (pH 5.6), phosphate buffer (pH 6.8) and glacial acetic acid (0.1% v/v)] in combination with different organic solvents (ACN and methanol) in various proportions were tried. For selection of the mobile phase the criteria employed was chromatographic peak properties of the drug (like retention time, asymmetric factor), sensitivity (height and area), ease of sample preparation and applicability of the method for various purposes.

(c) Calibration curve

Primary stock solution of 100 µg/ml of ZDV was prepared in selected mobile phase. A secondary stock of 10 µg/ml was prepared by taking an aliquot from the primary stock and diluting with the mobile phase. For developing the calibration curve, six different concentrations in the range of 100 – 1000 ng/ml were prepared (100, 200, 400, 600, 800 and 1000 ng/ml). The concentrations were prepared by taking appropriate aliquots of secondary stock solution in 10 ml standard flasks and diluting them suitably with the selected mobile phase. Calibration curve was plotted between peak area of ZDV against the concentration of the drug. To establish the linearity of the proposed method, eight separate series of drug solutions in the selected medium were prepared from the stock solutions and analyzed. The average area of each concentration was substituted in the regression equation to calculate corresponding predicted concentration. The data was subjected to least square regression analysis. ANOVA test (one-way) at 95% level of significance was performed based on the peak area values observed for each pure drug concentration during the replicate measurement of the standard solutions.

(d) Analytical method validation

The developed method was validated for various parameters according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2007b; Bolton and Bon, 2004).

To study the selectivity of the method, ZDV stock solution of 100 µg/ml was prepared in mobile phase with and without excipients [excipients used: talc, magnesium stearate, HPMC (1000, 4000, 15000 and 100000 cPs), ethylcellulose, sodium carboxy

methylcellulose and carbopol 971P] separately. All the solutions were diluted suitably with the mobile phase to get a drug concentration of 400 ng/ml and analyzed. Blank solutions containing only the excipients were also injected separately and interference near the drug peak was checked.

To determine the accuracy of the proposed method, different quality control levels of drug concentration (Lower quality control [LQC] sample – 150 ng/ml, Medium quality control [MQC] sample – 500 ng/ml and High quality control [HQC] sample – 900 ng/ml) were prepared independently from stock solution and analyzed (n = 6). Further, different concentrations of the pure drug (100, 200 and 400 ng/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 5) to check the accuracy. Accuracy was assessed as the mean percentage recovery and percentage bias (% Bias = 100×[(Predicted concentration – Nominal concentration)/Nominal concentration]).

Repeatability was determined by using different levels of drug concentration (same concentration levels as taken in accuracy study), prepared from independent stock solutions and analyzed (n = 6). Inter-day and intra-day variations were found out to determine the intermediate precision of the proposed method. Different quality control levels of drug concentration in triplicates were prepared at two different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (n = 18). The percentage relative standard deviation (% RSD) of the predicted concentration from the regression equation was taken as precision.

LOD and LOQ of ZDV by the proposed method were determined using signal to noise ratio. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where σ is the standard deviation of y-intercept of regression equation and S is the slope of the regression equation. Experiments were performed to determine the actual concentration that can be experimentally quantified using the proposed method.

Robustness of the proposed method was determined by changing the mobile phase composition (Aqueous phase:ACN composition of 58:42, 62:38 were tried) and checking the bench top stability of ZDV in the mobile phase at room temperature for 24 h.

(e) Estimation of drug content in formulations

Two different tablet brands (Retrovir® and Zidovir tablets) and one capsule brand (Retrovir® capsule) of ZDV that were commercially available were taken for estimation of total drug content per unit dosage form (tablet/capsule). In case of tablets, 20 tablets were weighed and pulverized. Amount of powder equivalent to 10 mg of ZDV was taken and

suitably dissolved in selected mobile phase to prepare a final concentration of 100 µg/ml. The resulting solution was filtered through Whatman filter paper no. 40. Further dilutions were made with selected mobile phase in order to obtain concentrations within the linearity range and the samples were analyzed using the proposed method (n = 5). In case of capsules, content of 20 capsules were emptied and mixed properly. Amount of powder equivalent to 10 mg of ZDV was taken and suitably dissolved in selected mobile phase and same steps were repeated as mentioned above to obtain concentration in linearity range and the final samples were analyzed (n = 5).

3.7.2. Results and discussion

(a) Selection of mobile phase

Mobile phase consisting of aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40 was selected based on the peak properties (retention time and asymmetric factor) and sensitivity (height and area). In the selected mobile phase ZDV was found to have a retention time of 5.08 ± 0.09 min with an asymmetric factor of 1.03 ± 0.04 (Figure 3.3). The retention time of ZDV increased to 6.11 min with decrease in the proportion of acetonitrile from 40 % v/v to 35% v/v in the mobile phase. No significant change was observed in the peak area, peak height and asymmetric factor. Use of methanol (40% v/v) instead of acetonitrile in the mobile phase decreased the retention time to 4.94 min. When water (60% v/v) was used instead glacial acetic acid in the mobile phase, the peak area and peak height were found to decrease while the asymmetric factor was found to increase. Thus, aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40 was finally selected as mobile phase.

(b) Calibration curve

Different standard concentrations and their corresponding peak area are shown in the Table 3.5. At all the concentration levels the standard deviation (SD) of the area was low and the percentage relative standard deviation (% RSD) did not exceed 1.55. Overlaid chromatograms of blank, 200, 400 and 800 ng/ml is shown in Figure 3.3. The retention time of ZDV was 5.08 ± 0.09 min in the selected mobile phase. Total run time for single injection was 8 min for the proposed method. The predicted concentrations were nearly matching with the nominal concentrations. In selected medium the linearity range was found to be 100 – 1000 ng/ml. The linear regression equation obtained was: Peak Area =

188.29×Concentration (ng/ml) - 985.95; with excellent regression coefficient of 0.9999. The standard error of slope, intercept and estimate were found to be 9.01×10^{-1}, 252.65 and 5.82 respectively. The individual values of slope and intercept were found to be within 95% confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9999), low standard error of estimate (5.82), MSSR (2.76×10^{-4}) and low F_{cal} (7.21×10^{-4}) than F_{crit} (2.25) [$P < 0.05$, F_{crit} (7,40) = 2.25]. Lower values of parameters like MSSR, standard error of slope, intercept and estimate indicated high precision of the proposed method.

(c) Analytical method validation

The overlaid chromatograms of ZDV and solid admixture of ZDV with HPMC 15000 cPs in 1:1 proportion is shown in Figure 3.4. Estimation of ZDV in formulations and comparison of pure drug peak with that of the drug peak in presence of common as well as special excipients used in the formulations confirmed the lack of interference at the retention time of ZDV. The blank samples of excipients did not show any interference near the drug peak. In the presence of excipients, peak characteristics of the drug like retention time, peak area and asymmetric factor were not affected. This indicated that there was no significant interference of excipients in the estimation of ZDV by the proposed method. This confirmed the specificity and selectivity of the method.

The accuracy (% Bias) of the proposed method at all the three QC levels ranged from -1.04 to 1.20 (Table 3.6). The high mean percentage recovery values (nearly 100) and their low standard deviation (SD) values (SD < 1.83) indicated high accuracy. The validity and reliability of the proposed method was evaluated by recovery studies from standard addition method. The mean percentage recovery (± SD, SD is given in the parenthesis) for 100, 200 and 400 ng/ml concentrations in the standard addition method studies were found to be 101.56 (± 1.45), 100.76 (± 1.32) and 99.46 (± 1.27) respectively. These results revealed the accuracy of the proposed method.

In repeatability study, % RSD values ranged from 1.20 to 1.53 at all the different levels of concentrations (Table 3.6). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 0.96 and inter-day variation not more than 1.05 (Table 3.7). Low % RSD values indicated the excellent precision of the proposed method.

LOD and LOQ were found to be 12.52 ng/ml and 37.95 ng/ml respectively. The mean percentage recovery (± SD) for 40 ng/ml (LOQ) in triplicate was found to be 100.43

(± 149) representing the accuracy and precision of the proposed method. The method was found to be robust as the variation in percentage of acetonitrile in the selected media by ± 2% v/v did not have any significant effect on retention time, peak height, peak area and asymmetric factor. Different concentrations of bench-top ZDV solutions showed % RSD values less than 1.53%, indicating stability of ZDV. These solutions exhibited no change in the chromatographic peak characteristics at least for a period of 24 h at room temperature. During this period no extra peaks were observed in the chromatograms across all concentrations.

(d) Estimation of drug content in formulations

The overlaid chromatograms of ZDV and sample of Retrovir® capsule is shown in Figure 3.5. The % recovery values for the estimation of ZDV in different commercially marketed pharmaceutical formulations ranged from 99.78 to 100.34% with standard deviation not more than 1.15. Assay values of formulations were found to be same as that mentioned in the labeled claim, suggesting that the interference of excipient matrix was insignificant in the estimation of ZDV by proposed method. The estimated drug content with low values of standard deviation establishes the precision of the proposed method (Table 3.8).

3.8. Analytical Method 3: RP-HPLC Method for Estimation of Zidovudine in Rabbit Serum

3.8.1. Experimental

(a) Collection of blood and separation of serum

Blood was collected from marginal ear vein of male New Zealand white rabbits weighing between 2.0–2.5 kg. Blood collection was done with the prior permission from Institutional Animal Ethics Committee (Protocol approval no. IAEC/RES/10/2). The collected blood was harvested for 45 min at room temperature and centrifuged at 2000 rpm for 20 min. The clear supernatant serum layer was collected to generate a drug free serum pool.

(b) Chromatographic conditions

Separation was performed on a reverse phase LiChroCART® RP-8 (250×4 mm, 5 µm) column. Mobile phase consisted of a mixture of aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40. The aqueous phase was filtered through 0.22 µm membrane filter before use. The organic phase (ACN) was degassed using a sonicator. Injection volume was 50 µl. Flow rate was adjusted to 0.6 ml/min. The sensitivity parameters were set at auxiliary range = 1.0 AU/V (Absorbance unit/volt) and recorder range = 1.0 AUFS (Absorbance unit full scale). The wavelength of the UV detector was set at 266 nm. The column oven temperature was maintained constant at 25 °C.

(c) Selection of mobile phase

Different aqueous media (water, phosphate buffer (pH 5.6), phosphate buffer (pH 6.8) and glacial acetic acid (0.1% v/v)) in combination with different organic solvents (ACN and methanol) in various proportions were investigated. For selection of the mobile phase the criteria employed were chromatographic peak properties of the drug (retention time and asymmetric factor), sensitivity (height and area), ease of sample preparation, non interference of the bio matrix and applicability of the method for in vivo studies in rabbits.

(d) Calibration curve

Primary stock solution of 100 µg/ml of ZDV was prepared in selected mobile phase. A secondary stock of 10 µg/ml was prepared by taking an aliquot from the primary stock and diluting with the mobile phase. Serum standards were prepared by spiking appropriate volume of secondary stock of ZDV in rabbit serum to obtain final concentrations of 100, 200, 400, 600, 800 and 1000 ng/ml.

A simple and efficient protein precipitation method was employed to prepare the serum standards. Frozen drug free serum samples were thawed at room temperature. An aliquot of different drug solutions were added to 90 µl of drug free serum and vortex mixed using a cylco mixer (Remi, India) for 2 min. Then 400 µl of acetonitrile was added as serum protein denaturating and precipitating agent. Samples were then kept for 10 min on bench top to allow complete precipitation. The samples were then vortex mixed for 1 min and centrifuged (Refrigerated Centrifuge, Model CPR 20, Remi, India) at 10000 rpm for 10 min at 4 °C. The supernatant was carefully taken and then transferred to clean and dry auto-sampler vials. Serum and analytical standards (50 µl) were injected on to the column for analysis. The peaks obtained for the serum and analytical standards were integrated and

peak area was calculated for each concentration. To establish the linearity of the proposed method, eight separate sets of serum standards were prepared and analyzed. Percent absolute drug recovery from serum sample was calculated by using the formula: [(Peak area of serum standard/Peak area of analytical standard of same concentration) × 100]. The calibration data was subjected to least square regression analysis. One way ANOVA test at 95% level of significance was performed based on the peak area values observed for each concentration during the replicate measurement of the serum standards.

(e) Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2007b; Bolton and Bon, 2004). Various validation parameters of the developed method were determined as per standard guidelines.

Selectivity of the method can be defined as non interference at the retention time of ZDV by the proteins and other impurities present in the bio matrix. Blank serum samples were processed in the similar manner to serum standards except that no drug was spiked into the serum and 100 µl of serum was used instead of 90 µl. Blank serum samples were analyzed by the proposed method to demonstrate specificity and selectivity.

To determine the accuracy of the proposed method, different quality control levels of drug concentration in serum (Lower quality control [LQC] sample – 150 ng/ml, Medium quality control [MQC] sample – 500 ng/ml and High quality control [HQC] sample – 900 ng/ml) were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed as the mean percentage recovery and percentage bias (% Bias = 100×[(Predicted concentration – Nominal concentration)/Nominal concentration]).

Repeatability was determined by using different quality control levels of drug concentration (same concentration levels as taken in accuracy study) (n = 6). Inter-day and intra-day variations were found out to determine the intermediate precision of the proposed method. Three quality control levels of drug concentrations in triplicates were prepared twice in a day and studied for intra day variation (n = 6). The same protocol was followed for three different days to study inter-day variation (n = 18). The percentage relative standard deviation (% RSD) of the predicted concentration from the regression equation was taken as precision.

Limit of quantification (LOQ) is defined as minimum concentration of ZDV in serum sample that can be quantified with less than 20% RSD (International Conference on

Harmonization, 1996). In order to determine LOQ, three independent serum samples containing 50 ng/ml of ZDV were prepared and analyzed using the proposed method. The peaks were integrated and concentrations were back calculated using calibration equation. Mean concentration and % RSD for three samples were determined. Freeze thaw stability of ZDV in rabbit serum was determined by preparing three quality control samples (LQC, MQC and HQC). Total of four sets were prepared in triplicates and one set of the prepared concentrations was analyzed on the day of preparation (no freeze thaw cycle) and the remaining three sets were frozen at -20 °C for 24 h by storing them in a deep freezer (Vestfrost, Australia). Frozen samples were thawed by keeping the sealed tubes at room temperature (25 ± 2 °C) for at least 60 min. One set in triplicate was analyzed and the remaining two sets were kept at -20 °C for freezing and were analyzed after two and three freeze thaw cycle. The percentage deviation from the mean concentrations observed on day of preparation was calculated. Stability of ZDV in the processed samples of rabbit serum (the supernatant collected after the protein precipitation) was investigated by preparing five sets of quality control samples (LQC, MQC and HQC) in triplicates. Processed samples were kept in the sample rack of auto-injector (25 ± 2 °C) and samples were analyzed in triplicate every 6 h for 24 h on the day of preparation. The percentage deviation from the mean concentrations observed at zero time for each of the quality control samples was calculated. Long term stability of ZDV in rabbit serum was determined by preparing four sets of quality control samples (LQC, MQC and HQC). Total of four sets were prepared in triplicates and one set of the prepared concentrations was analyzed on the day of preparation. The remaining three sets were frozen at -20 °C. One set each of stored samples was analyzed at 7, 14 and 30 days of sample preparation by thawing them at room temperature. The percentage deviation from the mean concentrations observed on day of preparation for each of the quality control samples was calculated.

3.8.2. Results and discussion

(a) Selection of mobile phase

Mobile phase consisting of aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40 was selected based on the peak properties (retention time and asymmetric factor) and sensitivity (height and area) and non interference of the serum protein impurities with the drug peak. . In the selected mobile phase ZDV was found to have a retention time of 4.92 ± 0.08 min with an asymmetric factor of 1.14 ± 0.09 (Figure 3.6). The retention time of ZDV increased to 5.79 min with decrease in the proportion of acetonitrile from 40 % v/v

to 35% v/v in the mobile phase. No significant change was observed in the peak area, peak height and asymmetric factor. Use of methanol (40% v/v) instead of acetonitrile in the mobile phase decreased the retention time to 4.23 min. When water (60% v/v) was used instead of glacial acetic acid in the mobile phase, the peak area and peak height were found to decrease while the asymmetric factor was found to increase. Thus, aqueous phase (glacial acetic acid 0.1% v/v) and ACN in the ratio of 60:40 was finally selected as mobile phase.

(b) Calibration curve

Different serum standard concentrations and their corresponding peak area are shown in the Table 3.9. At all the concentration levels the standard deviation of the area was low and the percentage relative standard deviation (% RSD) did not exceed 5.24. Overlaid chromatograms of blank serum, 200 and 400 ng/ml are shown in Figure 3.6. The retention time of ZDV was 4.92 ± 0.08 min in the selected mobile phase. The asymmetric factor was found to be 1.14 ± 0.09. Total run time for single injection was 8 min for the proposed method. In selected medium the linearity range was found to be 100 – 1000 ng/ml. The linear regression equation obtained was: Peak Area = 178.16×Concentration (ng/ml) – 897.03, with excellent regression coefficient of 0.9993. The standard error of slope, intercept and estimate were found to be 2.25, 290.27 and 6.95 respectively. The individual values of slope and intercept were found to be within 95% confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9993), low standard error of estimate (6.95), MSSR (2.04×10^{-4}) and low F_{cal} value (4.32×10^{-3}) than F_{crit} value (2.25) [$P < 0.05$, F_{crit} (7,40) = 2.25]. Lower values of parameters like MSSR, standard error of slope, intercept and estimate indicated high precision of the proposed method. The absolute recovery of ZDV from the spiked rabbit serum samples when compared with analytical standards of same concentration were within 93.65 to 96.01% with a maximum SD of 6.37 (Table 3.9). Thus, the proposed protein precipitation technique was found to be accurate and precise with high recovery values precluding the use of internal standard.

(c) Analytical method validation

The simple and efficient protein precipitation technique used in the proposed method was found to be suitable for estimation of ZDV in rabbit serum with no interference from serum protein impurities. The metabolites of ZDV are highly hydrophilic in nature than ZDV and hence are expected to elute along with the other protein impurities. In case of test

sample of pilot in vivo studies, no additional peaks resulting from metabolism or degradation were observed in the near vicinity of the peak (Figure 3.7). Thus, the proposed method is specific and selective for the estimation of ZDV in rabbit serum.

All three quality control samples (Lower quality control [LQC] sample – 150 ng/ml, Medium quality control [MQC] sample – 500 ng/ml and High quality control [HQC] sample – 900 ng/ml) showed an accuracy (% Bias) ranging from -1.86 to -0.94 (Table 3.10). The high (nearly 100%) mean percent recovery values and low SD values (SD < 3.04) further established the accuracy of the method.

In repeatability study, the % RSD values ranged from 1.87 to 2.49 at all the different levels of concentrations (Table 3.10). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 4.72 and inter-day variation not more than 4.46 (Table 3.11). Low % RSD values indicated the excellent precision of the proposed method.

The mean concentration of three independent samples of 50 ng/ml, calculated using calibration equation was found to be 48.45 ng/ml with % RSD value of 12.79. Hence, the concentration of 50 ng/ml was considered as limit of quantitation for the proposed method.

The stability of ZDV in rabbit serum was evaluated using quality control samples under different stress conditions and the results obtained are shown in Figure 3.8. In freeze thaw stability, no significant degradation of ZDV was observed up to three cycles over a period of three days. The deviation from the zero time concentration was found to be less than 6.31% at the end of three freeze thaw cycles as shown in Figure 3.8a. ZDV was found to be stable in the processed samples of rabbit serum for a period 24 h with a maximum deviation of 5.79% from the zero time concentration as shown in Figure 3.8b. In the long term stability studies, ZDV was found to be stable for 30 days when stored at -20 °C. The deviation in recoveries of ZDV after analysis at 7, 14 and 30 days of sample preparation was found to be within acceptable limits (Figure 3.8c). The results of this study indicated that storage temperature of -20 °C was adequate for storing the samples for at least 30 days.

3.9. Conclusions

The developed analytical methods were found to be accurate, precise, sensitive and suitable for the estimation of ZDV in bulk, formulations, in vitro release samples and in rabbit serum. The UV method was found to be simple (with fewer sample preparation steps and fewer reagents), rapid and more economical than reported methods and suitable for the estimation of ZDV in bulk, formulations, in vitro release samples.

Proposed HPLC method for the estimation of ZDV in bulk and formulations was found to be highly sensitive (low LOQ values) as compared to earlier reported values considering the same instrument and parameters (detector, injection volume). The proposed method was specific because of non interference of the commonly used as well as special excipients used in formulations. Proposed HPLC method for estimation of ZDV in rabbit serum was found to be highly sensitive (low LOQ values). The sensitivity and selectivity of this method was helpful in conducting pharmacokinetic study of the developed formulations in rabbits.

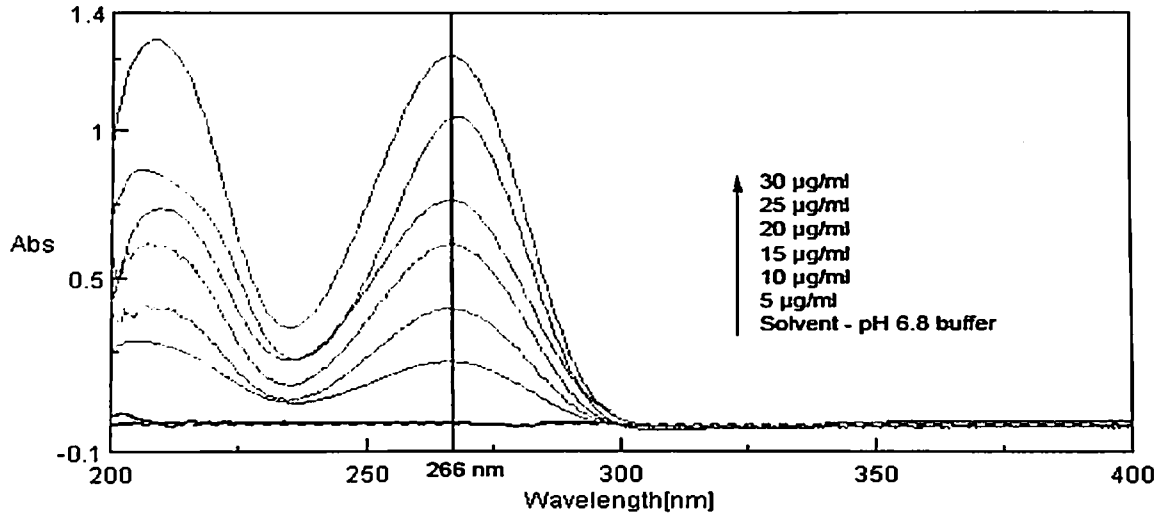


Figure 3.1: Overlaid UV absorption spectra of different concentrations of zidovudine in pH 6.8 phosphate buffer

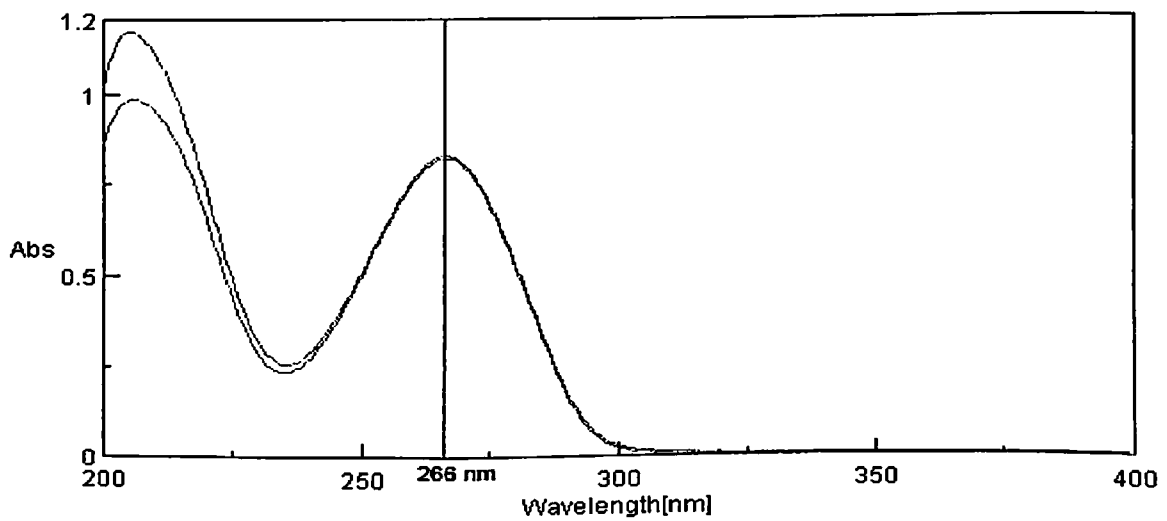


Figure 3.2: Overlaid UV absorption spectra of pure drug solution of zidovudine (20 µg/ml) and solution containing drug and HPMC 15000 cPs in 1:1 (20 µg/ml of zidovudine) ratio

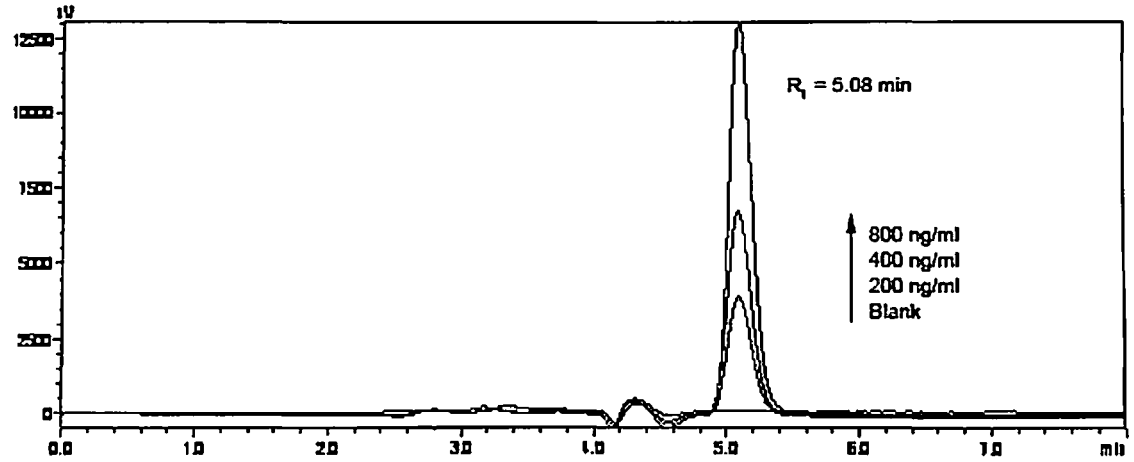


Figure 3.3: Overlaid chromatograms of blank, 200, 400 and 800 ng/ml of zidovudine in selected medium

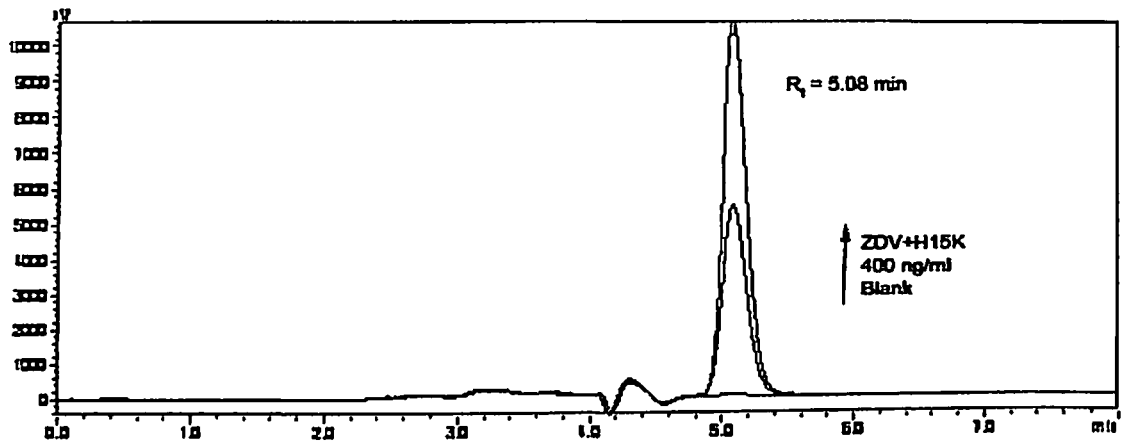


Figure 3.4: Overlaid chromatograms of blank, pure zidovudine (400 ng/ml) and solid admixture of zidovudine with HPMC 15000 cPs (ZDV+H15K) in 1:1 (700 ng/ml of zidovudine) ratio

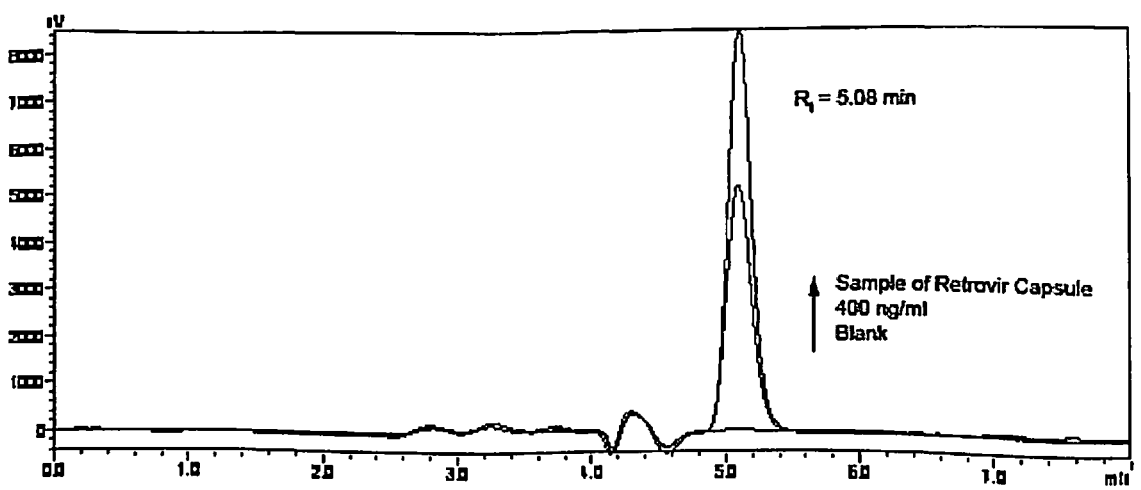


Figure 3.5: Overlaid chromatograms of blank, pure zidovudine (400 ng/ml) and marketed formulation of zidovudine (Retrovir capsules) in selected medium

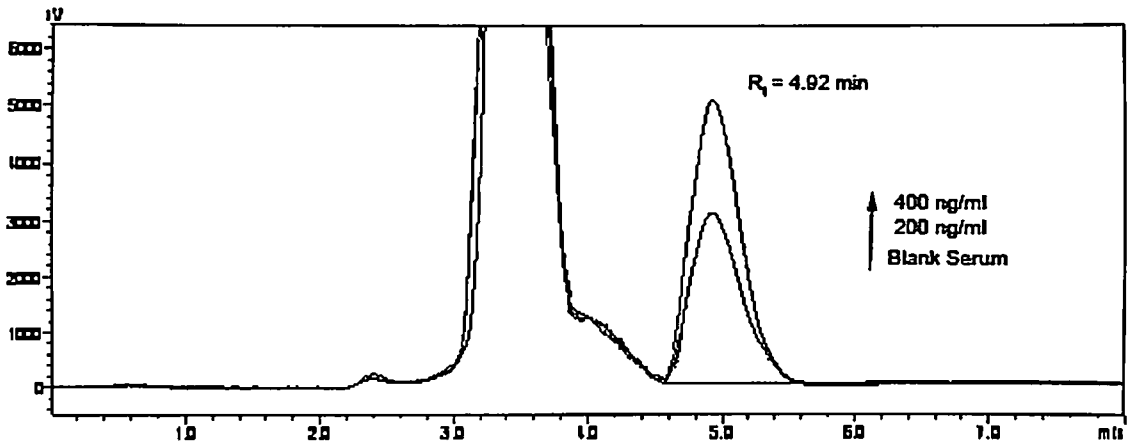


Figure 3.6: Overlaid chromatograms of blank serum, 200 and 400 ng/ml concentrations of zidovudine spiked in serum

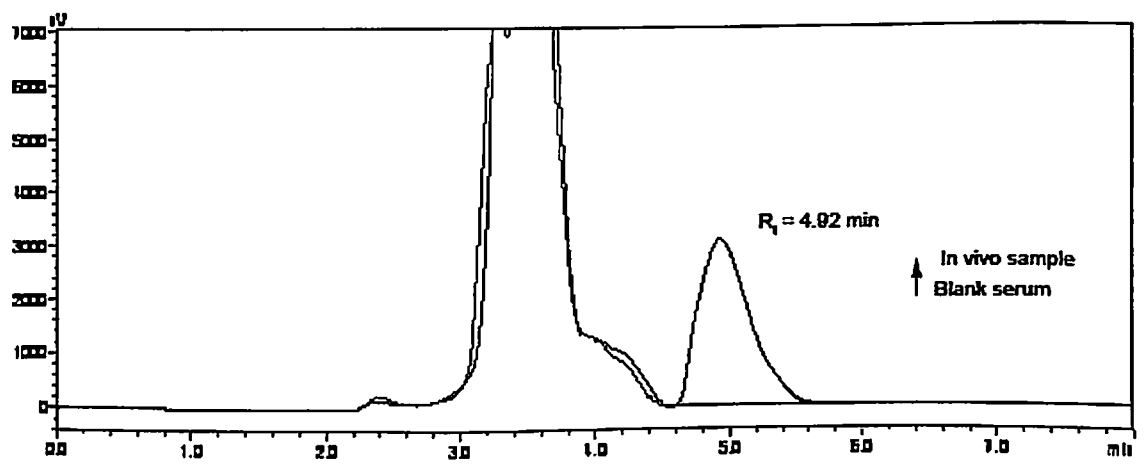
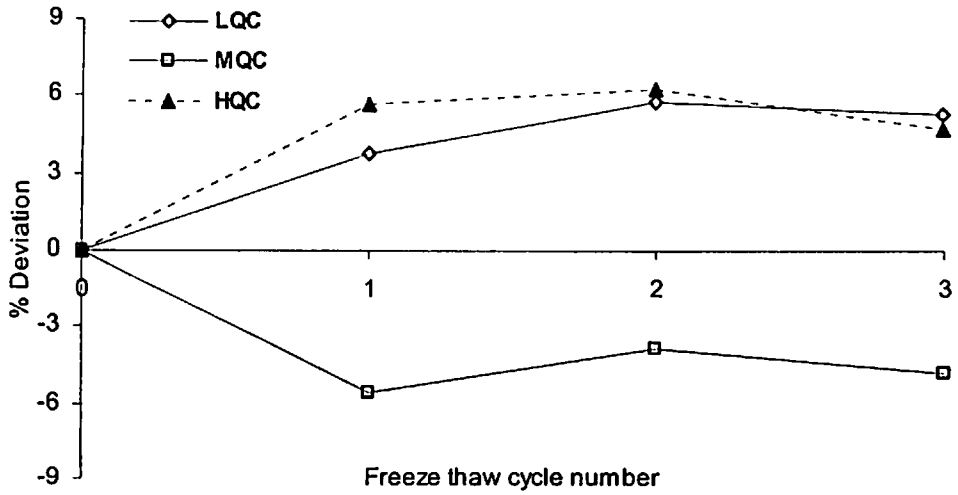
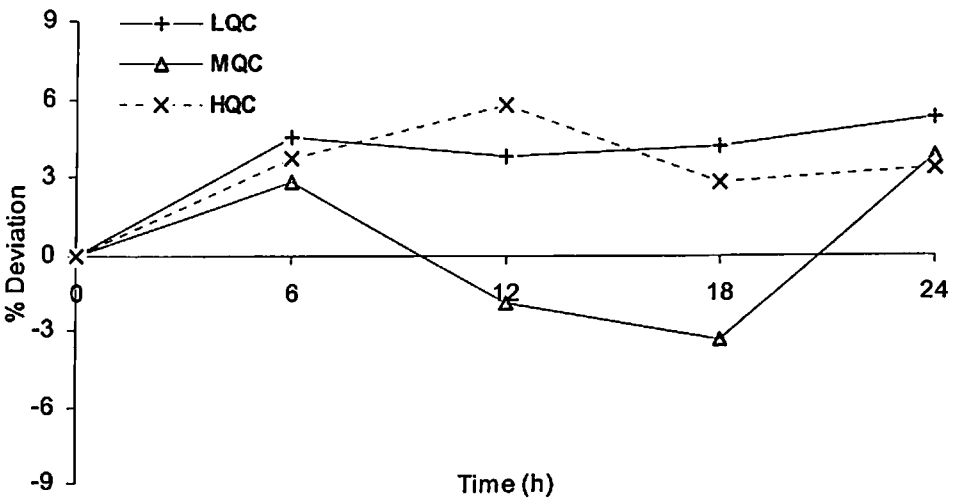


Figure 3.7: Overlaid chromatograms of blank serum and in vivo test sample obtained using bioanalytical method

(a)



(b)



(c)

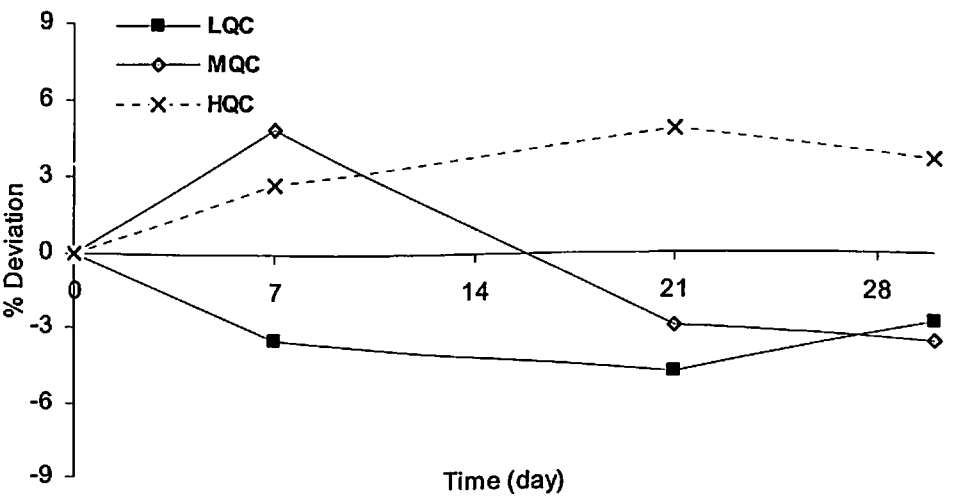


Figure 3.8: Stability studies of zidovudine in rabbit serum. (a) Freeze thaw stability, (b) Stability in processed samples and (c) Long term stability

Table 3.1: Calibration data for the estimation of zidovudine by UV spectrophotometric method

Zidovudine con. (µg/ml)	Absorbance[a] (Mean ± SD)	% RSD[b]	Predicted con.[c] (µg/ml)
5	0.2144 ± 0.0021	0.97	5.08
10	0.4087 ± 0.0033	0.81	9.95
15	0.6084 ± 0.0038	0.62	14.96
20	0.8049 ± 0.0059	0.73	19.88
25	1.0129 ± 0.0085	0.84	25.09
30	1.2087 ± 0.0094	0.78	30.01

[a] Each value is mean of nine separate determinations
[b] Percentage relative standard deviation
[c] Predicted concentration is calculated from the regression equation

Table 3.2: Accuracy and precision data for UV spectrophotometric method

Level	Predicted con.[a] (µg/ml)			% Recovery[b] (Mean ± SD)	Accuracy[c] (% Bias)
	Range	Mean[b] (± SD)	% RSD		
LQC	6.91 – 7.08	7.00 ± 0.08	1.14	99.88 ± 0.89	-0.12
MQC	12.43 – 12.61	12.51 ± 0.11	0.87	101.03 ± 0.76	1.03
HQC	27.42 – 27.58	27.49 ± 0.13	0.47	99.84 ± 1.01	-0.16

[a] Predicted concentration is calculated from the regression equation
[b] Each value is mean of six separate determinations
[c] Accuracy is given in terms of % Bias

Table 3.3: Intra-day and inter-day variability of UV spectrophotometric method

Level	Intra-day repeatability (% RSD) (n = 3)			Inter-day repeatability (% RSD) (n = 18)
	Day 1	Day 2	Day 3	
LQC	0.38	0.52	0.46	0.97
	0.43	0.49	0.32	
MQC	0.62	0.51	0.44	1.03
	0.48	0.67	0.57	
HQC	0.38	0.41	0.33	0.92
	0.29	0.34	0.47	

Table 3.4: Determination of zidovudine in marketed pharmaceutical preparations by UV spectrophotometric method

Commercial Products	Label claim	Assay[a] (mg) (Mean ± SD)	% Recovery[a] (Mean ± SD)
Retrovir Tablets	300 mg/tablet	299.43 ± 1.45	99.81 ± 0.92
Retrovir Capsules	100 mg/capsule	100.87 ± 1.33	100.87 ± 0.79
Zidovir Tablet	300 mg/tablet	300.59 ± 0.98	100.19 ± 1.02

[a] Each value is mean of five separate determinations

Table 3.5: Calibration data for the estimation of zidovudine by RP-HPLC method

Zidovudine con. (ng/ml)	Area (μv.sec)[a] (Mean ± SD)	% RSD[b]	Predicted con.[c] (ng/ml)
100	17914.53 ± 187.99	1.05	100.38
200	36784.49 ± 378.79	1.03	200.59
400	74586.50 ± 874.81	1.17	401.36
600	111906.08 ± 1349.04	1.21	599.56
800	148301.75 ± 2300.14	1.55	792.86
1000	188312.88 ± 2885.71	1.53	1005.35

[a] Each value is mean of eight separate determinations
[b] Percentage relative standard deviation
[c] Predicted concentration is calculated from the regression equation

Table 3.6: Accuracy and precision data for RP-HPLC method

Level	Predicted Con.[a] (ng/ml)			% Recovery[b] (Mean ± SD)	Accuracy[c] (% Bias)
	Range	Mean[b] ± SD	% RSD		
LQC	147.05 – 153.23	151.24 ± 2.08	1.37	101.19 ± 1.83	1.20
MQC	492.62 – 508.48	499.36 ± 7.67	1.53	98.96 ± 1.27	-1.04
HQC	889.88 – 911.39	901.72 ± 10.83	1.20	101.07 ± 1.79	1.07

[a] Predicted concentration is calculated from the regression equation
[b] Each determination is a result of six separate determinations
[b] Accuracy is given in terms of % Bias

Table 3.7: Intra-day and inter-day variability of RP-HPLC method

Level	Intra-day repeatability (% RSD) (n = 3)			Inter-day repeatability (% RSD) (n = 18)
	Day 1	Day 2	Day 3	
LQC	0.79	0.93	0.88	1.05
	0.62	0.87	0.76	
MQC	0.52	0.96	0.47	0.79
	0.44	0.92	0.39	
HQC	0.28	0.66	0.41	0.68
	0.34	0.53	0.45	

Table 3.8: Determination of zidovudine in pharmaceutical preparations available in market by RP-HPLC method

Commercial Products	Label claim	Assay[a] (mg) (Mean ± SD)	% Recovery[a] (Mean ± SD)
Retrovir Tablets	300 mg/tablet	300.25 ± 1.12	100.08 ± 1.09
Retrovir Capsules	100 mg/capsule	100.34 ± 0.95	100.34 ± 0.93
Zidovir Tablet	300 mg/tablet	299.36 ± 1.01	99.78 ± 1.15

[a] Each determination is result of five separate determinations

Table 3.9: Calibration data for the estimation of zidovudine in rabbit serum by RP-HPLC bioanalytical method

Zidovudine con. (ng/ml)	Area (µv.sec)[a] (Mean ± SD)	% RSD[b]	% Recovery[c] (Mean ± SD)
100	16940.48 ± 515.73	3.05	94.56 ± 4.28
200	35315.75 ± 1478.63	4.21	96.01 ± 5.83
400	70806.56 ± 3404.68	4.81	94.93 ± 4.09
600	105499.11 ± 5527.42	5.24	94.27 ± 5.12
800	138880.21 ± 6009.85	4.33	93.65 ± 5.26
1000	179462.15 ± 6423.41	3.58	95.31 ± 6.37

[a] Each value is mean of eight separate determinations

[b] Percentage relative standard deviation

[c] Percent drug recovery = [(Peak area of serum standard/Peak area of analytical standard of same concentration) × 100]

Table 3.10: Accuracy and precision data for RP-HPLC bioanalytical method

Level	Predicted Con.[a] (ng/ml)			% Recovery[b] (Mean ± SD)	Accuracy[c] (% Bias)
	Range	Mean[b] ± SD	% RSD		
LQC	146.81 – 153.41	150.42 ± 3.74	2.49	98.14 ± 2.46	-1.86
MQC	492.43 – 508.72	499.79 ± 9.34	1.87	99.06 ± 2.75	-0.94
HQC	880.14 – 919.45	900.13 ± 20.04	2.22	98.27 ± 3.04	-1.73

[a] Predicted concentration is calculated from the regression equation
[b] Each determination is a result of six separate determinations
[c] Accuracy is given in terms of % Bias

Table 3.11: Intra-day and inter-day variability of RP-HPLC bioanalytical method

Level	Intra-day repeatability (% RSD) (n = 3)			Inter-day repeatability (% RSD) (n = 18)
	Day 1	Day 2	Day 3	
LQC	2.42	3.05	2.89	4.21
	3.13	3.29	2.76	
MQC	4.72	3.59	4.48	4.46
	3.97	3.27	4.17	
HQC	2.14	4.47	2.86	3.17
	3.11	4.01	2.93	

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Chapter 4

Preformulation Studies

4.1. Introduction

In any formulation development process, preformulation study is very important and critical for understanding the pharmaceutically significant physiochemical properties of the selected drug. The goal of preformulation studies is to choose correct form of the drug, evaluate its physical properties and have a thorough understanding of the drug's stability under various conditions. An adequate understanding of these properties of the drug minimizes the problems in formulation development process and helps in selection of compatible excipients and development of appropriate dosage form for the drug substance (Wadke and Jacobson, 1982; Fiese and Hagen, 1987; Ravin and Radebaugh, 1990). These preformulation studies generally include determination of solubility of drug in different pH media, pH stability, partition coefficient, interaction with various formulation excipients and stability of the drug in solution state and solid state.

Physicochemical properties of zidovudine (ZDV) like pH solubility, dissociation constant (Keith, 2000) and partition coefficient (Zimmerman et al., 1987) have been reported in the literature. However, the pH solubility data reported, in the above literature, does not have information about the solubility at various pH values ranging from 1.2 to 8.0 and the reported partition coefficient value was for octanol/water system only. Since the current research endeavor aims at designing oral controlled release formulations, the pH solubility profiles and pH stability profiles were required to be established with the pH range encountered in the gastro intestinal tract (pH range: 1.2 to 8.0). The solid state stability of ZDV was done in the presence of different polymers and excipients which were planned to be used in the design of oral controlled tablet formulations of ZDV. In addition, n-octanol/water, n-octanol/pH 6.8 buffer, chloroform/water and chloroform/pH 6.8 buffer partition coefficient values of ZDV were also determined.

4.2. Materials

Pure ZDV was obtained as gift sample from Strides Arcolab Limited, Bangalore, India. The polymers like hydroxypropyl methylcellulose (HPMC) [1000 cPs, 4000 cPs (METHOCEL K4M Premium), 15000 cPs (METHOCEL K15M Premium) and 100000 cPs (METHOCEL K100M Premium)], ethyl cellulose (ETHOCEL™ Standard Premium, 10 cPs), sodium carboxy methyl cellulose and carbopol (Carbopol 971P NF) were obtained as gift samples from IPCA Laboratories, Mumbai, India. All other chemicals used were of analytical grade and purchased from Qualigens, Mumbai. Triple distilled water (TDW) from

all quartz glass apparatus was used for the preparation of various aqueous phases used in the different studies.

4.3. Equipments/Instruments

A constant temperature water bath shaker (MAX Instruments, India) was used for solubility studies and partition coefficient determination. All pH measurements were carried out using pH meter (Elico, India) equipped with glass electrode filled with potassium chloride gel and with auto temperature compensation probe. Frost-free 200 L refrigerator (Godrej, India) was used for stability studies at refrigerated conditions. A humidity chamber (MAC Instruments, India) was used to maintain accelerated conditions (40 ± 2 °C; 75 ± 5% RH). Thermal analysis was performed using differential scanning calorimeter (DSC) (Shimadzu, Japan; model: DSC-60; integrator: TA-60WS thermal analyzer; integrating software: TA-60WS collection monitor, version 1.51; analysis software: TA-60; principle: heat flux type; temperature range: -150 to 600 °C; heat flow range: ± 40 mW; temperature program rate: 0 to 99 °C/min; atmosphere: nitrogen at 40 ml/min). A five digit analytical balance (Mettler Toledo, Switzerland) was used for all weighing purposes. Drug excipient compatibility studies were carried out using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu, Japan; model: IR Prestige 21; model software: IR Solutions, version 1.0). Thin layer chromatographic (TLC) plates were checked in UV Fluorescence chamber (Superfit, India). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

4.4. Reagents

(a) Preparation of buffered solutions

Different buffer systems with 0.1 M strength ranging from 1.2 to 9.8 pH were prepared according to the procedures given in USP (US Pharmacopoeia, 2007). TDW was used as the solvent in all the cases.

(b) Preparation of unbuffered solutions

Unbuffered solutions of pH ranging from 1.2 to 9.8 were prepared using variable volumes of 0.1 N NaOH and 0.1 N HCl solutions. TDW was used as the solvent in all the cases.

(c) Preparation of 0.2 M sodium hydroxide

0.8 g of sodium hydroxide was transferred carefully into a 100 ml volumetric flask and 50 ml of TDW was added into the flask to dissolve sodium hydroxide completely. The final volume was made up to 100 ml with TDW (US Pharmacopoeia, 2007).

(d) Preparation of pH 6.8 phosphate buffer

6.805 g of potassium dihydrogen orthophosphate was transferred carefully into a 1000 ml volumetric flask and 500 ml of TDW was added into the flask to dissolve potassium dihydrogen orthophosphate completely. Then 112 ml of 0.2 M sodium hydroxide was added into the volumetric flask and the final volume was made upto 1000 ml with TDW (US Pharmacopoeia, 2007).

4.5. Methods

UV spectrophotometric method mentioned in chapter 3 was used for analysis of all samples in solubility and partition coefficient determination studies. Liquid chromatographic method mentioned in 3 was used for analysis of all samples in solid state and solution state stability studies.

4.5.1. Determination of solubility

Solubility studies were carried out in TDW (measured pH 7.0), unbuffered and buffered solutions with pH ranging from 1.2 to 9.8 at 37 °C. In solubility studies, excess amount of ZDV was added to vials containing 10 ml of TDW/unbuffered/buffered pH solution. At various time points, vials were checked for presence of insoluble drug and more drug was added into those vials in which was found to be completely soluble. The sample vials were agitated for 48 h using the water bath shaker maintained at 37 ± 1 °C. After 48 h, samples were collected from each of the sample vials and filtered through Whatman filter paper (No. 40) separately. The filtrate obtained from each sample vials were diluted appropriately and analyzed using analytical method 1 in chapter 3. All the measurements were done in triplicate.

4.5.2. Determination of partition coefficient

Partition coefficient of ZDV was determined in n-octanol/water, n-octanol/pH 6.8 buffer, chloroform/water and chloroform/pH 6.8 buffer systems by shake flask method. n-octanol was presaturated with both water and pH 6.8 phosphate buffer separately by shaking

it with water and pH 6.8 phosphate buffer separately for 24 h at room temperature (25 ± 2 °C). n-octanol saturated with aqueous phase (water/pH 6.8 phosphate buffer) was separated from the aqueous phase using glass separating funnel. The presaturated n-octanol and aqueous phase were used for determining the partition coefficient in n-octanol/water and n-octanol/pH 6.8 buffer systems. Similar procedure was followed to obtain presaturated chloroform and aqueous phase (water/pH 6.8 phosphate buffer) to determine the partition coefficient in chloroform/water and chloroform/pH 6.8 buffer systems. To 25 ml of n-octanol, 25 ml of 50 µg/ml of ZDV in presaturated aqueous phase (water/pH 6.8 phosphate buffer) was added and kept for shaking on rotary flask shaker at room temperature. The initial concentration of the drug in aqueous phase was determined using analytical method 1 in chapter 3. Aqueous samples of 1 ml each were collected at 24 and 48 h of shaking and centrifuged at 2000 rpm for 5 min. After equilibrium at room temperature for 15 min, aqueous phase was separated, diluted and analyzed by method 1 mentioned in chapter 3. The entire experiment was carried out in triplicates. Equilibrium partition coefficient ($P_{o/w}$) was calculated using the equation given below and Log $P_{o/w}$ was then calculated by taking logarithm to base 10 of equilibrium partition coefficient.

$$P_{o/w} = C_o / C_i = (A_i - A_f) / A_f$$ (As volume of the two phases is same, 25 ml)

Where, $P_{o/w}$ = equilibrium partition coefficient; C_o = concentration of drug in oily/non-aqueous phase; C_a = concentration of drug in aqueous phase; A_i = initial amount of the drug in aqueous phase and A_f = final amount of the drug in aqueous phase.

4.5.3. Stability studies

(a) Solution state stability studies

Solution state stability of ZDV was determined in buffered and unbuffered of various pH (pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.4, 7.4, 8.4 and 9.8) at 25 ± 2 °C. A stock solution of 5 mg/ml of ZDV was prepared in TDW. From this stock, 125 µl was added to buffered and unbuffered solutions of varying pH separately and the final volume was made up to 25 ml to achieve a final concentration of 25 µg/ml in each of the solutions. The entire study was done in triplicate. Samples were collected at different time points and were analyzed by the analytical method 1 mentioned in chapter 3. At different time points the solutions were spotted on a TLC plate eluted using mixture of dichloromethane and methanol in the ratio of

90:10 for assessing the stability of the drug. ZDV dissolved in TDW was used as control in all TLC studies.

To establish the thermal stability, 25 μg/ml solution of ZDV was made in TDW. The samples were exposed to different temperature conditions (25 ± 2 °C, 40 ± 2 °C and 60 ± 2 °C) in closed containers. The study was done in triplicates. Samples were withdrawn at different time points and were analyzed by method 1 of chapter 3. At different time points the solutions were spotted on a TLC plate and eluted using mixture of dichloromethane and methanol in the ratio of 90:10 for assessing the stability of the drug. ZDV dissolved in TDW and stored at room temperature was used as control in all TLC studies.

(b) Solid state stability studies

Solid admixtures of ZDV and various excipients, which were planned to be used in the preparation of oral controlled tablets, were prepared by physically mixing the drug and each excipient in the ratio of 1:10. Various formulation excipients like hydroxypropyl methylcellulose (1000, 4000, 15000 and 100000 cPs), ethylcellulose, sodium carboxy methylcellulose, carbopol, talc and magnesium stearate were used in the study. The solid admixtures of the drug with each excipients were filled in the vials and stored at different temperature conditions like controlled room temperature (CRT: 25 ± 2 °C/60 ± 5% RH), refrigerated condition (FT: 5 ± 2 °C) and at accelerated condition (AT: 40 ± 2 °C/75 ± 5% RH). At predetermined time intervals, samples (in triplicates) were taken and analyzed for drug content by analytical method 1 of chapter 3 after suitable dilution.

(c) Compatibility studies of zidovudine in presence of excipients

To study the compatibility of ZDV with various excipients, solid admixtures were prepared by mixing the drug with each excipient separately in the ratio of 1:1 and stored at controlled room temperature condition (CRT: 25 ± 2 °C/60 ± 5% RH). The solid admixtures were characterized using DSC and FTIR every 6 months for a period of one year. In case of DSC studies, a weighed quantity (around 1.0 to 2.5 mg) of the solid admixture was taken and sealed in standard aluminum pan with lid. The temperature range of measurement was 30 to 170 °C with a heating rate of 10 °C/min. Nitrogen gas was purged at a flow rate of 40 ml/min to provide the inert environment. Similarly, thermograms were also recorded for pure drug as well as pure excipients. In case of FTIR studies, a small quantity (5 to 10 mg) of the solid admixture was taken in a mortar and pulverized. To this dried and powdered potassium bromide was added (10 to 20 mg) and mixed thoroughly. After thorough mixing

a small quantity of the mixture was taken in FTIR sample holder and the spectrum was recorded. FTIR spectrum was also recorded for pure drug as well as pure excipients.

4.6. Results and Discussion

4.6.1. Determination of solubility

The solubility of ZDV at 37 ± 2 °C in TDW and various buffered and unbuffered solution of pH ranging from 1.2 to 9.8 is given in Table 4.1. The pH solubility profiles of ZDV in both buffered and unbuffered systems are shown in Figure 4.1. ZDV was found to have pH independent solubility in the pH ranging from 1.2 to 9.8 in both buffered as well as unbuffered systems. The solubility values of ZDV in buffered systems in pH 1.2 to 9.8 ranged from 19.97 ± 0.85 to 23.66 ± 0.47 mg/ml, indicating no significant difference in the solubility of ZDV with the change in pH. The solubility values of ZDV in unbuffered systems in pH 1.2 to 9.8 ranged from 18.47 ± 1.65 to 21.14 ± 1.20 mg/ml, indicating no significant change in the solubility of ZDV. The solubility of ZDV in TDW was found to be 20.79 ± 0.75 mg/ml. The pH independent solubility of ZDV is mainly due to its poor ionization properties. This is because, though ZDV is a weakly acidic drug, the pK_a of the acidic hydrogen on the thymine moiety is 9.9 and so the ionization of the drug is very poor (Keith, 2000).

Due to this pH independent solubility property of ZDV, it is considered to be a very good candidate for the preparation of oral controlled tablet formulations, as the drug release will not be influenced by pH change through out the gastrointestinal tract.

4.6.2. Determination of partition coefficient

The equilibrium partition coefficient of ZDV was determined in n-octanol/water, n-octanol/pH 6.8 buffer, chloroform/water and chloroform/pH 6.8 buffer systems by shake flask method. The partition coefficient values of ZDV in n-octanol/water and n-octanol/pH 6.8 phosphate buffer were found to be same at both 24 and 48 h, indicating that equilibrium was reached by 24 h itself. No significant difference was observed in the partition coefficient values of ZDV in n-octanol/water and n-octanol/pH 6.8 phosphate buffer systems. The partition coefficient values of ZDV in n-octanol/water and n-octanol/pH 6.8 phosphate buffer at 24 h were found to be 1.159 ± 0.013 and 1.155 ± 0.014 respectively (Table 4.2). In case of chloroform/water and chloroform/pH 6.8 buffer systems, the partition coefficient values of ZDV were found to be different at 24 and 48 h indicating that

distribution equilibrium was not reached at 24 h. So, the partition coefficient determined at 48 h was considered as the equilibrium partition coefficient for the drug in these systems. The partition coefficient values of ZDV in chloroform/water and chloroform/pH 6.8 buffer systems at 48 h were found to be 0.485 ± 0.009 and 0.481 ± 0.012 respectively (Table 4.2).

The $P_{o/w}$ values of ZDV determined indicates the hydrophilic nature of the drug that can be correlated to rapid dissolution of the drug and thereby the absorption. So, controlling and sustaining the release of ZDV from the oral delivery systems using suitable polymers is essential to extend the duration of action of the drug.

4.6.3. Stability studies

(a) Solution state stability studies

The log percent remaining to be degraded (% RTD) versus time profiles for ZDV in various buffered and unbuffered systems are given in Figure 4.2 and Figure 4.3 respectively. First order kinetics was observed for the degradation of ZDV in various buffered and unbuffered systems. First order degradation rate constants obtained from the slope were used to determine $t_{90\%}$ (time taken for 90% of the drug remaining to be degraded) and $t_{50\%}$ (time taken for 50% of the drug remaining to be degraded) at various pH values (Table 4.3 and 4.4). Low MSSR values and high regression coefficient ($R^2 \approx 1$) further established linear relationship between log % RTD versus time.

No significant difference was observed in the degradation of ZDV in various buffered and unbuffered systems (Figure 4.4). The degradation rate constant (K_{deg}) values obtained were ranging from 4.66×10^{-4} to 5.25×10^{-4} h^{-1} and 4.42×10^{-4} to 5.29×10^{-4} h^{-1} in buffered and unbuffered pH systems respectively. The $t_{90\%}$ values obtained were ranging from 8.27 to 9.34 days and 8.23 to 9.91 days in buffered and unbuffered pH systems respectively.

The retention factor (R_f) of ZDV was found to be 0.54 in TLC analysis using mixture of dichloromethane and methanol in the ratio of 90:10 as the elution. There was no difference in the R_f values of freshly prepared pure drug solution and drug in various buffered and unbuffered pH systems at zero time. Only one spot corresponding to the R_f of ZDV was observed under UV light of 254 nm wavelength in various buffered and unbuffered pH systems till 24 h. One extra spot other than that of the pure drug was observed under UV light of 254 nm wavelength in various buffered and unbuffered pH systems after 48 h of the study.

In thermal stability at various temperature conditions (25 ± 2 °C; 40 ± 2 °C and 60 ± 2 °C), the log % RTD versus time profiles were linear for all the plot indicating first order kinetics. The first order degradation rate constants obtained from the slope of the curves were used to determine $t_{90\%}$ and $t_{50\%}$ at various temperature conditions (Table 4.5). Low MSSR values and high regression coefficient ($R^2 \approx 1$) further established linear relationship between log % RTD versus time. The degradation rate constant was found to be dependent on the temperature condition. The degradation rate constant (K_{deg}) value was lesser at 25 °C (5.22×10^{-4} h^{-1}) and higher at 60 °C (10.57×10^{-4} h^{-1}). The $t_{90\%}$ values decreased with increase in temperature condition. The $t_{90\%}$ value was found to be 8.44 days at 25 °C and 4.14 days at 60 °C. In the TLC studies, only one spot corresponding to the R_f of ZDV was observed under UV light of 254 nm wavelength till 48 h for the samples stored at 25 and 40 °C, indicating the stability of ZDV at these temperature conditions.

(b) Solid state stability studies

The solid admixtures of ZDV with various excipients used for the solid stability study, showed good content uniformity with values ranging from 98.56 to 101.93% with a maximum SD of 1.92. The degradation kinetics of pure ZDV and solid admixtures of ZDV with various excipients are given in Table 4.6. At refrigerated condition (FT: 5 ± 2 °C), pure ZDV and the solid admixtures of ZDV with various excipients were found to be stable for the entire period of study (12 months). The log % RTD versus time plots for pure ZDV and the solid admixtures were linear indicating first order kinetics. Low MSSR values and high regression coefficient ($R^2 \approx 1$) further established linear relationship between log % RTD versus time (Table 4.6). The degradation rate constant for pure ZDV was found to be 2.38×10^{-3} and 5.32×10^{-3} $month^{-1}$ at CRT and AT respectively. The $t_{90\%}$ values for pure ZDV at CRT and AT were found to be 44.24 and 19.75 months respectively.

The degradation rate constant (K_{deg}) values for the solid admixtures stored at CRT conditions were ranging from 2.41×10^{-3} to 2.96×10^{-3} $month^{-1}$. The $t_{90\%}$ values were ranging from 34.72 to 43.68 months. The K_{deg} values for the solid admixtures stored at AT conditions were ranging from 4.71×10^{-3} to 5.27×10^{-3} $month^{-1}$. The $t_{90\%}$ values were ranging from 20.05 to 22.27 months. ZDV was found to be stable both alone as well as in solid admixtures for at least a period of 12 months when stored at CRT conditions and at least for a period of 6 months when stored at AT conditions.

(c) Compatibility studies of zidovudine in presence of excipients

Characterization with DSC was carried out for pure ZDV, pure excipients and solid admixtures of ZDV with various excipients mixed in the ratio of 1:1. The content uniformity of all the solid admixtures was determined by analytical method 1 of chapter 3. The content uniformity of all the solid admixtures was ranging from 98.58 to 101.27 with a maximum SD of 1.23.

The DSC thermogram of pure ZDV showed a sharp melting endotherm at 123.98 °C with a normalized energy of 92.5 J/g. In the DSC thermograms of HPMC (1000, 4000, 15000 and 100000 cPs), EC, CP and talc, no peaks were observed. The thermograms of solid admixtures of ZDV with various excipients, except magnesium stearate, characterized after 6 months and 1 year of storage, also had shown similar endothermic peak at approximately 124 °C with almost the same normalized energy (values ranging from 88.56 to 93.64) (Table 4.7), indicating that ZDV was unaffected in the presence of various excipients selected for the study (Figure 4.5 to 4.13). A slight change in peak shape and peak position (shifting to higher or lower temperature) was observed in some solid admixtures (particularly in magnesium stearate and sodium carboxy methylcellulose admixtures), which could be due to the mixing process that lowers the purity of each component (Verma and Garg, 2004).

In the DSC thermogram of pure magnesium stearate, two merged endothermic peaks were observed in the region of 102.27 °C to 127.84 °C with a normalized energy of 48.89 J/g due to the melting of magnesium stearate and stearic acid (present as impurity in magnesium stearate). In the solid admixture of ZDV with magnesium stearate, two merged endothermic peaks were obtained in the region of 104.78 °C to 128.15 °C with a normalized energy of 129.63 J/g due to the melting of ZDV, magnesium stearate and stearic acid. So the normalized energy of the merged peak was higher (129.63 J/g) than the normalized energy of melting endotherm of pure ZDV (92.5 J/g). However, it should not be considered that there was an interaction between ZDV and magnesium stearate.

In the DSC thermogram of pure sodium carboxy methylcellulose, a broad endothermic peak was observed in the region of 144.56 °C to 157.26 °C with a normalized energy of 25.67 J/g due to the melting of sodium carboxy methylcellulose. The DSC thermogram of solid admixture of ZDV with sodium carboxy methylcellulose showed one sharp endothermic peak in the region of 118.75 °C to 124.63 °C and one broad melting endotherm in the region of 140.27 °C to 149.45 °C due to the melting of ZDV and sodium

carboxy methylcellulose respectively. The melting endotherm of ZDV in the solid admixture was observed at lesser temperature than compared to the pure drug.

The data obtained from the compatibility studies of ZDV with various excipients by DSC was further supported by FTIR studies. FTIR spectra of pure ZDV and solid admixtures of ZDV with various excipients mixed in the ratio of 1:1, characterized after 6 months of storage at CRT condition, are given in Figure 4.14. The characteristic peak of carbonyl group at 1694 cm^{-1} and azide group at 2012 cm^{-1} (Araujo et al., 2003; Rama et al., 2005), present in all the spectrum indicate the stable nature of ZDV in the solid admixtures. Similar results were obtained when the samples were analyzed after 1 year of storage at CRT condition. DSC and FTIR studies of ZDV and solid admixtures of the drug established the stable nature and compatibility of drug with various excipients at least for a period of 1 year when stored at CRT conditions.

4.7. Conclusions

Solubility studies in various buffered and unbuffered pH systems showed that ZDV has pH independent solubility. ZDV followed first order degradation kinetics in solution state with good stability over the entire pH range. Solid state stability studies showed that ZDV was stable and compatible with various excipients planned to be used in development of oral controlled release tablets. DSC and FTIR studies had further supported the data obtained in solid state stability studies. The results of preformulation studies were helpful in design and development of oral controlled tablets formulations of ZDV.

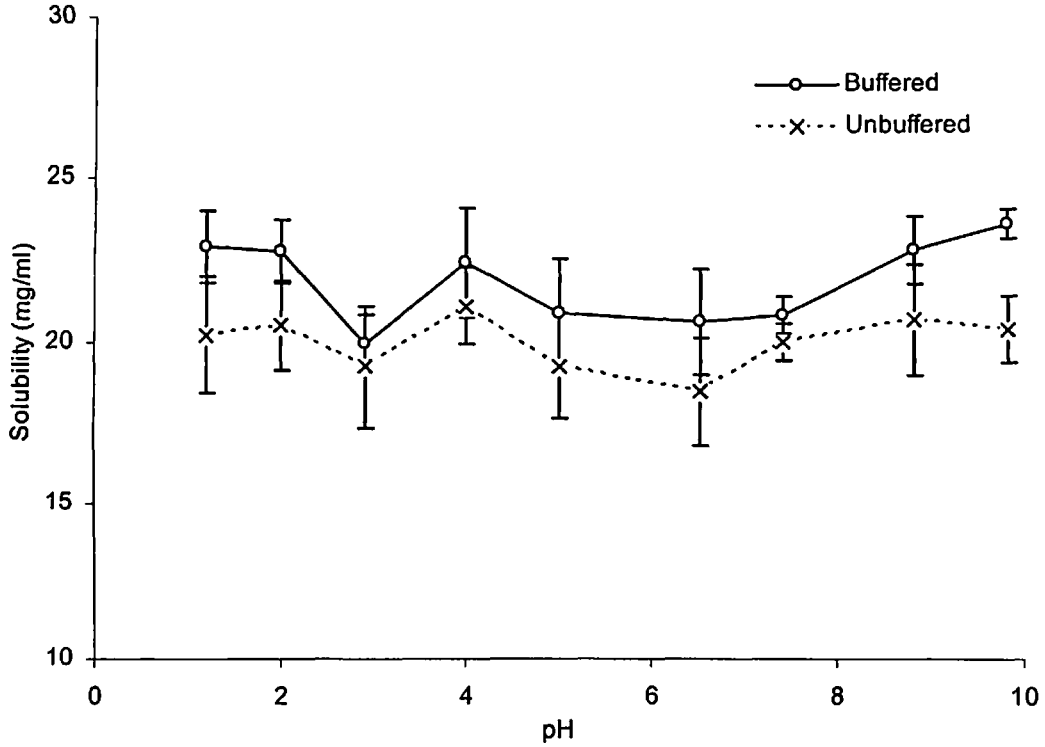

Figure 4.1: pH solubility profile of zidovudine in various buffered and unbuffered pH systems. Each point represents the mean value of three separate determinations with SD

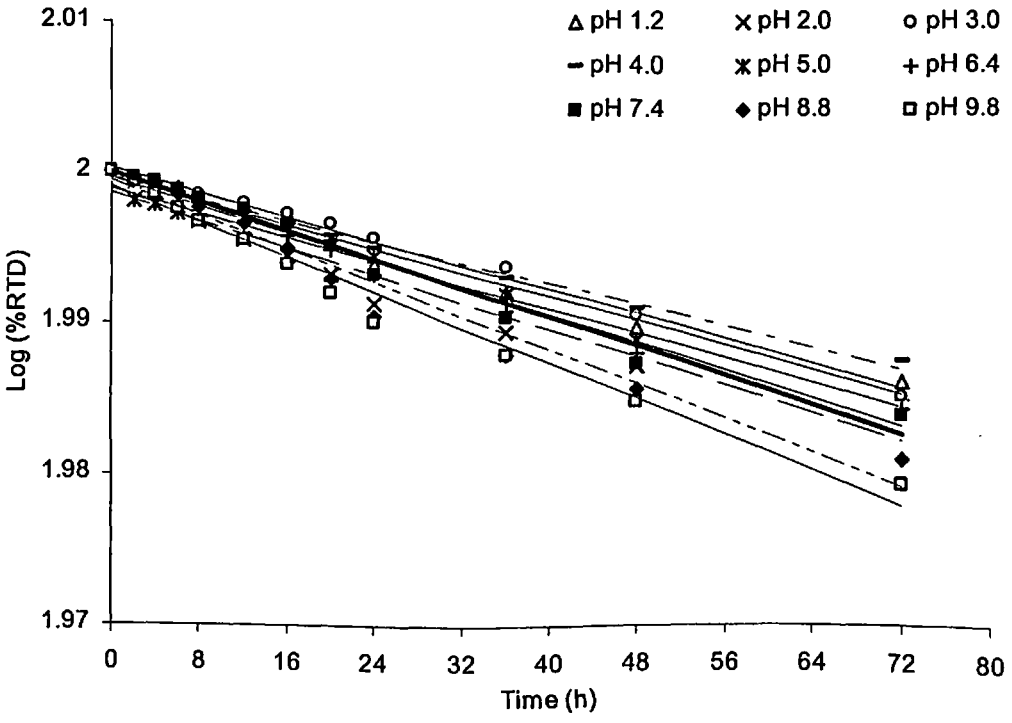

Figure 4.2: Stability study plots of zidovudine in various buffered pH solutions. Each point represents the mean value of three separate determinations with SD within ± 0.003

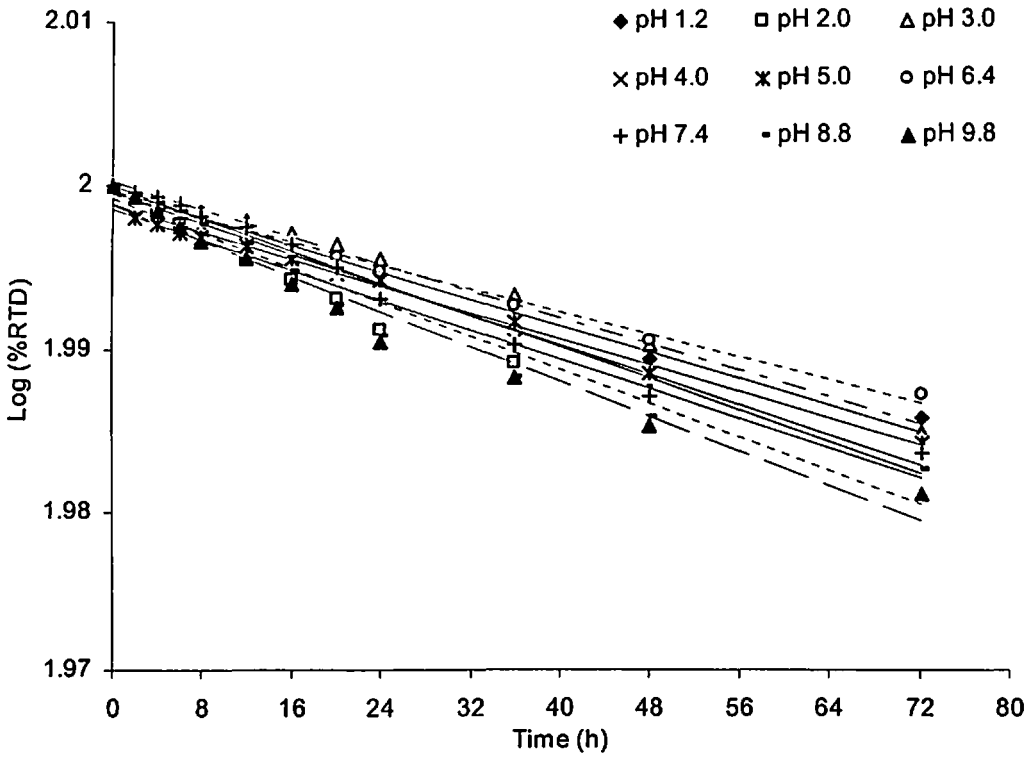

Figure 4.3: Stability study plots of zidovudine in various unbuffered pH solutions. Each point represents the mean value of three separate determinations with SD within ± 0.003

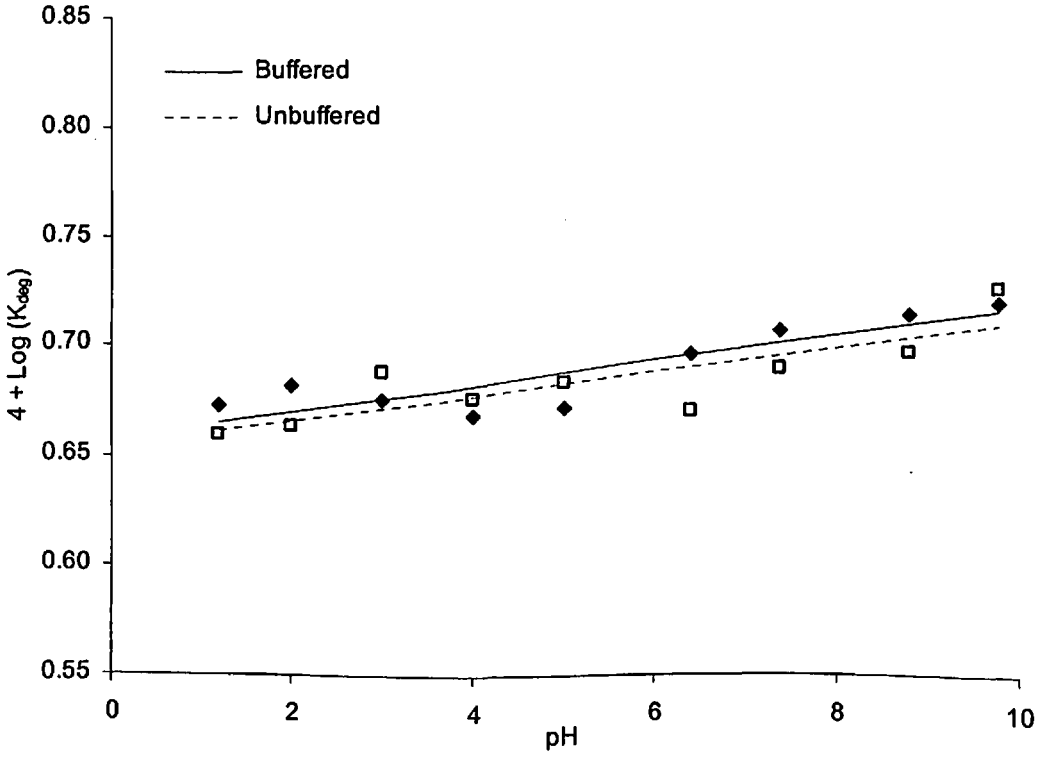

Figure 4.4: Comparative plots of degradation rate constants of zidovudine in various buffered and unbuffered systems

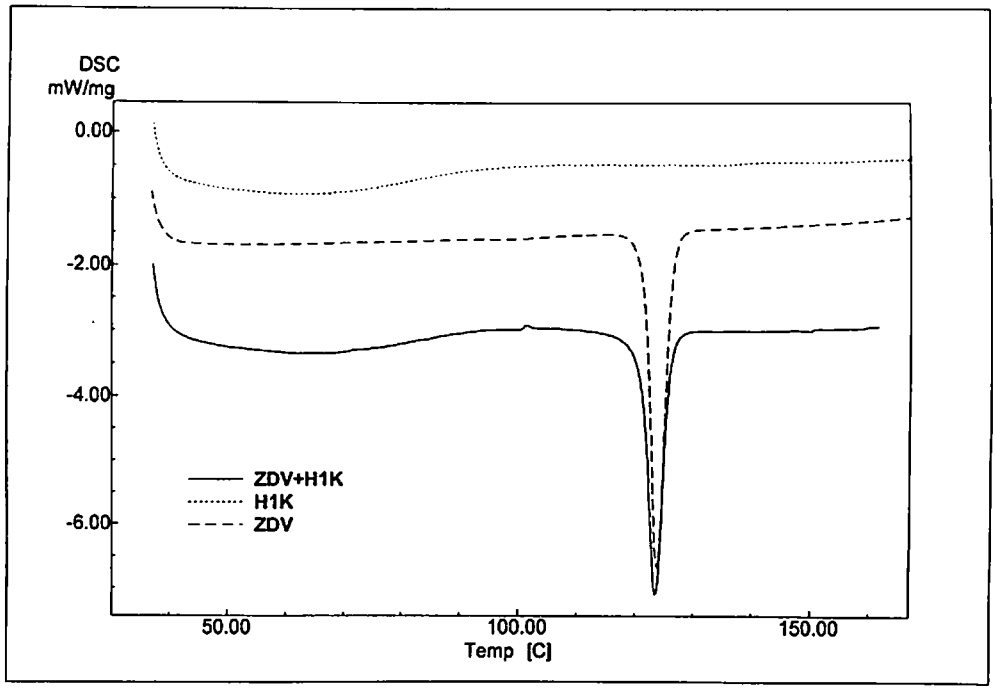

Figure 4.5: Thermograms of zidovudine (ZDV), hydroxypropyl methylcellulose 1000 cPs (H1K) and mixture of both (ZDV+H1K) in 1:1 ratio

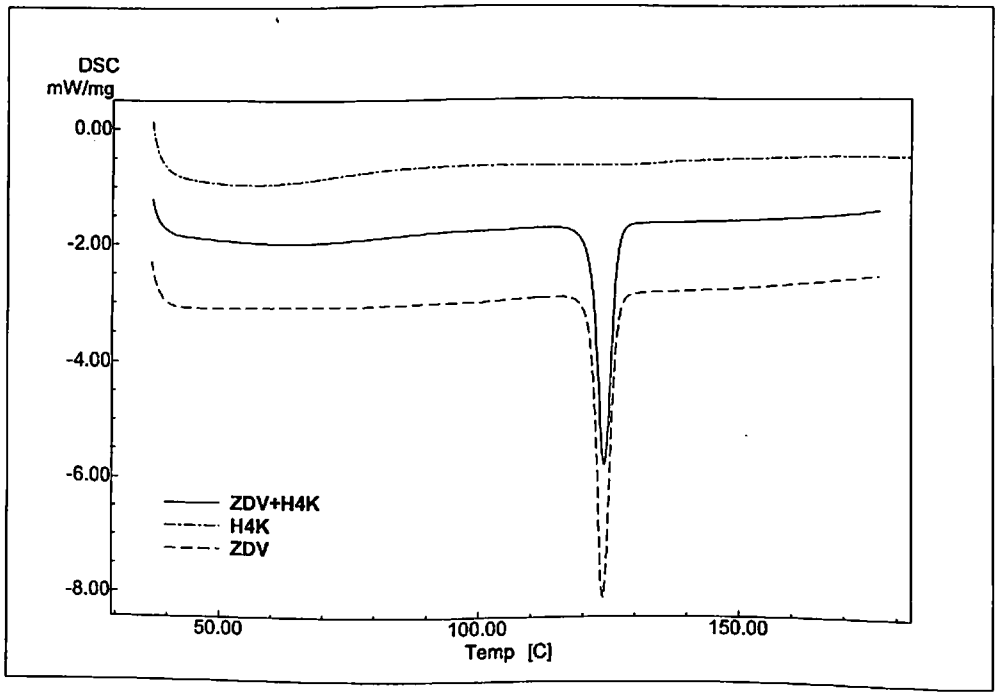

Figure 4.6: Thermograms of zidovudine (ZDV), hydroxypropyl methylcellulose 4000 cPs (H4K) and mixture of both (ZDV+H4K) in 1:1 ratio

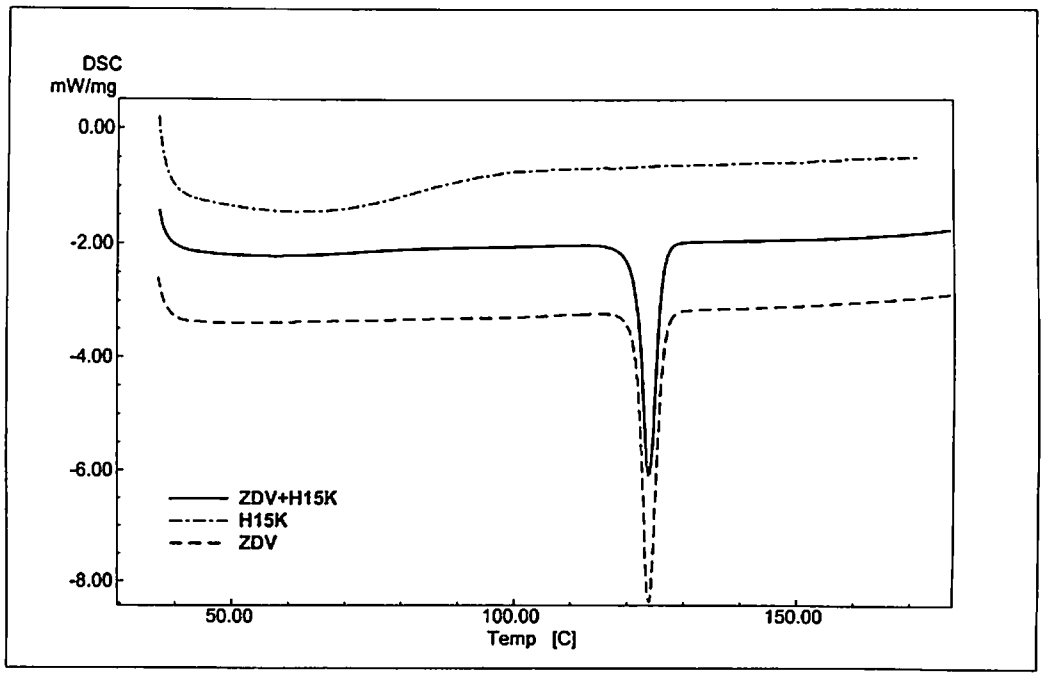

Figure 4.7: Thermograms of zidovudine (ZDV), hydroxypropyl methylcellulose 15000 cPs (H15K) and mixture of both (ZDV+H15K) in 1:1 ratio

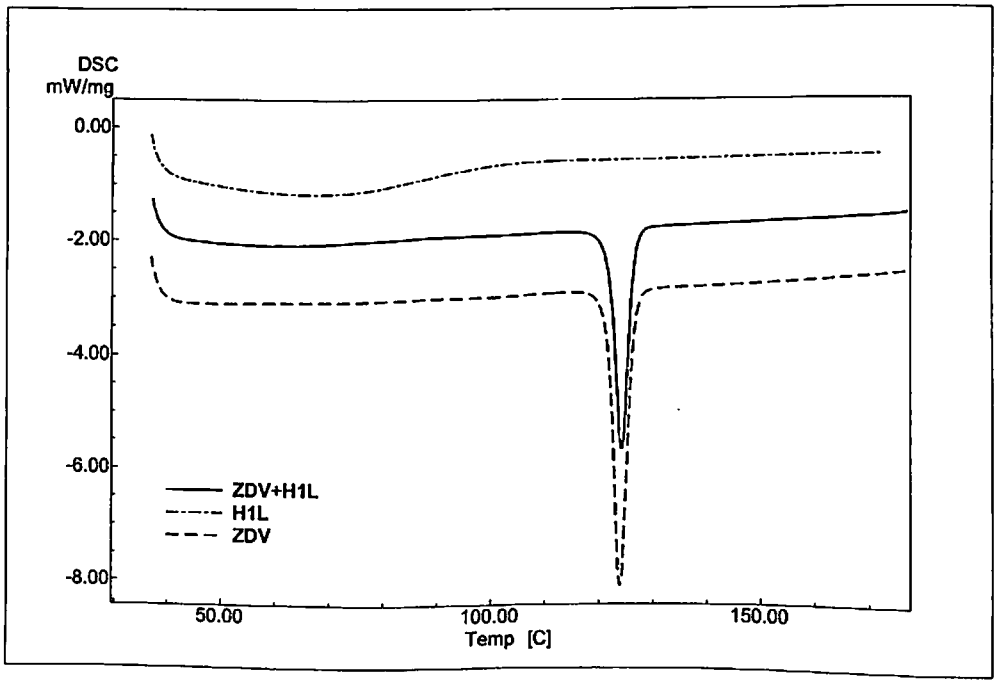

Figure 4.8: Thermograms of zidovudine (ZDV), hydroxypropyl methylcellulose 100000 cPs (H1L) and mixture of both (ZDV+H1L) in 1:1 ratio

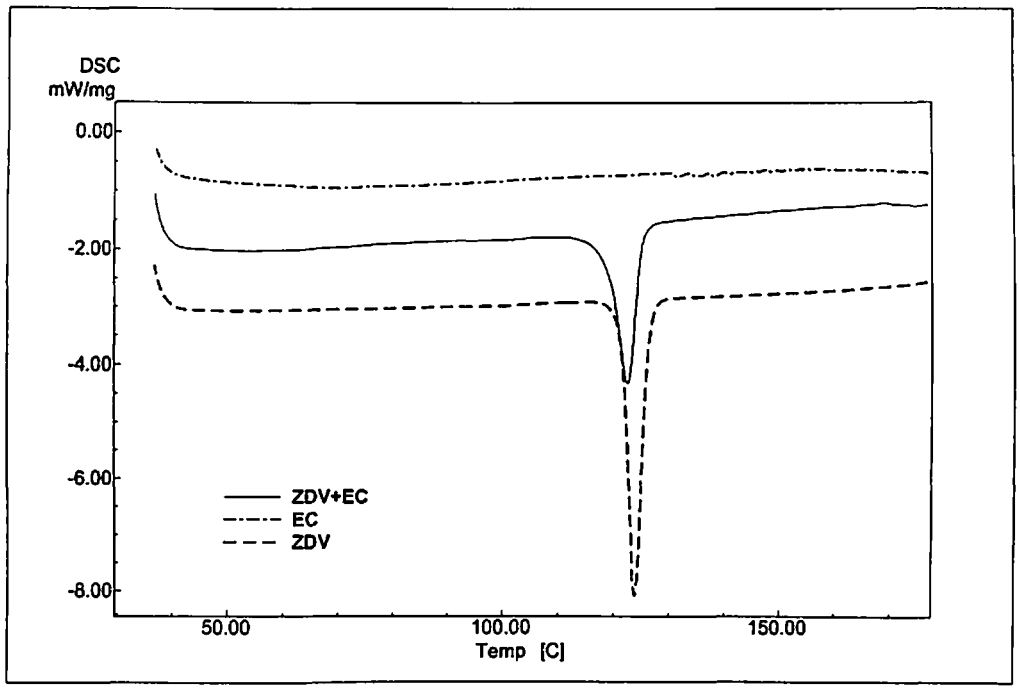

Figure 4.9: Thermograms of zidovudine (ZDV), ethylcellulose (EC) and mixture of both (ZDV+EC) in 1:1 ratio

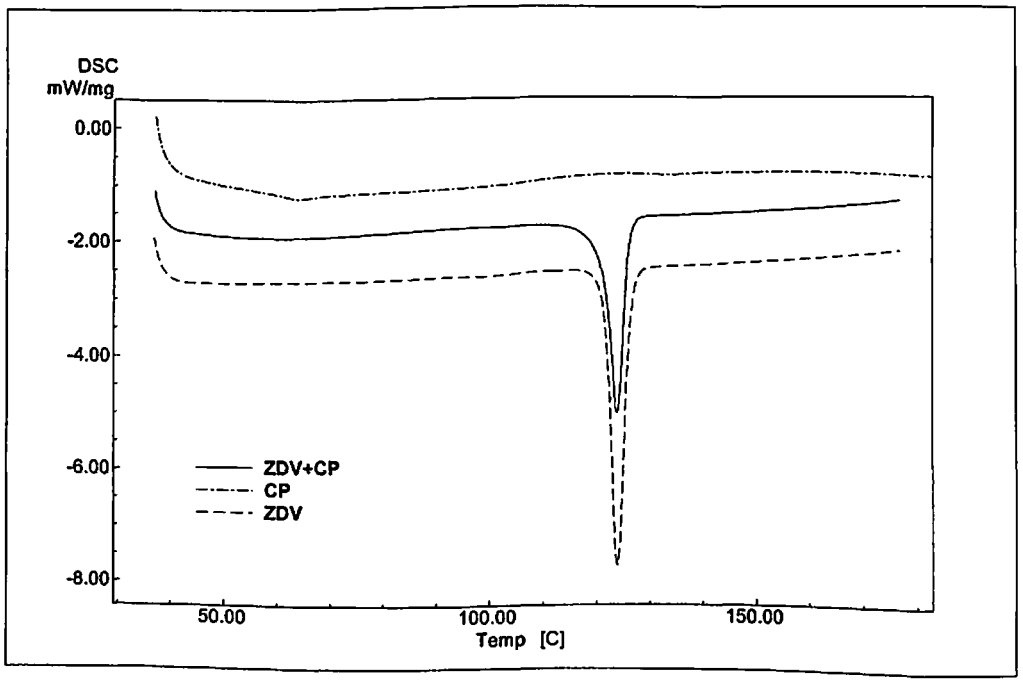

Figure 4.10: Thermograms of zidovudine (ZDV), carbopol 971P (CP) and mixture of both (ZDV+CP) in 1:1 ratio

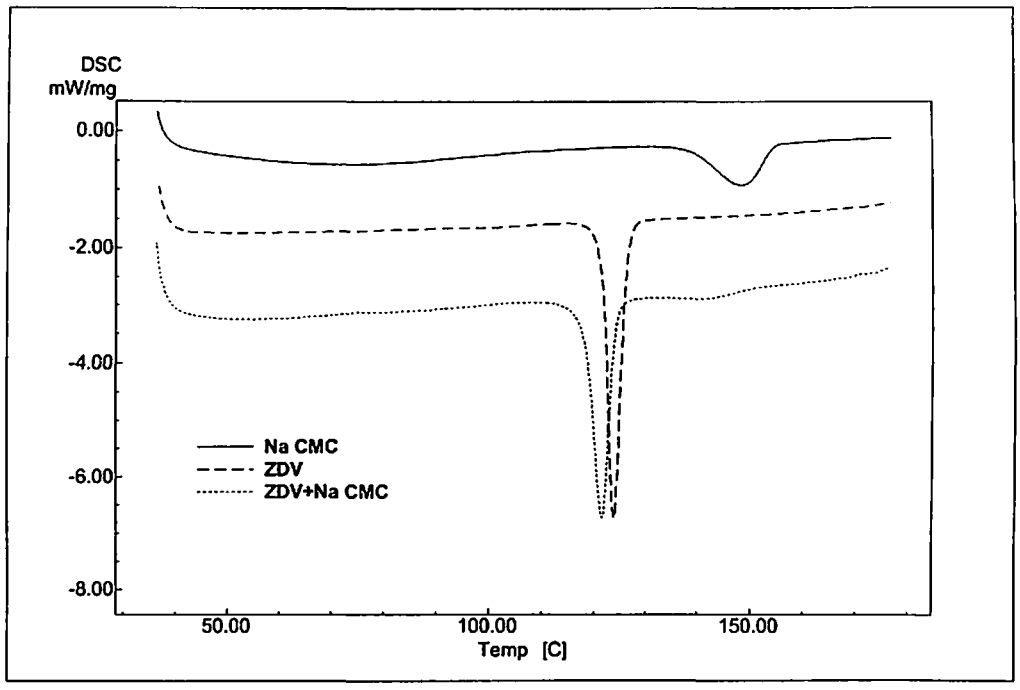

Figure 4.11: Thermograms of zidovudine (ZDV), sodium carboxy methylcellulose (Na CMC) and mixture of both (ZDV+Na CMC) in 1:1 ratio

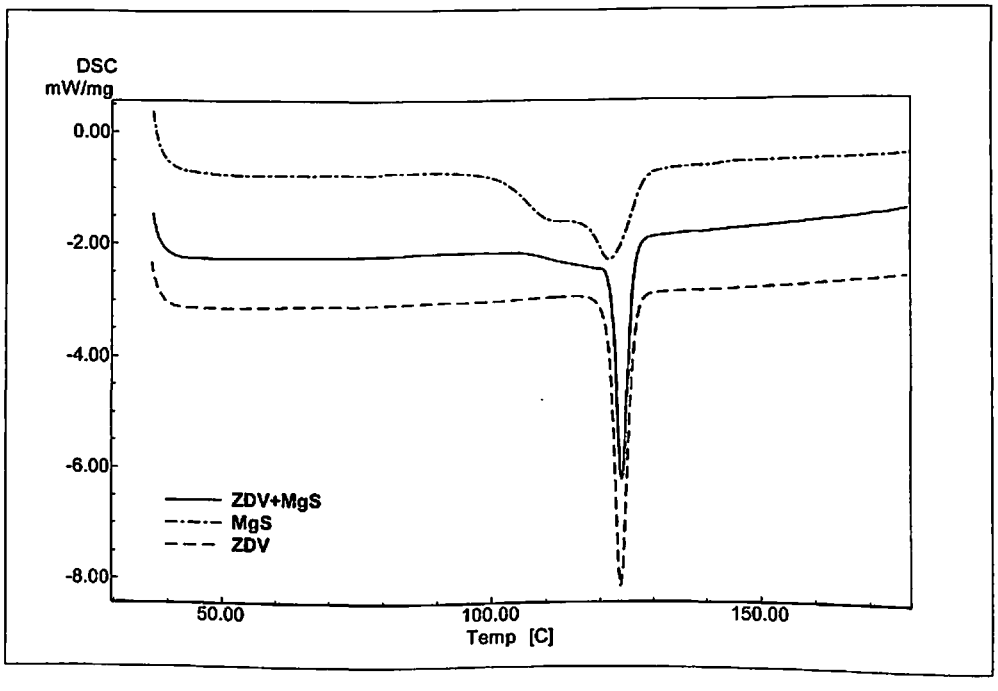

Figure 4.12: Thermograms of zidovudine (ZDV), magnesium stearate (MgS) and mixture of both (ZDV+MgS) in 1:1 ratio

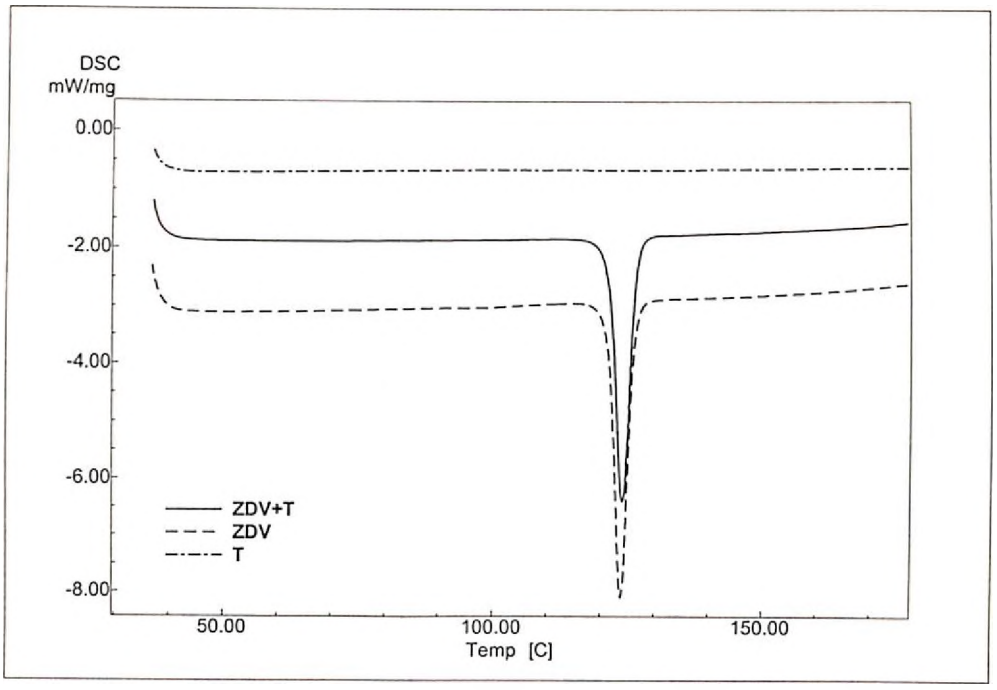

Figure 4.13: Thermograms of zidovudine (ZDV), talc (T) and mixture of both (ZDV+T) in 1:1 ratio

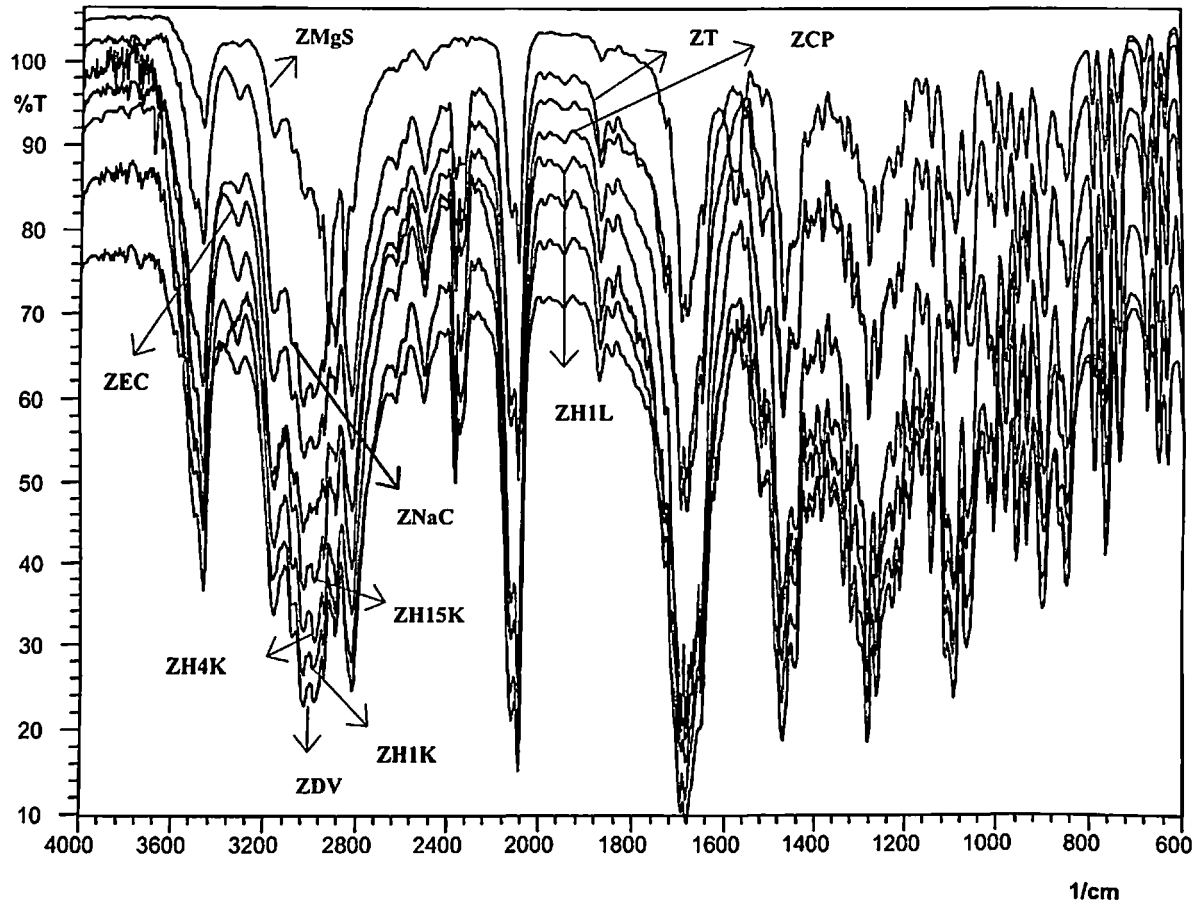

Figure 4.14: FTIR spectra of pure zidovudine (ZDV) and solid admixture of zidovudine with HPMC 1000 cPs (ZH1K), HPMC 4000 cPs (ZH4K), zidovudine with HPMC 15000 cPs (ZH15K), zidovudine with HPMC 100000 cPs (ZH1L), zidovudine with ethylcellulose (ZEC), zidovudine with carbopol 971P (ZCP), zidovudine with sodium carboxy methylcellulose (ZNaC), zidovudine with magnesium stearate (ZMgS) and zidovudine with talc (ZT) in the ratio of 1:1

Table 4.1: Solubility of zidovudine at 37 °C in buffered, unbuffered systems of various pH and TDW

pH	Solubility[a] (mg/ml) (Mean ± SD)	
	Buffered Systems	Unbuffered Systems
1.2	22.95 ± 1.11	20.21 ± 1.81
2.0	22.81 ± 0.98	20.52 ± 1.37
3.0	19.97 ± 0.85	19.22 ± 1.93
4.0	22.49 ± 1.72	21.14 ± 1.20
5.0	20.94 ± 1.63	19.28 ± 1.57
6.4	20.65 ± 1.59	18.47 ± 1.65
7.4	20.87 ± 0.43	20.01 ± 0.58
8.8	22.88 ± 1.04	20.73 ± 1.71
9.8	23.66± 0.47	20.41 ± 1.03
TDW	20.79 ± 0.75	

[a] Each value is mean of three separate determinations with SD

Table 4.2: Equilibrium partition coefficient data of zidovudine at 37 °C at different time points in different systems

Time (h)	n-octanol/water system[a] (Mean ± SD)	n-octanol/pH 6.8 phosphate buffer system[a] (Mean ± SD)
	$P_{o/w}$	$P_{o/w}$
24	1.159 ± 0.013	1.155 ± 0.014
48	1.164 ± 0.016	1.161 ± 0.018
	Chloroform/water system[a] (Mean ± SD)	**Chloroform/pH 6.8 phosphate buffer system[a] (Mean ± SD)**
	$P_{o/w}$	$P_{o/w}$
24	0.360 ± 0.011	0.357 ± 0.013
48	0.485 ± 0.009	0.481 ± 0.012

[a] Each value is mean of three separate determinations with SD

Table 4.3: pH stability data of zidovudine in buffered systems at room temperature

pH	K_{deg}[a] (x 10^{-4} h^{-1}) Mean ± SD	$t_{90\%}$[b] (days)	$t_{50\%}$[c] (days)	R^2	MSSR
1.2	4.72 ± 0.02	9.25	61.14	0.9786	5.22 x 10^{-5}
2.0	4.81 ± 0.03	8.85	59.74	0.9973	6.17 x 10^{-5}
3.0	4.74 ± 0.02	9.31	60.94	0.9982	3.82 x 10^{-5}
4.0	4.66 ± 0.02	9.34	61.99	0.9973	4.46 x 10^{-5}
5.0	4.69 ± 0.03	9.12	61.26	0.9958	5.78 x 10^{-5}
6.4	4.95 ± 0.03	8.79	58.27	0.9967	3.88 x 10^{-5}
7.4	5.07 ± 0.03	8.66	56.98	0.9971	4.46 x 10^{-5}
8.8	5.15 ± 0.02	8.34	55.40	0.9989	3.77 x 10^{-5}
9.8	5.25 ± 0.03	8.27	54.92	0.9873	4.05 x 10^{-5}

[a] Each value is mean of three separate determinations with SD
[b] Time taken for 90% of the drug remaining to be degraded
[c] Time taken for 50% of the drug remaining to be degraded

Table 4.4: pH stability data of zidovudine in unbuffered systems at room temperature

pH	K_{deg}[a] ($\times 10^{-4}$ h^{-1}) Mean ± SD	$t_{90\%}$[b] (days)	$t_{50\%}$[c] (days)	R^2	MSSR
1.2	4.58 ± 0.03	9.55	63.01	0.9979	4.38×10^{-5}
2.0	4.42 ± 0.03	9.91	65.32	0.9981	3.77×10^{-5}
3.0	4.88 ± 0.02	9.03	59.20	0.9975	6.05×10^{-5}
4.0	4.74 ± 0.03	9.23	60.86	0.9945	5.19×10^{-5}
5.0	4.81 ± 0.04	9.06	59.95	0.9988	3.56×10^{-5}
6.4	4.51 ± 0.03	9.71	63.97	0.9894	5.24×10^{-5}
7.4	4.68 ± 0.02	9.37	61.76	0.9982	3.07×10^{-5}
8.8	4.95 ± 0.03	8.81	58.27	0.9975	4.19×10^{-5}
9.8	5.29 ± 0.04	8.23	54.68	0.9959	5.79×10^{-5}

[a] Each value is mean of three separate determinations with SD
[b] Time taken for 90% of the drug remaining to be degraded
[c] Time taken for 50% of the drug remaining to be degraded

Table 4.5: Thermal stability data of zidovudine in TDW at different temperature conditions

Temperature	K_{deg}[a] ($\times 10^{-4}$ h^{-1}) Mean ± SD	$t_{90\%}$[b] (days)	$t_{50\%}$[c] (days)	R^2	MSSR
25 °C	5.22 ± 0.009	8.44	55.50	0.9983	4.18×10^{-5}
40 °C	7.74 ± 0.011	5.66	37.31	0.9968	5.07×10^{-5}
60 °C	10.57 ± 0.014	4.14	27.31	0.9991	6.05×10^{-5}

[a] Each value is mean of three separate determinations with SD
[b] Time taken for 90% of the drug remaining to be degraded
[c] Time taken for 50% of the drug remaining to be degraded

Table 4.6: Stability data of zidovudine in different solid admixtures stored at controlled room temperature and accelerated temperature conditions

Sample	CRT: 25 ± 2 °C/60 ± 5% RH				AT: 40 ± 2 °C/75 ± 5% RH			
	K_{deg}[a] (x 10^{-3} $month^{-1}$)	$t_{90\%}$[b] (months)	R^2	MSSR	K_{deg}[a] (x 10^{-3} $month^{-1}$)	$t_{90\%}$[b] (months)	R^2	MSSR
ZDV	2.38 ± 0.02	44.24	0.9889	1.04 x 10^{-5}	5.32 ± 0.04	19.75	0.9945	1.53 x 10^{-5}
ZDV + H1K	2.51 ± 0.03	41.56	0.9978	1.28 x 10^{-5}	5.20 ± 0.02	20.42	0.9924	1.06 x 10^{-5}
ZDV + H4K	2.56 ± 0.02	41.01	0.9947	1.96 x 10^{-5}	4.83 ± 0.03	21.83	0.9817	1.22 x 10^{-5}
ZDV + H15K	2.96 ± 0.02	34.72	0.9881	0.89 x 10^{-5}	4.82 ± 0.03	22.71	0.9798	1.31 x 10^{-5}
ZDV + H1L	2.75 ± 0.03	37.73	0.9697	2.12 x 10^{-5}	5.27 ± 0.05	20.23	0.9792	0.97 x 10^{-5}
ZDV + EC	2.41 ± 0.02	43.68	0.9842	1.19 x 10^{-5}	4.93 ± 0.02	21.33	0.9893	0.86 x 10^{-5}
ZDV + CP	2.78 ± 0.02	37.07	0.9886	1.08 x 10^{-5}	5.08 ± 0.02	20.69	0.9694	1.28 x 10^{-5}
ZDV + Na CMC	2.86 ± 0.02	36.68	0.9974	0.92 x 10^{-5}	5.24 ± 0.03	20.05	0.9882	2.01 x 10^{-5}
ZDV + T	2.75 ± 0.03	38.16	0.9685	1.37 x 10^{-5}	5.01 ± 0.02	20.98	0.9869	1.25 x 10^{-5}
ZDV + MgS	2.71 ± 0.02	38.51	0.9979	1.44 x 10^{-5}	4.71 ± 0.03	22.27	0.9772	1.33 x 10^{-5}

[a] Each value is mean of three separate determinations with SD

[b] Time taken for 90% of the drug remaining to be degraded

Table 4.7: Thermal properties of zidovudine, various excipients and the solid admixtures of zidovudine with various excipients

Sample	Phase transition	Phase transition onset (°C)	Peak (°C)	Phase transition endset (°C)	Heat (J/g)
H1K	T_g	37.25	-	-	-
H4K	T_g	37.13	-	-	-
H15K	T_g	36.98	-	-	-
H1L	T_g	36.94	-	-	-
EC	T_g	37.22	-	-	-
CP	T_g	37.01	-	-	-
Na CMC	T_g	37.21	-	-	-
	Endo	144.56	149.15	157.26	25.67
MgS	Two merged endothermic peaks were obtained	102.27	121.38	127.84	48.89
T	-	-	-	-	-
ZDV	Endo	121.59	123.98	126.14	92.50
ZDV + H1L	T_g	37.29	-	-	-
	Endo	120.95	124.01	126.75	91.43
ZDV + H4K	T_g	37.16	-	-	-
	Endo	121.13	124.05	126.95	91.95
ZDV + H15K	T_g	37.20	-	-	-
	Endo	121.74	123.64	126.11	90.48
ZDV + H1L	T_g	37.23	-	-	-
	Endo	120.99	123.87	127.06	91.45
ZDV + EC	T_g	36.99	-	-	-
	Endo	119.95	122.51	125.57	89.74
ZDV + CP	T_g	37.15	-	-	-
	Endo	120.12	123.66	126.19	89.02
ZDV + Na CMC	T_g	37.21	-	-	-
	Endo	118.75	121.89	124.63	88.56
	Endo	140.27	144.47	149.45	9.24
ZDV + MgS	Two merged endothermic peaks were obtained	104.78	122.79	128.15	129.63
ZDV + T	Endo	117.37	124.09	129.59	93.04

T_g – Glass transition temperature; Endo – Endothermic peak due to melting

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Chapter 5

Controlled Release Matrix Tablets: Development and In vitro Characterization

5.1. Introduction

In this chapter, studies involving development and in vitro evaluation of oral controlled release (CR) tablets of zidovudine (ZDV) prepared by matrix embedded technique have been presented. Matrix embedded CR tablets of ZDV were prepared using various polymers either alone or in combination by wet granulation process. The effect of various formulation and dissolution parameters like polymer type, polymer proportion, polymer viscosity, compression force, pH of dissolution medium and agitation speed on the in vitro release characteristics were studied in order to optimize these variables. Physical characterization of the developed formulations was done by various quality control tests. Stability of the developed formulations was assessed at various temperature and humidity conditions. Batch reproducibility of the developed formulations was also assessed.

5.2. Materials

Pure ZDV was obtained as gift sample from Strides Arcolab Limited, Bangalore, India. The polymers like hydroxypropyl methylcellulose (HPMC) [1000 cPs, 4000 cPs (METHOCEL K4M Premium), 15000 cPs (METHOCEL K15M Premium) and 100000 cPs (METHOCEL K100M Premium)], ethyl cellulose (EC) (ETHOCEL™ Standard Premium, 10 cPs), sodium carboxy methyl cellulose (Na CMC) and carbopol (CP) (Carbopol 971P NF) were obtained as gift samples from IPCA Laboratories, Mumbai, India. All other chemicals and reagents used were of analytical grade and purchased from Qualigens, Mumbai. Conventional formulations of ZDV: Retrovir® tablets, labeled claim of 300 mg of ZDV per tablet (GlaxoSmithKline Pharmaceuticals Limited, Mumbai, India) and Zidovir tablets, labeled claim of 300 mg ZDV per tablet (Cipla Pharmaceuticals Limited, Mumbai, India) were obtained from local market.

5.3. Equipments/Instruments

A 16 station tablet compression machine (Rotary Tabletting Machine, Cadmach, Ahmedabad, India) using round, flat face, beveled edge punches of 7 and 10 mm was used for manufacturing the tablets. Standard screw gauge was used for measuring the thickness and diameter of the tablets. A five digit analytical balance (Mettler Toledo, Switzerland) was used for all the weighing purposes. Friability of the designed tablets was determined in a friability tester (Campbell Electronics, Mumbai, India). In vitro release studies were carried out using USP Type 1 dissolution apparatus (Dissolution Tester (USP), TDT-08L, Electrolab, Mumbai, India). Tablet hardness was determined using a Monsanto tablet

hardness tester (Campbell Electronics, Mumbai, India). All pH measurements were carried out using pH meter (Elico, India) equipped with glass electrode filled with potassium chloride gel and with auto temperature compensation probe. Frost-free 200 L refrigerator (Godrej, India) was used for stability studies at refrigerated conditions. A humidity chamber (MAC Instruments, India) was used to maintain accelerated conditions (40 ± 2 °C; 75 ± 5% RH). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

5.4. Methods

5.4.1. Formulation of zidovudine matrix embedded CR tablets

Matrix embedded CR tablets of ZDV were prepared using various polymers like HPMC of different viscosity grades (1000 cPs, 4000 cPs, 15000 cPs and 100000 cPs), EC, CP and Na CMC either alone or in combination. The tablets were manufactured by wet granulation process using isopropyl alcohol (IPA) as the binding agent. The drug and polymer(s) (passed through sieve 60#) were mixed uniformly and granulated with IPA and dried in a tray drier at 50 °C. The dried granules were then passed through sieve 20#. The final granules were blended with talc (3% w/w of the dried granules weight) and magnesium stearate (1% w/w of the dried granules weight) and compressed on 16 station tablet compression machine using round, flat face, beveled edge punches of 10 mm diameter in order to obtain tablets containing 300 mg of ZDV. Three batches of tablets were prepared for each formulation to check the batch reproducibility. Composition of the prepared matrix embedded tablets containing 300 mg of ZDV and compressed using 10 mm diameter punches is given in Table 5.1a to 5.1c. On the basis of in vitro evaluation of designed formulations, six different selected formulations containing 100 mg of ZDV were prepared using the same manufacturing method, but were compressed using 7 mm diameter punches, for the purpose of in vivo bioavailability studies in rabbits. Composition of these selected formulations is given in Table 5.1d.

Matrix tablets containing 100 mg of ZDV and compressed using a 7 mm diameter punches were prepared to carry out in vivo bioavailability studies in rabbits, as matrix tablets containing 300 mg of ZDV (10 mm diameter) were difficult to administer orally in rabbits. Thus, the dose and the dimensions of the tablets were reduced to make it convenient for the oral administration of the designed tablets.

5.4.2. Effect of various formulation parameters

(a) Effect of polymer type and polymer proportion

Matrix embedded CR tablets of ZDV were prepared using various proportions of polymers like HPMC (various viscosity grades), EC, CP and Na CMC separately to study the effect of polymer type, polymer viscosity (in case of HPMC) and polymer proportion on tablets' physical and in vitro release characteristics. To study the effect of combination of polymers on physical and in vitro drug release characteristics, formulations were prepared using various proportions of combination of HPMC (1000 cPs) and EC.

(b) Effect of compression force

Formulations were prepared using different compression forces, keeping the polymer type and polymer proportion same, for studying the effect of compression force on the in vitro drug release.

5.4.3. Evaluation of the designed zidovudine matrix tablets

5.4.3.1. Physical characteristics

The thickness and diameter of 20 tablets of each batch were measured using a screw gauge. The weight variation was determined for each batch by taking weight of 20 tablets using an electronic balance. Tablet hardness was determined for 10 tablets using a Monsanto tablet hardness tester. Friability was determined by testing 20 tablets in a friability tester for falling shocks for 4 min at 25 rpm. Percentage friability was calculated using the initial and final weight of 20 tablets taken for testing.

5.4.3.2. Drug content

The drug content of the manufactured tablets of each batch was determined in triplicate. For each batch 20 tablets were taken, weighed and finely powdered. An accurately weighed quantity of this powder was taken and suitably dissolved in pH 6.8 phosphate buffer, and analyzed using UV spectrophotometric method of chapter 3 after making appropriate dilutions.

5.4.3.3. In vitro drug release studies

In vitro drug release studies for all the designed formulations carried upto 24 h using USP Type 1 dissolution apparatus (basket method) in 900 ml of pH 6.8 phosphate buffer at 37.5 ± 0.5 °C. The agitation speed was set at 100 rpm. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. After appropriate dilution the samples were analyzed using UV spectrophotometric method of chapter 3. Cumulative percent of the drug released was calculated and the mean of six tablets from three different batches were used in data analysis. To study the effect of agitation speed, in vitro release studies were also carried out at 50 rpm for some selected formulations and keeping the remaining test parameters same as mentioned above. To study the effect of dissolution media, in vitro release rate studies of some selected formulations from each polymer type were carried out in 900 ml of pH 1.2 (0.1N HCl solution) and pH 4.5 phosphate buffer, maintaining the same agitation speed (100 rpm) and bath temperature as mentioned above.

5.4.3.4. Characterization of release kinetics

The order and mechanism of ZDV release from the CR matrix tablets were determined by fitting the release rate studies data into various kinetic equations described in section 1.7.3.3.(a) of chapter 1.

The Zero order, First order and Higuchi's square root kinetics generally fail to explain the drug release mechanism from polymeric matrices that undergo swelling and/or erosion during the dissolution process. In such cases based on the value of n obtained by fitting the data into Ritger-Peppas' empirical equation, the mechanism of drug release from the formulation can be described (Atul et al., 2006). In case of Fickian release mechanism, the rate of drug release is much lesser than that of polymer relaxation (swelling/erosion). So the drug release is chiefly dependent on the diffusion through the matrix. In the non-Fickian (anomalous) case, the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation. Case II release generally refers to the polymer relaxation (Li et al., 2006).

The values of K (release rate constant for Ritger-Peppas' empirical equation), n (diffusional exponent indicative of release mechanism), $t_{50\%}$ (time required for 50% of drug release) R^2 (regression coefficient) and mean sum of square residuals (MSSR) were determined. Nature of drug release from the designed CR matrix tablets was inferred based on the correlation coefficients obtained from the plots of various kinetic models.

5.4.3.5. Release profiles comparison and statistical analysis

The drug release profiles were compared using a model-independent method (Costa and Lobo, 2001), by determining the mean dissolution time (MDT) and similarity factor (f_2) values of the formulations being compared. The MDT values were subjected to one-way ANOVA for analyzing the statistical difference. A confidence limit of $P < 0.05$ was fixed and the theoretical and calculated values of F (F_{crit} and F_{cal}) were compared for the interpretation of results. ANOVA was determined using software 'PRISM' (Graphpad, San Diego, USA). The MDT and f_2 values were calculated based on the Equations V and VI respectively, described in section 1.7.3.3.(b) of chapter 1.

5.4.3.6. Swelling and eroding behavior

The release of a drug from hydrophobic polymer matrix (EC) involves solvent penetration through the pore network of the matrix, dissolution of the drug in solvent and finally diffusion of the drug through solvent filled capillaries within the pore network of the matrix (Neau et al., 1999). The mechanism of drug release from hydrophilic polymeric matrices (HPMC or CP or Na CMC) involves solvent penetration, hydration and swelling of the polymer, diffusion of the dissolved drug in the matrix and erosion of the gel layer. Initially, the diffusion coefficient of drug in the dehydrated polymer matrix will be less and increases significantly as the polymer matrix imbibes more and more water, and forms a gel, as the time progresses. The hydration rate of the polymer matrix and thereby the gel formation and subsequent erosion depends significantly on polymer proportion, viscosity and to a less degree on polymer particle size (Jelena et al., 2004). So swelling and erosion studies were carried out according the method reported by Al-Taani and Tashtoush (Al-Taani and Tashtoush, 2003), to understand the swelling and erosion properties of the formulations prepared using various polymers, to understand the influence of swelling and erosion behavior on drug release and also to determine the effect of polymer viscosity (in case of various viscosity grade HPMC) on the swelling and erosion process.

Selected CR matrix tablets of ZDV prepared using various polymers were introduced into the dissolution apparatus under the standard set of conditions as specified in the release rate studies. The tablets were removed using a small basket and swollen weight of each tablet was determined. To determine matrix erosion, swollen tablets were dried in a vacuum oven at 45 °C to a constant weight. Swelling (%) and erosion (%) were calculated according to the following formula:

$$\%Swelling = S/R \times 100 \qquad \text{(I)}$$

$$\%Erosion = (T - R)/T \times 100 \qquad \text{(II)}$$

Where, S is the weight of the matrix after swelling; R is the weight of the eroded matrix; and T is the initial weight of the matrix.

5.4.3.7. Batch reproducibility

To study batch reproducibility, three batches of each formulation were manufactured and evaluated for physical and in vitro release characters as described in the previous sections. Triplicate samples from each batch were evaluated for all quality parameters discussed above.

5.4.3.8. Stability studies

Two best formulations from each polymer type and viscosity grade, showing desired in vitro drug release profiles were selected for stability studies. The selected formulations were packed in airtight cellophane packets and stored at different temperature and humidity conditions like controlled room temperature (CRT: 25 ± 2 °C/60 ± 5% RH), refrigerated condition (FT: 5 ± 2 °C) and at accelerated condition (AT: 40 ± 2 °C/75 ± 5% RH) as per the ICH guidelines (International Conference on Harmonization, 1996). Samples in triplicate were withdrawn from each batch at predetermined time intervals (0, 0.5, 1, 3 and 6 months for AT conditions; 0, 1, 3, 6, 12 and 24 months for CRT and FT conditions) and analyzed for physical characters and in vitro release behavior. The physical characters such as weight variation, hardness and friability were evaluated as per the specifications enlisted in the previous sections and compared with the results obtained at initial (zero time) values. The samples (in triplicates) were analyzed for drug content by UV spectrophotometric method of chapter 3 after suitable dilution. The percentage drug remaining to be degraded (% RTD) was plotted against time, and the degradation rate constant (K_{deg}) and time taken for 90% of the drug remaining to be degraded ($t_{90\%}$) values were calculated for all the formulations stored at different conditions.

5.5. Results and Discussion

5.5.1. Formulation of zidovudine matrix embedded CR tablets

Matrix embedded CR tablets of ZDV were prepared using various polymers alone or in combination were found to be of good quality. Wet granulation process using IPA was

found to be suitable for manufacturing quality tablets. Wet granulation method was employed instead of direct compression process because the tablets manufactured using direct compression process in most of the polymers studied, produced tablets with high friability and disintegrated immediately during the in vitro release studies. Wet granulation method produced quality matrix tablets of ZDV with good physical characters. Results obtained from the physical characterization of the prepared matrix embedded tablets are given in Table 5.2a to 5.2d.

5.5.2. Physical characteristics and drug content

The physical appearance, tablet hardness, friability, weight variation and drug content uniformity of all tablets were found to be satisfactory and reproducible as observed from the data in Table 5.2a to 5.2d. The tablets were found to have a smooth flat face with beveled edges and were found to be white to off white in color. The thickness of the tablets varied depending on the weight of tablet, bulk density of the dried granules and the compression force used. The diameter of tablets was found to be 7 and 10 mm for tablets compressed using 7 and 10 mm punches respectively. Tablet hardness was found to be good (between 3.5-12.0 kg/cm^2) depending on the compression force applied and friability was less than 0.5% (w/w). The manufactured tablets showed low weight variation and a high degree of drug content uniformity indicating that the wet granulation method was a suitable method for preparing good quality matrix tablets of ZDV.

5.5.3. In vitro drug release studies

Dissolution test is the only in vitro quality control test that is available till date, which can provide an insight to predict in vivo behavior of a formulation. The value of dissolution test as a quality control tool is significantly enhanced if the dissolution test conditions like volume of dissolution media, pH and composition of dissolution media, type of dissolution apparatus, agitation speed and temperature of bath can simulate the conditions prevalent in the gastro-intestinal tract (GIT) during the in vivo absorption process of a drug in a formulation (Skelley et al., 1990; Abuzarur et al., 1998).

In order to optimize the dissolution test conditions and to maintain proper sink conditions during the in vitro dissolution/release studies, preliminary dissolution studies were carried out for 300 mg of pure ZDV and conventional formulations of ZDV (Retrovir® tablets and Zidovir tablets) available in the market. It was observed that 100% drug released/dissolved within 30 min (Figure 5.1). In order to maintain proper sink conditions,

900 ml of dissolution media was taken in all the in vitro release studies of the developed CR matrix tablets. Since, the drug has pH independent solubility and any oral controlled release formulation with continuous release is going to have larger residence time in the intestine, phosphate buffer of pH 6.8 was selected as the dissolution medium. The bath temperature was maintained at 37.5 ± 0.5 °C. The agitation speed was set at 100 rpm to simulate the peristaltic movements in GIT. USP Type 1 dissolution apparatus (basket method) was selected based on the initial in vitro release studies carried out for different formulations prepared using various polymers being studied. It was observed that some formulations (particularly those containing HPMC) were found to float after initial hours of dissolution (0.5 to 2 h). So in order to keep the formulations immersed in the dissolution media for the complete duration of dissolution study, USP Type 1 dissolution apparatus (basket method) was selected. So the selected dissolution test conditions were able to provide good sink conditions and simulate the conditions prevalent in the GIT.

5.5.3.1. Effect of polymer proportion

(a) HPMC formulations

The kinetic parameters and MDT values for all the CR matrix tablets prepared using HPMC (various viscosity grades) alone as the retarding polymer are given in Table 5.3a to 5.3b. Release data of all the HPMC based formulations were analyzed and fitted with various kinetic equations and based on the R^2 values ($R^2 > 0.986$), best fit was observed with Ritger-Peppas' empirical equation.

A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (20, 40, 60, 80, 100 and 120%) of HPMC 1000 cPs, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.2. The profiles of matrix tablets containing HPMC 1000 cPs less than 20% produced extension of release much less than 8 h, and are not shown in the Figure 5.2.

The percent released for the first hour varied between 11-29% for all the formulations. However, in the later stages the release was found to be slower and more controlled in the tablets with higher proportion of the polymer. The release of the drug from the tablets extended as the polymer proportion was increased from 20% to 120%. The release extended till 10 h in case of 20% (H1K-1) to more than 24 h in case of 120% (H1K-6) polymer proportion. In case of H1K-6, around 93% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate

was significantly dependent on the proportion of polymer. Release rate decreased with an increase in polymer proportion. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations containing 20, 40, 60, 80, 100 and 120% polymer proportion were found to be 29.41% $h^{-0.552}$, 25.51% $h^{-0.563}$, 22.45% $h^{-0.574}$, 19.16% $h^{-0.583}$, 16.28% $h^{-0.619}$ and 12.24% $h^{-0.641}$ respectively. The $t_{50\%}$ values for these formulations were found to be 2.62, 3.30, 4.04, 5.18, 6.21 and 8.98 h. The MDT values were found to be 3.30, 3.97, 4.92, 6.36, 7.32 and 9.62 h. Statistically significant increase ($P < 0.05$, F_{crit} (5,30) = 2.53 and F_{cal} = 4079.78) was observed in the MDT values of formulations, as the polymer proportion increased from 20% to 120%.

A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (5, 10, 20, 40 and 60%) of HPMC 4000 cPs, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.3. The percent released for the first hour varied between 11-25% for all the formulations. However, in the later stages the release was found to be slower and more controlled in the tablets with higher proportion of the polymer. The release of the drug from the tablets extended as the polymer proportion was increased from 5% to 60%. The release extended till 12 h in case of 5% (H4K-1) to more than 24 h in case of 60% (H4K-5) polymer proportion. In case of H4K-5, around 91% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate was significantly dependent on the proportion of polymer. Release rate decreased with an increase in polymer proportion. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations containing 5, 10, 20, 40 and 60% polymer proportion were found to be 25.19% $h^{-0.569}$, 21.72% $h^{-0.581}$, 18.27% $h^{-0.603}$, 14.72% $h^{-0.644}$ and 12.05% $h^{-0.675}$ respectively. The $t_{50\%}$ values for these formulations were found to be 3.33, 4.19, 5.33, 6.66 and 8.23 h. The MDT values were found to be 3.94, 5.11, 6.36, 7.68, and 9.86 h. Statistically significant increase ($P < 0.05$, F_{crit} (4,25) = 2.76 and F_{cal} = 3199.38) was observed in the MDT values of formulations, as the polymer proportion increased from 5% to 60%.

A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (5, 10, 20 and 40%) of HPMC 15000 cPs, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.4. The percent released for the first hour varied between 10-21% for all the formulations. However, in the later stages the release was found to be slower and more controlled in the tablets with higher proportion of the polymer. The release of the drug from the tablets extended as the polymer proportion was increased from 5% to 40%. The release extended till 16 h in case of 5% (H15K-1) to

more than 24 h in case of 40% (H15K-4) polymer proportion. In case of H15K-4, around 90% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate was significantly dependent on the proportion of polymer. Release rate decreased with an increase in polymer proportion. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations containing 5, 10, 20 and 40% polymer proportion were found to be 23.76% $h^{-0.581}$, 18.33% $h^{-0.598}$, 14.91% $h^{-0.634}$ and 11.28% $h^{-0.693}$ respectively. The $t_{50\%}$ values for these formulations were found to be 3.61, 5.35, 6.74 and 8.39 h. The MDT values were found to be 4.33, 6.44, 7.87 and 9.90 h. Statistically significant increase ($P < 0.05$, F_{crit} (3,20) = 3.10 and F_{cal} = 3840.34) was observed in the MDT values of formulations, as the polymer proportion increased from 5% to 40%.

A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (5, 10, 20 and 40%) of HPMC 100000 cPs, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.5. The initial release for the first hour varied between 7-19% depending on polymer proportion. However, the release was found to be much slower and controlled, extending it beyond 24 h in the tablets even with 10% (HL-1) of polymer proportion. The release of the drug from the tablets extended as the polymer proportion was increased from 5% to 40%. The release extended till 20 h in case of 5% (H1L-1) to beyond 24 h in case of 40% (H1L-4) polymer proportion. In case of H1L-4, around 67% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate was significantly dependent on the proportion of polymer. Release rate decreased with an increase in polymer proportion. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations containing 5, 10, 20 and 40% polymer proportion were found to be 20.17% $h^{-0.582}$, 15.52% $h^{-0.606}$, 11.49% $h^{-0.655}$ and 8.46% $h^{-0.699}$ respectively. The $t_{50\%}$ values for these formulations were found to be 4.77, 6.89, 9.42 and 12.66 h. The MDT values were found to be 5.54, 8.36, 11.75 and 15.99 h. Statistically significant increase ($P < 0.05$, F_{crit} (3,20) = 3.10 and F_{cal} = 15641.62) was observed in the MDT values of formulations, as the polymer proportion increased from 5% to 40%.

In general, increase in polymer proportion resulted in a decrease in drug release rate from the matrix tablets prepared using HPMC, irrespective of the viscosity grade used. Similar results were reported in the literature by several research groups, when they studied the effect of polymer proportion on the release of drugs like propranolol hydrochloride, aminophylline and indomethacin from matrix tablets of HPMC (Ford et al., 1985a,b). The

release rate of the drug from the matrix tablets decreased with increase in polymer proportion because of an increase in the gel strength as well as the formation of a gel layer with a longer diffusional path. This could have caused a decrease in the effective diffusion coefficient of the drug and therefore a reduction in the drug release rate (Manuel and Leopoldo, 2004; Shah et al., 1993; Katzhendler et al., 2000)

The release rate was fastest from the formulation containing HPMC 1000 cPs at 20% w/w of the drug weight (H1K-1) with a K value of 29.41% $h^{-0.552}$ and $t_{50\%}$ value of 2.62 h. The release rate was slowest from the formulation containing HPMC 100000 cPs at 40% w/w of the drug weight (H1L-4) with a K value of 8.46% $h^{-0.699}$ and $t_{50\%}$ value of 12.66 h. The release rates of H4K-3 and H15K-2 were almost similar, and no significant difference ($P < 0.05$, F_{crit} (1,10) = 4.96) was found between K (F_{cal} = 1.87), $t_{50\%}$ (F_{cal} = 0.35), MDT (F_{cal} = 0.76), initial release in first hour (F_{cal} = 2.14) and duration of release (F_{cal} = 0.15) values of these two formulations. Similarly, no significant difference ($P < 0.05$, F_{crit} (2,15) = 3.68) was observed in the values of K (F_{cal} = 3.57), $t_{50\%}$ (F_{cal} = 2.93), MDT (F_{cal} = 3.63), initial release in first hour (F_{cal} = 3.02) and duration of release (F_{cal} = 3.17) values for formulations H1K-5, H4K-4 and H15K-3, indicating that they showed similar release profiles. These results prove that the release profiles obtained with higher proportions of low viscosity HPMC (1000, 4000 cPs) can be achieved with lower proportions of high viscosity HPMC (15000 or 100000 cPs).

The n values for all the formulations prepared using various viscosity grades of HPMC (1000 cPs, 4000 cPs. 15000 cPs and 100000 cPs) ranged from 0.541 to 0.699 indicating that the release mechanism was non-Fickian or anomalous release ($0.45 < n < 0.89$). Based on the values of n obtained, it can be inferred that the release was dependent on both drug diffusion as well as polymer relaxation.

Among HPMC 1000 cPs formulations, desired release profile, with a first hour release of 15 to 25% and release extension up to 16 to 20 h, was observed in H1K-3 and H1K-4. Formulations H4K-2 and H4K-3 have shown the desired release profiles among HPMC 4000 cPs based matrix tablets. In HPMC 15000 cPs based matrix tablets, H15K-1 and H15K-2 while in HPMC 100000 cPs matrix tablets, H1L-1 have shown the desired release profiles. So addition of free drug in the form of loading dose was not required in these formulations as they were able to produce sufficient release in the first hour to produce a target concentration.

(b) Ethylcellulose formulations

The kinetic parameters and MDT values for all the formulations prepared using EC alone as the retarding polymer are given in Table 5.3b. A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (10, 20, 40, 60, 80 and 100%) of EC, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.6.

In matrix embedded CR tablets containing EC as the retarding polymer, the percent released for the first hour varied between 14-37% depending on polymer proportion. The release of the drug from the tablets extended from 10 h in case of 10% (EC-1) to beyond 24 h as in case of 100% (EC-6) polymer. In case of EC-6, around 89% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate decreased with increase in polymer proportion. Release data of all the EC formulations were analyzed and fitted with various kinetic equations and based on the R^2 values ($R^2 > 0.988$), best fit was observed with Ritger-Peppas' empirical equation. The release rate constants, obtained from Ritger-Peppas' empirical equation, for the formulations containing 10, 20, 40, 60, 80 and 100% polymer proportion were found to be 33.78% $h^{-0.491}$, 28.74% $h^{-0.507}$, 23.47% $h^{-0.538}$, 18.22% $h^{-0.575}$, 15.43% $h^{-0.598}$ and 12.99% $h^{-0.609}$ respectively. The $t_{50\%}$ values for these formulations were found to be 2.24, 2.98, 4.08, 5.69, 7.12 and 9.39 h. The MDT values were found to be 3.12, 3.96, 5.19, 6.93, 8.44 and 10.94 h. Statistically significant increase ($P < 0.05$, F_{crit} (5,30) = 2.53 and F_{cal} = 7163.13) was observed in the MDT values of the formulations, as the polymer proportion was increased from 10% to 100%.

In matrix embedded CR tablets containing EC, the release rate decreased with increase in polymer proportion because of an increase in tortuous pathway and/or a decrease in porosity inside the matrix. The formulations prepared using EC remained intact over the drug release period and little/no swelling was observed. The *n* values for EC based formulations ranged from 0.491 to 0.609. Based on the *n* values obtained, the drug release was found to follow non-Fickian release mechanism. The drug release was chiefly dependent on drug diffusion than on polymer relaxation. This observation was in agreement with the other reported works, in which the drug release decreased from the matrix tablets of different drugs as the proportion of EC increased (Rekhi and Jambhelkar, 1995; Zabeed et al., 2002; Anjali et al., 2003).

Among EC based formulations, desired release profile, with a first hour release of 15 to 25% and release extension up to 16 to 20 h was observed in EC-3 and EC-4. In case of

EC-3, the first hour release was around 24% and the release extended up to 16 h, while in case of EC-4 the first hour release was around 19% and the release extended up to 20 h. So addition of free drug in the form of loading dose was not required in these formulations as they were able to produce sufficient release in the first hour to produce a target concentration.

(c) Carbopol 971P formulations

The kinetic parameters and MDT values for all the formulations prepared using CP alone as the retarding polymer are given in Table 5.3b. A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (5, 10, 15, 20 and 25%) of CP, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.7.

In matrix embedded CR tablets containing CP as the retarding polymer, the percent released for the first hour varied between 4-17% depending on polymer proportion. The release of the drug from the tablets extended from 12 h in case of 5% (CP-1) to beyond 24 h as in case of 25% (CP-5) polymer. In case of CP-5, around 86% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate decreased with increase in polymer proportion. Release data of all the CP formulations were analyzed and fitted with various kinetic equations and based on the R^2 values ($R^2 > 0.991$), best fit was observed with Ritger-Peppas' empirical equation. The release rate constants, obtained from Ritger-Peppas' empirical equation, for the formulations containing 5, 10, 15, 20 and 25% polymer proportion were found to be 18.57% $h^{-0.750}$, 13.62% $h^{-0.762}$, 10.38% $h^{-0.793}$, 7.62% $h^{-0.834}$ and 5.33% $h^{-0.903}$ respectively. The $t_{50\%}$ values for these formulations were found to be 3.68, 5.51, 7.26, 9.53 and 11.91 h. The MDT values were found to be 4.65, 5.97, 7.83, 10.03 and 12.75 h. Statistically significant increase ($P < 0.05$, F_{crit} (4,25) = 2.76 and F_{cal} = 8315.22) was observed in the MDT values of the formulations, as the polymer proportion was increased from 5% to 25%.

Formulations containing CP were found to undergo rapid swelling process and form rigid gel like structures during the process of dissolution (Khan and Zhu, 1999; Marcos et al., 1996). The n values for CP based formulations ranged from 0.750 to 0.903. The release mechanism was found to change from non-Fickian release to Super Case II release as the proportion of polymer was increased from 5% to 25%, indicating that polymer relaxation had a greater influence on drug release mechanism than diffusion of drug through the matrix. At higher proportion of the polymer (25%), the drug release was totally based on

polymer relaxation. The initial release (first 1 h) was found to be less than 17% in all the CP formulations. This was due to the rapid swelling process of the CP matrix and formation of a gel structure that prevented the initial release of the drug through the pores present in the matrix gel structure. Similar results were obtained when Jelena et al. have studied the effect of proportion of CP on the release of paracetamol from matrix based tablets (Jelena et al., 2004).

(d) Sodium carboxy methylcellulose formulations

The kinetic parameters and MDT values for all the formulations prepared using Na CMC alone as the retarding polymer are given in Table 5.3c. A plot of cumulative percent drug released versus time for matrix embedded CR tablets of ZDV prepared using different proportions (10, 20, 40, 60 and 80%) of Na CMC, with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.8.

In matrix embedded CR tablets containing Na CMC as the retarding polymer, the percent released for the first hour varied between 5-16% depending on polymer proportion. The release of the drug from the tablets extended from 12 h in case of 10% (Na CMC-1) to beyond 24 h as in case of 80% (Na CMC-5) polymer. In case of Na CMC-5, around 85% drug release was observed at 24 h of dissolution because of the high polymer proportion used in the formulation. The release rate decreased with increase in polymer proportion. Release data of all the CP formulations were analyzed and fitted with various kinetic equations and based on the R^2 values ($R^2 > 0.989$), best fit was observed with Ritger-Peppas' empirical equation. The release rate constants, obtained from Ritger-Peppas' empirical equation, for the formulations containing 10, 20, 40, 60 and 80% polymer proportion were found to be 14.58% $h^{-0.786}$, 12.46% $h^{-0.802}$, 10.04% $h^{-0.817}$, 8.12% $h^{-0.839}$ and 6.19% $h^{-0.849}$ respectively. The $t_{50\%}$ values for these formulations were found to be 4.79, 5.65, 7.14, 8.73 and 11.14 h. The MDT values were found to be 5.03, 6.01, 7.33, 8.99 and 11.63 h. Statistically significant increase ($P < 0.05$, F_{crit} (4,25) = 2.76 and F_{cal} = 5591.82) was observed in the MDT values of the formulations, as the polymer proportion was increased from 10% to 80%.

The n values for Na CMC based formulations ranged from 0.786 to 0.849 indicating that the release mechanism was non-Fickian or anomalous release ($0.45 < n < 0.89$). Based on the values of n obtained, it can be inferred that the release was dependent on both drug diffusion as well as polymer relaxation, but more so on polymer relaxation as the values were more towards higher values (0.89). Similar results were obtained when some research

groups have studied the effect of proportion of Na CMC on the release of drugs like lithium carbonate, metronidazole and diltiazem from matrix based tablets (Ranga et al., 1988; Amal et al., 2002; Chowdary et al., 2003; Emami et al., 2004).

The percent released in the first hour was found to be less than 16% in all the Na CMC formulations. This was due to the quick swelling process of the Na CMC matrix and formation of a gel structure that prevented the initial release of the drug through the pores present in the matrix gel structure. But the drug release was found to higher from 2 h to 8 h as the matrix porosity was increased rapidly during this period. However the release was found to be slightly more controlled in the later stages due to no further change in the porosity and increase in the diffusional path length.

5.5.3.2. Effect of viscosity of HPMC

The effect of viscosity of HPMC on the drug release was studied by comparing the dissolution profiles of matrix tablets of ZDV prepared using same proportion of polymer and same compression force, but with different viscosity grades. The effect of viscosity of HPMC on the drug release from formulations containing same proportion of polymer (20% w/w of the drug weight), with hardness 7.0-8.0 kg/cm^2 is shown in Figure 5.9. As the viscosity of HPMC was increased from 1000 cPs (H1K-1) to 100000 cPs (H1L-3) the release rate extended from 10 h to beyond 24 h; the values of K decreased from 29.41% $h^{-0.552}$ to 11.49% $h^{-0.655}$; and the values of $t_{50\%}$ increased from 2.62 h to 9.42 h. In case of H1L-3, around 81% drug release was observed at 24 h of dissolution. The MDT values increased significantly ($P < 0.05$, F_{crit} (3,20) = 3.10 and F_{cal} = 7360.95) with increase in polymer viscosity. This observation was in agreement with other reported works (Gao et al., 1996; Kim and Fassihi, 1997; Eyjolfsson, 1999). The release rate was faster with lower viscosity grades of HPMC probably due to lesser polymer entanglement and lesser gel strength and also larger effective molecular diffusional area at lower viscosity as compared to higher viscosity grades of HPMC (Kim and Fassihi, 1997). The values of n for H1K-1, H4K-3, H15K-3 and H1L-3 were found to be 0.552, 0.603, 0.634 and 0.655 respectively. The values of n increased as the viscosity of polymer was increased. So, it can be inferred that the influence of polymer relaxation on the mechanism of drug release increased while that of drug diffusion on mechanism of drug release decreased as the viscosity of polymer increased.

It was observed from the swelling (Figure 5.10) and erosion (Figure 5.11) studies that the % swelling and % erosion of the matrix tablets was totally dependent on the

viscosity of the polymer used. The % swelling increased with increase in polymer viscosity, while % erosion decreased with increase in polymer viscosity. This was because higher viscosity grades HPMC have higher and faster water absorption capacities and tend to swell rapidly than compared to the lower viscosity grades (Lee and Peppas, 1987; Ju et al., 1995; Katzhendler, et al., 2000). Moreover the matrix formed by higher viscosity grades HPMC would have more gel strength than the one formed by lower viscosity grades because of which the erosion would be lesser. Due to these reasons the diffusional path length increased and the diffusion coefficient of the drug through the matrix decreased as the viscosity grade of HPMC was increased (Nicole and Owen, 2004).

5.5.3.3. Effect of type of polymer

The effect of type of polymer on the drug release was studied by comparing the dissolution profiles of matrix tablets of ZDV prepared using different polymers (EC, Na CMC, HPMC 4000 cPs, and CP), but with same polymer proportion and compression force. The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 20% of EC, Na CMC, HPMC 4000 cPs, and CP separately with hardness 7.0-8.0 kg/cm^2, is shown in Figure 5.12.

The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations containing EC, Na CMC, HPMC 4000 cPs, and CP separately were found to be 28.74% $h^{-0.507}$, 12.46% $h^{-0.802}$, 18.27% $h^{-0.603}$ and 7.62% $h^{-0.834}$ respectively. The $t_{50\%}$ values for these formulations were found to be 2.98, 5.65, 5.33 and 9.53 h. The MDT values were found to be 3.96, 6.01, 6.36 and 10.03 h. Statistically significant increase ($P < 0.05$, F_{crit} (3,20) = 3.10 and F_{cal} = 4543.04) was observed in the MDT values of the formulations. The release rate was fastest from formulations containing EC with a K value of 28.74% $h^{-0.507}$ and $t_{50\%}$ value of 2.98 h. The release rate was slowest from formulations containing CP with a K value of 7.62% $h^{-0.834}$ and $t_{50\%}$ value of 9.53 h.

The values of n for the formulations containing EC, Na CMC, HPMC 4000 cPs, and CP separately were found to be 0.507, 0.802, 0.603 and 0.834 respectively. Based on the values n, it can be inferred that the mechanism of drug release from each of the polymers used was different. The formulations prepared using EC recovered at the end of dissolution process were intact with no swelling or erosion (indicating that release was controlled mainly due to diffusion of dissolved drug through pore network in polymer matrix), which was evident from the value of n being closer to 0.5. The formulations prepared using HPMC, CP and Na CMC recovered at the end of dissolution process were swollen and

formed gel like structures (indicating that release was controlled mainly due to drug diffusion and/or polymer relaxation or disentanglement). The intensity of swelling was more and the swollen mass was more firm in case of CP than compared to HPMC and Na CMC. The higher value of *n* (0.834) in case of CP was mainly due to the rapid swelling properties of the polymer.

In case of HPMC and Na CMC matrices that were recovered at the end of dissolution study, it was observed that the Na CMC matrices were soft and more eroded than compared to the HPMC matrices. The percent drug released from the Na CMC based formulations in the initial stages (till 4 h) was found to be lesser than HPMC based formulations while in the later stages (beyond 6 h), the drug release was found to be higher in Na CMC formulations than compared to HPMC formulations. The first hour release in Na CMC and HPMC formulations were found to be 13% and 18% respectively. But the release extended upto 16 h in case of Na CMC while it was extended upto 20 h in case of HPMC. Moreover it was observed from the swelling (Figure 5.13) and erosion (Figure 5.14) studies that Na CMC matrices swell more rapidly than compared to HPMC matrices in the initial stages (till 6 h) while they undergo rapid erosion process beyond 6 h due to the lesser gel strength of the matrix. This explains the lesser percent release from the Na CMC based formulations in the initial stages and faster release in the later stages. So, in case of Na CMC formulations, polymer relaxation or disentanglement played a greater role in the drug release than compared to drug diffusion (n = 0.802 for Na CMC) while drug diffusion had more dominant effect than polymer relaxation or disentanglement in HPMC formulations (n = 0.603 for HPMC) in the drug release.

The release rate was fastest from formulations containing EC when compared to HPMC or CP or Na CMC, because of its low viscosity and smaller diffusional path of the drug inside the matrix due to its lesser swelling properties. In formulations containing CP the release of the drug was more controlled and retarded because of its faster hydration/swelling, longer diffusional path length, higher strength and rigidity of the gel as compared to HPMC or Na CMC, which forms a less rigid gel structure which can erode over the dissolution period.

5.5.3.4. Effect of combination of HPMC and EC

The effect of combination of HPMC 1000 cPs and EC on the drug release was studied by comparing the dissolution profiles of matrix tablets of ZDV prepared using 100% HPMC 1000 cPs and 100% EC alone with the release profiles of formulations prepared

using various combinations of HPMC and EC (25/75, 50/50 and 75/25). The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 100% HPMC 1000 cPs and 100% EC alone and various combinations of HPMC 1000 cPs and EC (HPMC 1000 cPs/EC - 25/75, 50/50 and 75/25), is shown in Figure 5.15.

The release rate constants, obtained from Ritger-Peppas' empirical equation, for the formulations containing 100% HPMC 1000 cPs, 100% EC, 25/75 HPMC 1000cPs/EC, 50/50 HPMC 1000cPs/EC and 75/25 HPMC 1000cPs/EC were found to be 16.28% $h^{-0.619}$, 12.99% $h^{-0.610}$, 13.69% $h^{-0.607}$, 14.54% $h^{-0.616}$ and 15.33% $h^{-0.619}$ respectively. The $t_{50\%}$ values for these formulations were found to be 6.21, 9.39, 8.43, 7.38 and 6.69 h. The MDT values were found to be 7.32, 10.94, 10.07, 8.84 and 7.77 h. Statistically significant increase ($P < 0.05$, F_{crit} (4,25) = 2.76 and F_{cal} = 2361.22) was observed in the MDT values of the formulations. The release rate was fastest from formulations containing 100% HPMC 1000 cPs with a K value of 16.28% $h^{-0.614}$ and $t_{50\%}$ value of 6.21 h. The release rate was slowest from formulations containing 100% EC with a K value of 12.99% $h^{-0.610}$ and $t_{50\%}$ value of 10.94 h.

In formulations containing the combination of HPMC 1000 cPs and EC, the release rate was found to decrease with increase in the proportion of EC in the formulations. The increase in the proportion of EC has decreased the diffusion of the dissolution media into the matrix thereby reducing the release rate from the formulations. The values of n were found to decrease with increase in proportion of EC in the formulations containing combination of HPMC 1000 cPs and EC, because EC being a hydrophobic polymer did not undergo any swelling or erosion during the process of dissolution. So as the proportion of EC was increased, the effect of drug diffusion on the release mechanism was increased thereby reducing the values of n. Similar results were reported in the literature by several research groups when they studied the effect of combination of HPMC and EC on the release of drugs like atenolol and celecoxib from matrix tablets (Lotfipour et al., 2004; Sajeev et al., 2006).

5.5.3.5. Effect of compression force

Several authors have reported the significance and influence of compression force on the hardness, apparent density and porosity of the tablet (Dahl et al., 1990; Lui et al., 1995). Increase in the compression force increases the hardness and the apparent density of matrix tablet, thereby reducing the matrix porosity in the tablet (Hiremath and Saha, 2004).

The relationship between pressure-density was reported to be dependent on material, compression speed, size and shape of the tooling (York, 1979).

(a) HPMC formulations

The effect of compression force on the release of drugs like promethazine and propranolol hydrochloride from matrix tablets prepared using HPMC of different viscosity grades was studied by Ford et al. (Ford et al., 1985a,b). It was reported that the drug release rate from the HPMC matrix tablets decreased with increase in compression force and the effect of compression force is more pronounced in lower viscosity grade HPMC than compared to higher viscosity grade HPMC, because lower viscosity grade HPMC polymers deform more readily to fill interparticulate voids than higher viscosity grade HPMC polymers. This difference in the compressibility/deformity of different viscosity grades of HPMC was responsible for the difference in the porosity and thereby the drug release obtained for the HPMC matrix tablets when compressed at different hardness levels.

The effect of compression force on the drug release was studied by preparing tablets using same polymer proportion and viscosity but with different compression forces to get tablets with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2. The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 20% of HPMC 4000 cPs with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2, is shown in Figure 5.17. The release rate decreased with increase in compression force. Statistically significant difference was observed in the MDT values ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 2171.39) of the formulations prepared using different compression forces. The release of the drug from formulations prepared with less compression force (H4K-3:3.5-4.5) was found to be significantly much faster ($P < 0.05$) (K value 25.05% $h^{-0.568}$; $t_{50\%}$ value 3.37 h) than compared to formulations prepared with higher compression forces (K values are 18.27% $h^{-0.603}$ and 15.80% $h^{-0.622}$ for hardness 7.0-8.0 kg/cm^2 (H4K-3:7-8) and 11.0-12.0 kg/cm^2 (H4K-3:11-12) respectively; $t_{50\%}$ values are 5.33 h and 6.38 h for hardness 7.0-8.0 kg/cm^2 and 11.0-12.0 kg/cm^2 respectively). The effect of compression force on the release rate was found to be more pronounced at lesser compression forces than at higher compression forces. Similar results were obtained by another research group, when they studied the effect of compression force on drug release from binary polymer matrix systems. The drug release was found to be faster at less compression forces than at higher because of the relatively larger matrix porosity of the

tablet, which allowed greater penetration of dissolution fluid into the matrix, thus enhancing polymer disentanglement and drug dissolution (Kim and Fassihi, 1997).

Compression force was found to have no effect on the release mechanism as the values of n varied from 0.568 to 0.622, indicating that release mechanism still followed anomalous, non-Fickian diffusion, which is in agreement with earlier reported works (Velasco et al., 1999). Similarly, compression force was found to have no effect on the drug release mechanism and the drug release followed non-Fickian diffusion from formulations prepared using HPMC 1000 cPs (Figure 5.16) (n varied from 0.567 to 0.618 from formulation prepared using 80% HPMC 1000 cPs as the compression force was increased from 3.5-4.5 kg/cm^2 to 11.0-12.0 kg/cm^2), HPMC 15000 cPs (Figure 5.18) (n varied from 0.560 to 0.646 from formulation prepared using 10% HPMC 15000 cPs as the compression force was increased from 3.5-4.5 kg/cm^2 to 11.0-12.0 kg/cm^2) as well as HPMC 100000 cPs (Figure 5.19) (n varied from 0.541 to 0.614 from formulation containing 5% HPMC 100000 cPs as the compression force was increased from 3.5-4.5 kg/cm^2 to 11.0-12.0 kg/cm^2). Based on the above results obtained, it can be inferred that compression force had no effect on the drug release mechanism irrespective of the viscosity of HPMC used in the CR matrix tablets.

(b) Ethylcellulose formulations

The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 60% of EC with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2, is shown in Figure 5.20. The release rate decreased with increase in compression force from EC matrix tablets. The value of n varied from 0.562 to 0.590 in formulations containing EC, indicating that the release mechanism was unaffected with the increase in compression force. Release rate was found to decrease with increase in compression forces because of increase in hardness and apparent density of the tablet thereby a reduction in porosity and/or increase in tortuosity (Katikaneni et al., 1995; Anjali et al., 2003). Since the drug release from EC based matrix tablets was primarily dependent on diffusion process, the reduction in porosity of the EC matrix, with increase in compression force, had decreased the diffusion of the drug and thereby the drug release.

The release of the drug from EC based formulations prepared with less compression force (Hardness 3.5-4.5 kg/cm^2) was found to be significantly much faster (K value 22.52 h$^{-0.561}$; $t_{50\%}$ value 4.13 h) than compared to EC based formulations prepared with higher compression forces (K values are 18.22% h$^{-0.575}$ and 16.33% h$^{-0.590}$ for hardness 7.0-8.0

kg/cm^2 and 11.0-12.0 kg/cm^2 respectively; $t_{50\%}$ values are 5.69 h and 6.65 h for hardness 7.0-8.0 kg/cm^2 and 11.0-12.0 kg/cm^2 respectively). Significant difference was observed in the release profiles of tablets compressed with different hardness till 7.0-8.0 kg/cm^2, but less significant difference was observed in the release profiles of tablets with different hardness beyond 7.0-8.0 kg/cm^2. This could be because of significant decrease in the porosity of the matrix with increase in hardness from 3.5-4.5 kg/cm^2 to 7.0-8.0 kg/cm^2, but beyond hardness level of 7.0-8.0 kg/cm^2 there was no significant change in the porosity of the matrix.

(c) Carbopol 971P formulations

The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 15% of CP with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2, is shown in Figure 5.21. The release rate decreased with increase in compression force from CP matrix tablets. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2 were found to be 15.98% $h^{-0.754}$, 10.38% $h^{-0.793}$ and 8.46% $h^{-0.822}$ respectively. The $t_{50\%}$ values for these formulations were found to be 4.53, 7.26 and 8.67 h. The MDT values were found to be 4.96, 7.83 and 9.27 h. Statistically significant increase ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 3290.49) was observed in the MDT values of the formulations. The value of n varied from 0.754 to 0.822 in formulations containing CP, indicating that the release mechanism still followed non-Fickian release and was unaffected with the increase in compression force. These results were in agreement with the work reported by Huang and Schwartz, in which they studied the effect of compression force on the drug release from matrix tablets, prepared using various carbomer polymers (Huang and Schwartz, 1995).

(d) Sodium carboxy methylcellulose formulations

The in vitro drug release profiles of matrix embedded CR tablets of ZDV, in pH 6.8 phosphate buffer, prepared using 40% of Na CMC with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2, is shown in Figure 5.22. The release rate decreased with increase in compression force from Na CMC matrix tablets. The release rate constants, obtained from Ritger-Peppas empirical equation, for the formulations with different hardness levels, 3.5-4.5, 7.0-8.0 and 11.0-12.0 kg/cm^2 were found to be 13.69% $h^{-0.799}$, 10.04% $h^{-0.817}$ and 8.52% $h^{-0.825}$ respectively. The $t_{50\%}$ values for these formulations were

found to be 5.05, 7.14 and 8.54 h. The MDT values were found to be 5.75, 7.33 and 9.07 h. Statistically significant increase ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 1943.93) was observed in the MDT values of the formulations. The value of n varied from 0.799 to 0.825 in formulations containing Na CMC, indicating that the release mechanism still followed non-Fickian release and was unaffected with the increase in compression force. Similar results were reported in the literature by some research groups when they studied the effect of compression force on the release of drugs like centperazine and losartan potassium from matrix tablets prepared using Na CMC (Baveja and Ranga, 1986; Aithal et al., 1992).

5.5.3.6. Effect of dissolution media

The effect of dissolution media (pH 1.2, 4.5 and 6.8) on the drug release from formulations prepared using 40% HPMC (4000 cPs), 80% EC, 20% CP and 40% Na CMC separately, compressed with hardness of 7.0-8.0 kg/cm^2, are shown in Figure 5.23, 5.24, 5.25 and 5.26 respectively. No significant difference was observed in the drug release from formulations containing HPMC in different dissolution media based on the MDT and f_2 values obtained (Table 5.3d).

No statistically significant difference ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 3.03) was observed in the MDT values for formulations containing 40% HPMC in different dissolution media. Similarly no statistically significant difference ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 3.51) was observed in the MDT values for formulations containing 80% EC in different dissolution media. The f_2 values determined by comparing drug release profiles in pH 1.2 with pH 6.8, pH 4.5 with pH 6.8 and pH 1.2 with 4.5 were found to be greater than 70, 83 and 82 respectively for HPMC based formulations; 75, 85 and 84 respectively for EC based formulations.

Statistically significant difference was observed in the drug release from formulations containing CP ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 7469.67) and Na CMC ($P < 0.05$, F_{crit} (2,15) = 3.68 and F_{cal} = 1742.68). In case of CP formulations, the drug release was fastest in pH 1.2 with a K value of 20.21% h$^{-0.661}$ and $t_{50\%}$ value of 3.90 h. The drug release was slowest in pH 6.8 with a K value of 7.62% h$^{-0.834}$ and $t_{50\%}$ value of 9.53 h. The values of n for CP formulations in pH 1.2 and 4.5 were found to be 0.661 and 0.750 respectively, indicating that the mechanism of drug release from the formulations in pH 1.2 and 4.5 was different compared to pH 6.8 (n value 0.834). The order of swelling for CP formulations recovered at different time points during the study was found to be pH 6.8 > pH 4.5 > pH 1.2.

In case of Na CMC formulations, the release rate constants, obtained from Ritger-Peppas empirical equation, in pH 1.2, 4.5 and 6.8 were found to be 17.16% $h^{-0.531}$, 9.15% $h^{-0.783}$ and 10.04% $h^{-0.817}$ respectively. The $t_{50\%}$ values for these formulations were found to be 7.48, 8.74 and 7.14 h. The MDT values were found to be 10.33, 9.32 and 7.33 h. The drug release was fastest in pH 6.8 with a *K* value of 10.04% $h^{-0.817}$ and MDT value of 7.33 h and the release was extended upto 20 h (100% drug release at 24 h). The drug release was slowest in pH 1.2 with a *K* value of 17.16% $h^{-0.531}$ and MDT value of 10.33 h and the release was extended to beyond 24 h (only 85% drug release at 24 h). The values of *n* for Na CMC formulations in pH 1.2, 4.5 and 6.8 were found to be 0.531, 0.783 and 0.817 respectively. The Na CMC formulations recovered at different time points during the study from pH 1.2 were found to have less % swelling than compared to pH 4.5 than compared to pH 6.8. While the % erosion for the formulations recovered was more in pH 6.8 than compared to pH 4.5 than compared to pH 1.2.

Whatever the possible difference in dissolution profiles of ZDV CR matrix tablets due to difference in dissolution media that would be observed, it should be possibly due to differences in polymer characteristics in different media than due to the drug. This is because ZDV has pH independent solubility (Keith, 2000). No significant difference was observed in the dissolution profiles of formulations prepared using HPMC as well as EC in different dissolution media. Because HPMC, a cellulose derivative with methoxyl and hydroxypropyl substituents on a β-*o*-glucopyranosyl ring backbone, was very resistant to changes in pH or ionic content of the medium. At pH values from 2 to 13, HPMC was relatively stable and the swelling or erosion properties of HPMC were unaffected by the pH changes in dissolution media (Marcos et al., 1996). So the diffusional path length and porosity of the matrix were same in all the pH media, hence the release rate was found to be same. Similarly EC, a cellulose derivative with ethoxyl substitution on anhydroglucose ring backbone, was insoluble in water and its release properties were less affected by the pH changes (Atsuko et al., 2006). So for a drug like ZDV, which has pH independent solubility, release profiles from either HPMC or EC matrices were found to be independent of pH changes in dissolution media.

The difference in the dissolution profiles of CP based formulations was due to the difference in the ionization of CP in different dissolution media because of which there was a difference in swelling, gel strength and diffusional path length of the matrix. The carboxylic groups that make up the carbomer backbone (pK_a = 6.0) ionized very little in acidic media and polymer chain repulsion, by the negative charges of carboxylic groups,

was at minimum (Maichel et al., 2001). So the swelling and diffusional path length of the CP matrix was lesser in pH 1.2. As the pH of media increased, the ionization of carboxylic groups was increased and thereby the swelling and diffusional path length of the CP matrix increased. Since the diffusional path length was increased, the dissolution rate decreased. Therefore, the drug release was fastest in pH 1.2 and slowest in pH 6.8. The values of *n* for CP formulations were found to be lesser in lower pH conditions because the effect of drug diffusion was more on the drug release mechanism in lower pH conditions than at higher pH conditions.

In case of Na CMC based formulation, difference in the dissolution profiles was due to the difference in solubility of Na CMC and ionization of the carboxymethyl (CH_2COO^-) groups, present on the β-glucopyranose units in the polymer chain, in different dissolution media because of which there was a difference in swelling, gel strength, diffusional path length and erosion of the matrix. The carboxymethyl groups present on the Na CMC polymeric chain ($pK_a = 3.0$) ionized very little in lower pH conditions as compared to pH 4.5 or pH 6.8 and polymer chain repulsion, by the negative charges of carboxymethyl groups, was at minimum (Buri and Doelkar, 1980; Aqualon®, 1999). The solubility of Na CMC in pH 1.2 was less and so the erosion of the matrix in pH 1.2 was minimum than compared to higher pH conditions where the solubility of Na CMC was high. So in pH 1.2, the formulations were found to have minimal swelling and the diffusional path length was lesser in matrix because of which the drug release was faster in pH 1.2 in the initial hours. But the release was more controlled and extended for longer hours in pH 1.2 because of the lesser erosion of the matrix in pH 1.2. At higher pH conditions, the ionization of carboxymethyl groups was increased and thereby the swelling and diffusional path length of the Na CMC matrix increased. Since the diffusional path length was increased, the percent drug release for the initial hours was lesser in higher pH conditions. Since the solubility of Na CMC was more in higher pH conditions and as more and more water was imbibed in to the matrix, the matrix was found to undergo more erosion and drug release rate was higher at later stages (Conti et al., 2007a,b).

5.5.3.7. Effect of agitation speed

The effect of agitation speed on the *in vitro* release profiles of drug from formulations prepared using 40% HPMC (4000 cPs), 60% EC, 20% CP and 40% Na CMC separately with similar hardness (7.0-8.0 kg/cm^2), in 900 ml of pH 6.8 phosphate buffer are shown in Figure 5.27, 5.28, 5.29 and 5.30 respectively. No significant difference was

observed in the release rate with decrease in agitation speed from 100 rpm to 50 rpm from formulations containing EC as indicated by MDT values ($P < 0.05$, F_{crit} (1,10) = 4.96 and F_{cal} = 0.19) and f_2 values ($f_2 > 84$). Statistically significant decrease was observed in the release rate with a decrease in agitation speed from 100 rpm to 50 rpm in case of HPMC ($P < 0.05$, F_{crit} (1,10) = 4.96 and F_{cal} = 1529.02), CP ($P < 0.05$, F_{crit} (1,10) = 4.96 and F_{cal} = 249.46) and Na CMC ($P < 0.05$, F_{crit} (1,10) = 4.96 and F_{cal} = 1664.77) based formulations. The value of K decreased from 14.78% $h^{-0.646}$ to 11.23% $h^{-0.700}$ and the value of $t_{50\%}$ increased from 6.59 h to 8.43 h in case of HPMC; value of K decreased from 7.62% $h^{-0.834}$ to 4.62% $h^{-0.966}$ and the value of $t_{50\%}$ increased from 9.53 h to 11.76 h in case of CP formulations; and value of K decreased from 10.04% $h^{-0.817}$ to 8.17% $h^{-0.822}$ and the value of $t_{50\%}$ increased from 7.14 h to 9.46 h in case of Na CMC formulations, as the agitation speed decreased from 100 to 50 rpm (Table 5.3d).

The decrease in release rate with decrease in agitation speed in HPMC, CP and Na CMC based formulations was mainly due to decrease in the attrition of matrix structure formed by such swellable polymers at the swelling/dissolution front. So the gel structure that could be formed by HPMC or CP or Na CMC during the process of dissolution would swell and /or erode quickly at 100 rpm compared to 50 rpm because of which the release of drug due to diffusion and/or erosion was faster at 100 rpm than 50 rpm (Kim and Fassihi, 1997). Whereas in case of EC, no significant difference was observed in release rates because it is insoluble in water and does not undergo swelling or erosion processes. Moreover the release of drug from EC matrices is mainly due to diffusion of the drug through the pores of the matrix. The drug diffusion process was unaffected by the change in the agitation speed as the drug has good solubility in the dissolution medium.

5.5.3.8. In vitro release studies of selected formulations containing 100 mg of zidovudine

The in vitro drug release profiles of some selected matrix embedded CR tablets containing 100 mg of ZDV, in pH 6.8 phosphate buffer, prepared using HPMC 1000 cPs and EC alone and in combination and compressed using 7 mm diameter punches with hardness of 7.0-8.0 kg/cm^2, is shown in Figure 5.31. The kinetic parameters and MDT values are given Table 5.3e.

In case of formulations containing 20% and 40% of HPMC 1000 cPs, the K values were found to be 33.05% $h^{-0.544}$ and 29.41% $h^{-0.558}$ respectively. The $t_{50\%}$ values were 2.14 and 2.59 h. Statistically significant difference was observed in the MDT values ($P < 0.05$,

F_{crit} (1,10) = 4.96 and F_{cal} = 73.13) of the formulations containing 20% and 40% of HPMC 1000 cPs. The release extended from 8 to 10 h as the polymer proportion was increased from 20 to 40%. The values of n for 20% and 40% of HPMC 1000 cPs were found to be 0.544 and 0.558, indicating that the drug release was found to follow non-Fickian or anomalous release mechanism. Based on these results and those obtained from the in vitro studies of formulations containing 300 mg of ZDV prepared using 20 and 40% of HPMC 1000 cPs, it can be inferred that the drug release mechanism was unaffected by the compression weight and diameter of the punches used.

In case of formulations containing 20% and 40% of EC, the K values were found to be 30.72% $h^{-0.511}$ and 28.42% $h^{-0.520}$ respectively. The $t_{50\%}$ values were 2.58 and 2.89 h. Statistically significant difference was observed in the MDT values ($P < 0.05$, F_{crit} (1,10) = 4.96 and F_{cal} = 145.35) of the formulations containing 20% and 40% of EC. The release extended from 10 to beyond 12 h (96% drug release was observed at 12 h) as the polymer proportion was increased from 20 to 40%. The values of n for 20% and 40% of EC were found to be 0.511 and 0.520, indicating that the drug release was found to be influence by drug diffusion than polymer relaxation. The drug release mechanism observed for matrix tablets 100 mg of ZDV prepared using 20 and 40% EC and compressed using 7 mm diameter punches was similar to that of the tablets containing 300 mg of ZDV prepared using 20 and 40% EC and compressed using 10 mm diameter punches.

Release rate of the drug from formulations containing combination of HPMC 1000 cPs and EC at 10/10 proportion was found to be lesser than that of formulations containing 20% HPMC 1000 cPs alone but higher than that of formulations containing 20% EC alone. Similar results were obtained for the release rate of drug from formulations containing combination of HPMC 1000 cPs and EC at 20/20 proportion when compared to formulations containing 40% HPMC and 40% EC alone.

It was observed that substituting a part of HPMC 1000 cPs proportion with EC in the formulations containing combination of HPMC 1000 cPs and EC, the release rate decreased. These results were in agreement with that of the results obtained with formulations containing 300 mg of ZDV prepared using combination of HPMC 1000 cPs and EC and compressed using 10 mm diameter punches.

5.5.4. Swelling and erosion studies

(a) Formulations prepared using various viscosity grades HPMC

The results obtained from the swelling and erosion studies of CR matrix tablets of ZDV prepared using HPMC (20%) of various viscosity grades are given in Figure 5.10 and Figure 5.11 respectively. It was observed from the swelling and erosion studies that the % swelling and % erosion of the matrix tablets was totally dependent on the viscosity of the polymer used. The % swelling increased with increase in polymer viscosity, while % erosion decreased with increase in polymer viscosity. This was because higher viscosity grades HPMC have higher and faster water absorption capacities and tend to swell rapidly than compared to the lower viscosity grades (Lee et al., 1999; Katzhendler et al., 2000). Moreover the matrix formed by higher viscosity grades HPMC would have more gel strength than the one formed by lower viscosity grades because of which the erosion would be lesser. Due to these reasons the diffusional path length increased and the diffusion coefficient of the drug through the matrix decreased as the viscosity grade of HPMC was increased. The results obtained from these swelling and erosion studies further support the data obtained in effect of viscosity of HPMC on drug release studies, where it was observed that for the same proportion of polymer, the drug release rate decreased with increase in viscosity of HPMC used in the formulation.

(b) Formulations prepared using different types of polymer

The results obtained from the swelling and erosion studies of CR matrix tablets of ZDV prepared using similar proportion (20%) of HPMC (4000 cPs), Na CMC and CP are given in Figure 5.13 and Figure 5.14 respectively. No significant change was observed in the % swelling and % erosion of the EC based formulations for the entire duration of study and hence the data was not included in the figures. In CP based formulations % swelling was found to higher and the % erosion was found to be lesser than compared to HPMC or Na CMC formulations for the entire duration of study. In case of Na CMC formulations, % swelling was higher in the initial hours than compared to HPMC formulations. This was the reason for the higher percent drug release in the first hour in HPMC (18% in 1 h) formulations than compared to Na CMC (13% in 1 h) formulations. But as the time progressed (beyond 6 h), the increase in % swelling was increasing with time in HPMC formulations while the % swelling was found to decrease in Na CMC formulations beyond 10 h. Moreover the % erosion was found to be higher in Na CMC formulations in later

stages (beyond 6 h) than compared to HPMC formulations because of which faster drug release (drug release extended up to 16 h) in Na CMC formulations than compared to HPMC formulations (drug release extended up to 20 h). The values of n in case of CP (0.834) and Na CMC (0.802) formulations the values of n were higher because of the rapid swelling process which played a dominant role than diffusion in the drug release mechanism. These swelling and erosion studies have provided necessary information in understanding the effect of type of polymer on drug release from CR matrix tablets.

5.5.5. Batch reproducibility

Batch to batch variability and reproducibility of the manufacturing process was studied based on the evaluation of the physical properties and release characteristics in triplicate from three batches of each of the designed formulations. Low values of standard deviation (SD) of drug content, weight variation, hardness, friability and thickness for three independently prepared batches, indicated that the manufacturing process employed was reliable and reproducible (Table 5.2a to 5.2d). No significant difference was observed in the in vitro release profiles as indicated by the low MSSR values and low SD for $t_{50\%}$ and MDT values (Table 5.3a, 5.3b, 5.3c and 5.3e), confirming excellent batch to batch reproducibility.

5.5.6. Stability studies

The results of stability studies carried out for the selected formulations at different conditions of temperature and humidity are given in Table 5.4. At refrigerated condition (5 ± 2 °C), all the selected formulations were found to be stable for the entire period of study (24 months). Hence the data is not presented in the Table 5.4 for this condition. The log percent drug remaining to be degraded (log % RTD) versus time plots for all the selected formulations were linear indicating first order kinetics. Low mean sum of square residuals (MSSR) values and high regression coefficient ($R^2 \approx 1$) further established linear relationship between log % RTD versus time (Table 5.4).

At accelerated condition, the maximum degradation rate constant for ZDV was found to be 6.15×10^{-3} $month^{-1}$ for formulations prepared using Na CMC with a $t_{90\%}$ value of 17.11 months. The minimum degradation rate constant for ZDV was found to be 4.76×10^{-3} $month^{-1}$ for formulations prepared using EC with a $t_{90\%}$ value of 22.54 months. These values were almost comparable to the degradation rate constant (5.32×10^{-3} $month^{-1}$) and $t_{90\%}$ (19.75 months) values of pure ZDV obtained from the preformulation studies

(section 4.6.3.(b) of chapter 4). All the formulations were stable for entire study duration (6 months) with no apparent change in physical characteristics and in vitro release behavior.

In the formulations stored at CRT, the maximum degradation rate constant for ZDV was found to be 2.91×10^{-3} $month^{-1}$ for formulations prepared using Na CMC with a $t_{90\%}$ value of 35.36 months. The minimum degradation rate constant for ZDV was found to be 2.45×10^{-3} $month^{-1}$ for formulations prepared using HPMC 15000 cPs with a $t_{90\%}$ value of 42.89 months. These values were almost comparable to the degradation rate constant (2.38×10^{-3} $month^{-1}$) and $t_{90\%}$ (44.24 months) values of pure ZDV obtained from the preformulation studies (section 4.6.3.(b) of chapter 4). All the formulations were stable for entire study duration (24 months) with no apparent change in physical characteristics and in vitro release behavior.

5.6. Conclusions

The designed CR matrix tablets of ZDV were found to possess good physical characteristics indicating that wet granulation technique was suitable for manufacturing good quality tablets using different polymers. Weight variation and content uniformity of all the designed formulations were found to be highly satisfactory. Acceptable values of friability, good hardness and low batch to batch variation further confirmed the suitability of the adopted method.

Release of the drug from matrix embedded CR tablets was dependent on type of polymer used. Drug release rate decreased with increase in polymer proportion. Increase in compression force was found to decrease the release rate from the designed formulations irrespective of the polymer used. Formulations prepared using HPMC and EC formulations showed pH independent drug release, while formulations prepared using CP and Na CMC showed pH dependent drug release. Agitation speed was found to effect the release rate from formulations prepared using swellable hydrophilic polymers (like HPMC, CP and Na CMC) but no significant effect was observed on formulations prepared using hydrophobic polymer like EC. The drug release was chiefly dependent on diffusion of drug through the matrix in case of EC formulations, while drug diffusion as well as polymer relaxation had an influence on drug release mechanism in case of HPMC formulations. In case of CP and Na CMC formulations, polymer relaxation had a dominating influence on drug release mechanism than drug diffusion. Designed CR matrix tablets, with pH independent drug release and a good initial release (15-25% in first hour) and extending the release up to 16-20 h, can overcome the disadvantages associated with conventional tablets of ZDV.

The designed formulations were found to be stable for at least 2 years when stored at CRT. This indicated that the excipients, process and packaging materials used were compatible with the drug. The tablet manufacturing method was relatively simple and can be easily adopted in industries on a commercial scale.

Further, to carry out the in vivo pharmacokinetic studies of the designed CR matrix tablets of ZDV in rabbits, formulations containing 20% HPMC (H1K/7mm-1) and 20% EC (EC/7mm-1) alone and combination of HPMC 1000 cPs and EC at 10/10 proportion (H1K-EC/7mm-1) containing 100 mg of ZDV compressed using 7 mm diameter punches were selected as they were able to extend the release for 8 to 10 h with good initial release.

Table 5.1a: Formulation components of designed controlled release matrix tablets (10 mm) of zidovudine prepared using HPMC 1000, 4000 and 15000 cPs

Formulation	Components in the CR matrix formulation[a]				Theoretical tablet weight (mg)
	ZDV (mg)	HPMC 1000 cPs (%)[b]	HPMC 4000 cPs (%)[b]	HPMC 15000 cPs (%)[b]	
H1K-1	300	20	-	-	374.4
H1K-2	300	40	-	-	436.8
H1K-3	300	60	-	-	499.2
H1K-4	300	80	-	-	561.6
H1K-4:3.5-4.5	300	80	-	-	561.6
H1K-4:11-12	300	80	-	-	561.6
H1K-5	300	100	-	-	624.0
H1K-6	300	120	-	-	686.4
H4K-1	300	-	5	-	327.6
H4K-2	300	-	10	-	343.2
H4K-3	300	-	20	-	374.4
H4K-3:3.5-4.5	300	-	20	-	374.4
H4K-3:11-12	300	-	20	-	374.4
H4K-4	300	-	40	-	436.8
H4K-5	300	-	60	-	499.2
H15K-1	300	-	-	5	327.6
H15K-2	300	-	-	10	343.2
H15K-2:3.5-4.5	300	-	-	10	343.2
H15K-2:11-12	300	-	-	10	343.2
H15K-3	300	-	-	20	374.4
H15K-4	300	-	-	40	436.8

[a] Also contains talc and magnesium stearate at 3% and 1% w/w of the dry granules weight respectively as manufacturing additives and isopropyl alcohol was used as binding solvent, [b] Percent w/w of the drug weight

Table 5.1b: Formulation components of designed controlled release matrix tablets (10 mm) of zidovudine prepared using HPMC 100000 cPs, EC and CP

Formulation	Components in the CR matrix formulation[a]				Theoretical tablet weight (mg)
	ZDV (mg)	HPMC 100000 cPs (%)[b]	EC (%)[b]	CP (%)[b]	
H1L-1	300	5	-	-	327.6
H1L-1:3.5-4.5	300	5	-	-	327.6
H1L-1:11-12	300	5	-	-	327.6
H1L-2	300	10	-	-	343.2
H1L-3	300	20	-	-	374.4
H1L-4	300	40	-	-	436.8
EC-1	300	-	10	-	343.2
EC-2	300	-	20	-	374.4
EC-3	300	-	40	-	436.8
EC-4	300	-	60	-	499.2
EC-4:3.5-4.5	300	-	60	-	499.2
EC-4:11-12	300	-	60	-	499.2
EC-5	300	-	80	-	561.6
EC-6	300	-	100	-	624.0
CP-1	300	-	-	5	327.6
CP-2	300	-	-	10	343.2
CP-3	300	-	-	15	358.8
CP-3:3.5-4.5	300	-	-	15	358.8
CP-3:11-12	300	-	-	15	358.8
CP-4	300	-	-	20	374.4
CP-5	300	-	-	25	390.0

[a] Also contains talc and magnesium stearate at 3% and 1% w/w of the dry granules weight respectively as manufacturing additives and isopropyl alcohol was used as binding solvent, [b] Percent w/w of the drug weight

Table 5.1c: Formulation components of designed controlled release matrix tablets (10 mm) of zidovudine prepared using Na CMC alone and combination of HPMC 1000 cPs and EC

Formulation	Components in the CR matrix formulation[a]				Theoretical tablet weight (mg)
	ZDV (mg)	Na CMC (%)[b]	HPMC 1000 cPs (%)[b]	EC (%)[b]	
Na CMC-1	300	10	-	-	343.2
Na CMC-2	300	20	-	-	374.4
Na CMC-3	300	40	-	-	436.8
Na CMC-3:3.5-4.5	300	40	-	-	436.8
Na CMC-3:11-12	300	40	-	-	436.8
Na CMC-4	300	60	-	-	499.2
Na CMC-5	300	80	-	-	561.6
H1K-EC/25:75	300	-	25	75	624.0
H1K-EC/50:50	300	-	50	50	624.0
H1K-EC/75:25	300	-	75	25	624.0

[a] Also contains talc and magnesium stearate at 3% and 1% w/w of the dry granules weight respectively as manufacturing additives and isopropyl alcohol was used as binding solvent

[b] Percent w/w of the drug weight

Table 5.1d: Formulation components of some selected controlled release matrix tablets (7 mm) of zidovudine prepared using HPMC 1000 cps, EC and combination of HPMC 1000 cPs and EC

Formulation	Components in the CR matrix formulation[a]			Theoretical tablet weight (mg)
	ZDV (mg)	HPMC 1000 cPs (%)[b]	EC (%)[b]	
H1K/7mm-1	100	20	-	124.8
H1K/7mm-2	100	40	-	145.6
EC/7mm-1	100	-	20	124.8
EC/7mm-2	100	-	40	145.6
H1K-EC/7mm-1	100	10	10	124.8
H1K-EC/7mm-2	100	20	20	145.6

[a] Also contains talc and magnesium stearate at 3% and 1% w/w of the dry granules weight respectively as manufacturing additives and isopropyl alcohol was used as binding solvent

[b] Percent w/w of the drug weight

Table 5.2a: Physical characteristics of designed controlled release matrix tablets (10 mm) of zidovudine prepared using HPMC 1000, 4000 and 15000 cPs

Formulation	Physical characteristics					
	Tablet weight (mg)[a]	Weight variation (%)[b]	Drug content (mg/tablet)[c]	Hardness (Kg/cm^2)[d]	Friability (%)	Thickness (mm)[e]
H1K-1	374.2	± 2.0	299.5 ± 1.2	7.5 ± 0.3	< 0.5	3.09 ± 0.02
H1K-2	437.6	± 1.7	300.1 ± 1.1	7.4 ± 0.4	< 0.5	3.31 ± 0.03
H1K-3	499.8	± 1.5	300.9 ± 0.8	7.6 ± 0.4	< 0.5	3.58 ± 0.02
H1K-4	562.7	± 1.1	299.8 ± 1.3	7.5 ± 0.2	< 0.5	3.81 ± 0.02
H1K-4:3.5-4.5	563.4	± 1.8	301.1 ± 1.7	3.9 ± 0.3	< 0.5	3.91 ± 0.03
H1K-4:11-12	562.5	± 1.2	300.4 ± 1.4	11.5 ± 0.4	< 0.5	3.70 ± 0.03
H1K-5	624.5	± 1.6	299.1 ± 1.3	7.4 ± 0.2	< 0.5	4.02 ± 0.03
H1K-6	687.3	± 2.3	299.7 ± 1.4	7.5 ± 0.4	< 0.5	4.21 ± 0.02
H4K-1	325.6	± 1.8	300.4 ± 1.5	7.4 ± 0.4	< 0.5	2.81 ± 0.02
H4K-2	342.7	± 0.9	298.5 ± 1.2	7.5 ± 0.3	< 0.5	2.93 ± 0.02
H4K-3	373.9	± 1.5	301.7 ± 0.9	7.6 ± 0.3	< 0.5	3.12 ± 0.03
H4K-3:3.5-4.5	374.5	± 1.3	300.1 ± 2.1	3.9 ± 0.3	< 0.5	3.24 ± 0.03
H4K-3:11-12	373.4	± 2.5	301.5 ± 0.8	11.5 ± 0.5	< 0.5	3.01 ± 0.02
H4K-4	437.6	± 1.4	299.4 ± 1.5	7.5 ± 0.4	< 0.5	3.39 ± 0.04
H4K-5	497.5	± 0.9	297.2 ± 1.9	7.3 ± 0.3	< 0.5	3.65 ± 0.03
H15K-1	327.5	± 1.6	301.4 ± 0.8	7.6 ± 0.3	< 0.5	2.83 ± 0.03
H15K-2	344.2	± 2.0	301.3 ± 0.6	7.6 ± 0.2	< 0.5	2.95 ± 0.03
H15K-2:3.5-4.5	343.7	± 1.8	299.4 ± 1.1	4.0 ± 0.3	< 0.5	3.04 ± 0.03
H15K-2:11-12	345.5	± 1.6	299.8 ± 1.4	11.4 ± 0.3	< 0.5	2.84 ± 0.02
H15K-3	376.7	± 2.1	302.6 ± 1.0	7.4 ± 0.4	< 0.5	3.14 ± 0.03
H15K-4	438.5	± 2.3	299.5 ± 0.8	7.4 ± 0.2	< 0.5	3.40 ± 0.04

[a] Mean of 20 tablets, [b] ± Maximum variation from the mean tablet weight, [c] Mean of triplicate with SD, [d] Mean of 10 tablets with SD, [e] Mean of 20 tablets with SD

Table 5.2b: Physical characteristics of designed controlled release matrix tablets (10 mm) of zidovudine prepared using HPMC 100000 cPs, EC and CP

Formulation	Physical characteristics					
	Tablet weight (mg)[a]	Weight variation (%)[b]	Drug content (mg/tablet)[c]	Hardness (Kg/cm^2)[d]	Friability (%)	Thickness (mm)[e]
H1L-1	326.4	± 2.1	299.8 ± 1.3	7.5 ± 0.3	< 0.5	2.83 ± 0.03
H1L-1:3.5-4.5	325.7	± 1.8	300.4 ± 1.5	4.0 ± 0.3	< 0.5	2.94 ± 0.02
H1L-1:11-12	326.8	± 1.6	301.5 ± 1.3	11.5 ± 0.4	< 0.5	2.72 ± 0.03
H1L-2	342.6	± 2.0	300.9 ± 1.1	7.4 ± 0.4	< 0.5	2.95 ± 0.03
H1L-3	374.3	± 2.2	300.5 ± 0.8	7.5 ± 0.2	< 0.5	3.14 ± 0.02
H1L-4	436.5	± 2.1	301.6 ± 0.4	7.6 ± 0.3	< 0.5	3.38 ± 0.03
EC-1	343.8	± 2.0	301.2 ± 1.3	7.5 ± 0.3	< 0.5	2.92 ± 0.02
EC-2	374.1	± 2.1	301.8 ± 1.6	7.6 ± 0.4	< 0.5	3.08 ± 0.02
EC-3	438.2	± 1.8	300.7 ± 1.2	7.4 ± 0.3	< 0.5	3.31 ± 0.03
EC-4	498.3	± 0.9	299.3 ± 0.7	7.6 ± 0.2	< 0.5	3.52 ± 0.03
EC-4:3.5-4.5	499.1	± 1.1	300.4 ± 1.7	4.0 ± 0.3	< 0.5	3.64 ± 0.02
EC-4:11-12	497.2	± 2.1	301.8 ± 1.2	11.4 ± 0.3	< 0.5	3.39 ± 0.02
EC-5	560.8	± 1.6	302.1 ± 2.1	7.4 ± 0.4	< 0.5	3.80 ± 0.05
EC-6	623.7	± 1.2	298.5 ± 1.4	7.5 ± 0.4	< 0.5	4.11 ± 0.04
CP-1	326.3	± 2.0	299.1 ± 0.9	7.3 ± 0.3	< 0.5	2.79 ± 0.03
CP-2	343.5	± 1.6	301.7 ± 0.8	7.6 ± 0.3	< 0.5	2.90 ± 0.02
CP-3	357.7	± 1.3	300.3 ± 1.1	7.5 ± 0.5	< 0.5	2.99 ± 0.03
CP-3:3.5-4.5	356.5	± 2.2	301.4 ± 1.9	3.9 ± 0.4	< 0.5	3.08 ± 0.02
CP-3:11-12	358.1	± 0.9	302.6 ± 1.8	11.6 ± 0.3	< 0.5	2.89 ± 0.03
CP-4	375.7	± 1.5	298.8 ± 1.7	7.6 ± 0.3	< 0.5	3.11 ± 0.04
CP-5	391.3	± 1.7	301.7 ± 1.4	7.5 ± 0.4	< 0.5	3.21 ± 0.03

[a] Mean of 20 tablets, [b] ± Maximum variation from the mean tablet weight, [c] Mean of triplicate with SD, [d] Mean of 10 tablets with SD, [e] Mean of 20 tablets with SD

Table 5.2c: Physical characteristics of designed controlled release matrix tablets (10 mm) of zidovudine prepared using Na CMC alone and combination of HPMC 1000 cPs and EC

Formulation	Physical characteristics					
	Tablet weight (mg)[a]	Weight variation (%)[b]	Drug content (mg/tablet)[c]	Hardness (Kg/cm^2)[d]	Friability (%)	Thickness (mm)[e]
Na CMC-1	344.5	± 2.1	301.2 ± 1.3	7.4 ± 0.3	< 0.5	2.92 ± 0.02
Na CMC-2	374.9	± 1.4	300.5 ± 1.6	7.5 ± 0.4	< 0.5	3.13 ± 0.02
Na CMC-3	437.2	± 2.1	300.7 ± 1.2	7.6 ± 0.3	< 0.5	3.31 ± 0.03
Na CMC-3:3.5-4.5	436.1	± 1.3	299.3 ± 0.7	3.9 ± 0.3	< 0.5	3.39 ± 0.03
Na CMC-3:11-12	437.4	± 0.8	300.4 ± 1.7	11.4 ± 0.4	< 0.5	3.22 ± 0.02
Na CMC-4	499.5	± 2.0	301.8 ± 1.2	7.3 ± 0.3	< 0.5	3.49 ± 0.02
Na CMC-5	562.3	± 1.7	301.9 ± 2.1	7.4 ± 0.4	< 0.5	3.70 ± 0.04
H1K-EC/25:75	623.4	± 1.6	300.5 ± 0.8	7.5 ± 0.4	< 0.5	4.05 ± 0.04
H1K-EC/50:50	624.7	± 1.3	301.6 ± 0.4	7.3 ± 0.3	< 0.5	4.06 ± 0.03
H1K-EC/75:25	623.5	± 0.9	301.2 ± 1.3	7.6 ± 0.3	< 0.5	4.05 ± 0.03

[a] Mean of 20 tablets, [b] ± Maximum variation from the mean tablet weight, [c] Mean of triplicate with SD, [d] Mean of 10 tablets with SD, [e] Mean of 20 tablets with SD

Table 5.2d: Physical characteristics of designed controlled release matrix tablets (7 mm) of zidovudine prepared using HPMC 1000 cPs, EC and combination of HPMC 1000 cPs and EC

Formulation	Physical characteristics					
	Tablet weight (mg)[a]	Weight variation (%)[b]	Drug content (mg/tablet)[c]	Hardness (Kg/cm^2)[d]	Friability (%)	Thickness (mm)[e]
H1K/7mm-1	124.3	± 1.3	101.4 ± 0.8	7.4 ± 0.4	< 0.5	1.15 ± 0.02
H1K/7mm-2	144.8	± 0.8	100.8 ± 1.4	7.5 ± 0.3	< 0.5	1.26 ± 0.02
EC/7mm-1	123.6	± 0.9	100.5 ± 0.9	7.6 ± 0.4	< 0.5	1.11 ± 0.01
EC/7mm-2	145.2	± 1.1	99.3 ± 1.5	7.4 ± 0.3	< 0.5	1.22 ± 0.02
H1K-EC/7mm-1	125.2	± 1.5	100.4 ± 1.1	7.5 ± 0.4	< 0.5	1.13 ± 0.02
H1K-EC/7mm-2	144.6	± 1.0	99.8 ± 1.3	7.3 ± 0.3	< 0.5	1.24 ± 0.02

[a] Mean of 20 tablets, [b] ± Maximum variation from the mean tablet weight, [c] Mean of triplicate with SD, [d] Mean of 10 tablets with SD, [e] Mean of 20 tablets with SD

Table 5.3a: Release kinetics parameters and MDT values of designed controlled release matrix tablets of zidovudine prepared using HPMC 1000, 4000 and 15000 cPs in dissolution media of pH 6.8 at 100 rpm

Formulation	MDT[a] (h)	Ritger-Peppas' empirical equation				
		K[b] (% h^{-n})	n[c]	$t_{50\%}$[d] (h)	R^2	MSSR
H1K-1	3.30	29.41	0.5515	2.62	0.9957	1.12×10^{-5}
H1K-2	3.97	25.51	0.5629	3.30	0.9897	1.04×10^{-5}
H1K-3	4.92	22.45	0.5735	4.04	0.9983	2.08×10^{-5}
H1K-4	6.36	19.16	0.5831	5.18	0.9895	1.04×10^{-5}
H1K-4:3.5-4.5	4.64	23.32	0.5669	3.84	0.9874	2.17×10^{-5}
H1K-4:11-12	7.91	15.85	0.6177	6.36	0.9986	1.42×10^{-5}
H1K-5	7.32	16.28	0.6198	6.21	0.9892	1.01×10^{-5}
H1K-6	9.62	12.24	0.6409	8.98	0.9959	1.42×10^{-5}
H4K-1	3.94	25.19	0.5689	3.33	0.9948	1.98×10^{-5}
H4K-2	5.11	21.72	0.5808	4.19	0.9886	2.33×10^{-5}
H4K-3	6.36	18.27	0.6030	5.33	0.9895	2.58×10^{-5}
H4K-3:3.5-4.5	4.24	25.05	0.5678	3.37	0.9878	3.11×10^{-5}
H4K-3:11-12	7.33	15.79	0.6216	6.38	0.9858	2.95×10^{-5}
H4K-4	7.68	14.72	0.6440	6.66	0.9911	2.93×10^{-5}
H4K-5	9.86	12.05	0.6749	8.23	0.9894	2.38×10^{-5}
H15K-1	4.33	23.76	0.5811	3.61	0.9896	1.77×10^{-5}
H15K-2	6.44	18.33	0.5979	5.35	0.9888	1.86×10^{-5}
H15K-2:3.5-4.5	5.03	22.88	0.5598	4.04	0.9986	2.05×10^{-5}
H15K-2:11-12	7.85	14.72	0.6460	6.64	0.9895	2.12×10^{-5}
H15K-3	7.87	14.91	0.6342	6.74	0.9925	2.73×10^{-5}
H15K-4	9.90	11.28	0.6929	8.39	0.9946	1.99×10^{-5}

[a] Mean of 6 tablets with maximum SD within ± 0.18 h, [b] Release rate constant for Ritger-Peppas' empirical equation
[c] Diffusional exponent indicative of release mechanism, [d] Mean of 6 tablets with maximum SD within ± 0.11 h

Table 5.3b: Release kinetics parameters and MDT values of designed controlled release matrix tablets of zidovudine using HPMC 100000 cPs, EC and CP in dissolution media of pH 6.8 at 100 rpm

Formulation	MDT[a] (h)	Ritger-Peppas' empirical equation				
		K[b] (% h^{-n})	n[c]	$t_{50\%}$[d] (h)	R^2	MSSR
H1L-1	5.54	20.17	0.5821	4.77	0.9976	1.69×10^{-5}
H1L-1:3.5-4.5	4.42	24.86	0.5412	3.67	0.9896	1.55×10^{-5}
H1L-1:11-12	7.13	16.68	0.6135	5.98	0.9969	1.72×10^{-5}
H1L-2	8.36	15.52	0.6060	6.89	0.9913	1.38×10^{-5}
H1L-3	11.75	11.49	0.6552	9.42	0.9873	2.04×10^{-5}
H1L-4	15.99	8.46	0.6991	12.66	0.9899	1.97×10^{-5}
EC-1	3.12	33.78	0.4912	2.23	0.9945	1.56×10^{-5}
EC-2	3.96	28.74	0.5071	2.98	0.9968	1.85×10^{-5}
EC-3	5.19	23.47	0.5379	4.08	0.9952	2.37×10^{-5}
EC-4	6.93	18.22	0.5752	5.69	0.9937	2.08×10^{-5}
EC-4:3.5-4.5	5.13	22.52	0.5616	4.13	0.9945	1.86×10^{-5}
EC-4:11-12	7.75	16.33	0.5901	6.65	0.9939	2.07×10^{-5}
EC-5	8.44	15.43	0.5984	7.12	0.9893	2.33×10^{-5}
EC-6	10.94	12.99	0.6099	9.39	0.9888	2.49×10^{-5}
CP-1	4.65	18.57	0.7502	3.68	0.9957	2.15×10^{-5}
CP-2	5.97	13.62	0.7621	5.51	0.9938	1.96×10^{-5}
CP-3	7.83	10.38	0.7926	7.26	0.9955	1.39×10^{-5}
CP-3:3.5-4.5	4.96	15.98	0.7542	4.53	0.9985	2.22×10^{-5}
CP-3:11-12	9.27	8.46	0.8224	8.67	0.9910	2.48×10^{-5}
CP-4	10.03	7.62	0.8340	9.53	0.9976	2.57×10^{-5}
CP-5	12.75	5.33	0.9030	11.91	0.9961	2.34×10^{-5}

[a] Mean of 6 tablets with maximum SD within ± 0.18 h, [b] Release rate constant for Ritger-Peppas' empirical equation
[c] Diffusional exponent indicative of release mechanism, [d] Mean of 6 tablets with maximum SD within ± 0.11 h

Table 5.3c: Release kinetics parameters and MDT values of designed controlled release matrix tablets of zidovudine prepared using Na CMC alone and combination of HPMC 1000 cPs and EC in dissolution media of pH 6.8 at 100 rpm

Formulation	MDT[a] (h)	Ritger-Peppas' empirical equation				
		K[b] (% h^{-n})	n[c]	$t_{50\%}$[d] (h)	R^2	MSSR
Na CMC-1	5.03	14.58	0.7862	4.79	0.9925	2.04×10^{-5}
Na CMC-2	6.01	12.46	0.8020	5.65	0.9937	2.56×10^{-5}
Na CMC-3	7.33	10.04	0.8171	7.14	0.9894	2.13×10^{-5}
Na CMC-3:3.5-4.5	5.75	13.69	0.7991	5.05	0.9934	1.99×10^{-5}
Na CMC-3:11-12	9.07	8.52	0.8250	8.54	0.9917	1.82×10^{-5}
Na CMC-4	8.99	8.12	0.8389	8.73	0.9963	2.28×10^{-5}
Na CMC-5	11.63	6.19	0.8489	11.14	0.9945	2.44×10^{-5}
H1K-EC/25:75	10.07	13.69	0.6072	8.43	0.9894	1.87×10^{-5}
H1K-EC/50:50	8.84	14.54	0.6158	7.38	0.9943	1.46×10^{-5}
H1K-EC/75:25	7.77	15.33	0.6186	6.69	0.9989	2.06×10^{-5}

[a] Mean of 6 tablets with maximum SD within ± 0.18 h, [b] Release rate constant for Ritger-Peppas' empirical equation
[c] Diffusional exponent indicative of release mechanism, [d] Mean of 6 tablets with maximum SD within ± 0.11 h

Table 5.3d: Effect of pH of dissolution media and agitation speed on drug release kinetics of selected controlled release formulations of zidovudine

Formulation	Media pH	Agitation Speed	MDT[a] (h)	Ritger-Peppas' empirical equation				Similarity factor[e] (f_2)
				K^b (% h^{-n})	n^c	$t_{50\%}$[d] (h)	R^2	
H4K-4	1.2	100	8.05	14.21	0.6494	6.94	0.9787	pH 1.2/6.8 > 70
	4.5	100	7.98	14.47	0.6509	6.73	0.9915	pH 4.5/6.8 > 83
	6.8	100	7.88	14.78	0.6460	6.59	0.9811	pH 1.2/4.5 > 82
	6.8	50	10.02	11.23	0.6998	8.43	0.9758	rpm 100/50 < 48
EC-5	1.2	100	8.61	14.26	0.6216	7.52	0.9945	pH 1.2/6.8 > 75
	4.5	100	8.56	14.40	0.6225	7.38	0.9952	pH 4.5/6.8 > 85
	6.8	100	8.49	15.43	0.5984	7.12	0.9853	pH 1.2/4.5 > 84
CP-4	1.2	100	4.45	20.21	0.6614	3.90	0.9833	pH 1.2/6.8 < 35
	4.5	100	7.63	11.79	0.7501	6.96	0.9571	pH 4.5/6.8 < 48
	6.8	100	10.03	7.62	0.8340	9.53	0.9976	pH 1.2/4.5 < 26
	6.8	50	12.42	4.62	0.9664	11.76	0.9929	rpm 100/50 < 51
Na CMC-3	1.2	100	10.33	17.16	0.5310	7.48	0.9912	pH 1.2/6.8 < 28
	4.5	100	9.32	9.15	0.7829	8.74	0.9891	pH 4.5/6.8 < 44
	6.8	100	7.33	10.04	0.8171	7.14	0.9896	pH 1.2/4.5 < 38
	6.8	50	12.42	8.17	0.8222	9.46	0.9889	rpm 100/50 < 50
EC-4	6.8	100	6.90	18.23	0.5752	5.69	0.9937	rpm 100/50 > 84
	6.8	50	6.96	16.88	0.5837	5.86	0.9951	

[a] Mean of 6 tablets with maximum SD within ± 0.18 h, [b] Release rate constant for Ritger-Peppas' empirical equation, [c] Diffusional exponent indicative of release mechanism, [d] Mean of 6 tablets with maximum SD within ± 0.11 h, [e] Mean f_2 values calculated by comparing dissolution profiles

Table 5.3e: Release kinetics parameters and MDT values of designed controlled release matrix tablets (7 mm) of zidovudine prepared using HPMC 1000 cPs, EC and combination of HPMC 1000 cPs and EC in dissolution media of pH 6.8 at 100 rpm

Formulation	MDT[a] (h)	Ritger-Peppas' empirical equation				
		K[b] (% h^{-n})	n[c]	$t_{50\%}$[d] (h)	R^2	MSSR
H1K/7mm-1	2.74	33.02	0.5435	2.14	0.9986	1.39×10^{-5}
H1K/7mm-2	3.11	29.41	0.5575	2.59	0.9948	1.67×10^{-5}
EC/7mm-1	3.43	30.72	0.5106	2.58	0.9879	1.52×10^{-5}
EC/7mm-2	3.91	28.42	0.5203	2.89	0.9963	1.64×10^{-5}
H1K-EC/7mm-1	3.07	31.94	0.5281	2.34	0.9992	1.98×10^{-5}
H1K-EC/7mm-2	3.65	29.48	0.5168	2.78	0.9885	1.36×10^{-5}

[a] Mean of 6 tablets with maximum SD within ± 0.12 h, [b] Release rate constant for Ritger-Peppas' empirical equation
[c] Diffusional exponent indicative of release mechanism, [d] Mean of 6 tablets with maximum SD within ± 0.09 h

Table 5.4: Stability data of zidovudine in selected controlled release formulations stored at controlled room temperature and accelerated temperature conditions

Sample	CRT: 25 ± 2 °C/60 ± 5% RH				AT: 40 ± 2 °C/75 ± 5% RH			
	K_{deg}[a] (x 10^{-3} $month^{-1}$)	$t_{90\%}$[b] (months)	R^2	MSSR	K_{deg}[a] (x 10^{-3} $month^{-1}$)	$t_{90\%}$[b] (months)	R^2	MSSR
H1K-3	2.57 ± 0.03	40.82	0.9976	2.07×10^{-5}	5.12 ± 0.03	20.62	0.9885	1.83×10^{-5}
H1K-4	2.54 ± 0.02	41.46	0.9896	1.84×10^{-5}	5.69 ± 0.03	18.55	0.9898	1.56×10^{-5}
H4K-2	2.63 ± 0.03	39.93	0.9887	1.45×10^{-5}	5.46 ± 0.02	19.43	0.9925	1.78×10^{-5}
H4K-3	2.67 ± 0.02	39.35	0.9952	2.09×10^{-5}	5.80 ± 0.02	18.15	0.9826	2.01×10^{-5}
H15K-1	2.45 ± 0.02	42.89	0.9917	1.52×10^{-5}	4.93 ± 0.03	21.38	0.9882	1.28×10^{-5}
H15K-2	2.60 ± 0.03	40.54	0.9924	1.63×10^{-5}	5.13 ± 0.03	20.52	0.9779	1.08×10^{-5}
H1L-1	2.76 ± 0.02	38.14	0.9796	1.28×10^{-5}	5.71 ± 0.03	18.61	0.9784	1.44×10^{-5}
H1L-2	2.62 ± 0.03	40.21	0.9879	2.15×10^{-5}	5.32 ± 0.02	19.94	0.9927	1.85×10^{-5}
EC-3	2.47 ± 0.02	42.63	0.9882	1.48×10^{-5}	4.97 ± 0.03	21.32	0.9923	2.17×10^{-5}
EC-4	2.51 ± 0.02	42.08	0.9868	1.33×10^{-5}	4.76 ± 0.02	22.54	0.9882	1.94×10^{-5}
CP-2	2.76 ± 0.03	37.96	0.9798	2.12×10^{-5}	5.82 ± 0.02	18.24	0.9914	1.57×10^{-5}
CP-3	2.71 ± 0.02	38.33	0.9892	1.29×10^{-5}	5.59 ± 0.03	18.99	0.9758	1.41×10^{-5}
Na CMC-2	2.91 ± 0.03	35.36	0.9876	2.46×10^{-5}	6.15 ± 0.03	17.11	0.9993	2.24×10^{-5}
Na CMC-3	2.82 ± 0.02	36.31	0.9781	1.93×10^{-5}	6.01 ± 0.02	17.88	0.9829	1.61×10^{-5}
H1K-EC/25-75	2.71 ± 0.03	38.32	0.9893	1.27×10^{-5}	5.68 ± 0.02	18.49	0.9887	1.65×10^{-5}
H1K-EC/75-25	2.55 ± 0.03	40.39	0.9976	1.42×10^{-5}	5.52 ± 0.03	18.41	0.9875	1.08×10^{-5}

[a] Each value is mean of three separate determinations

[b] Time taken for 90% of the drug remaining to be degraded

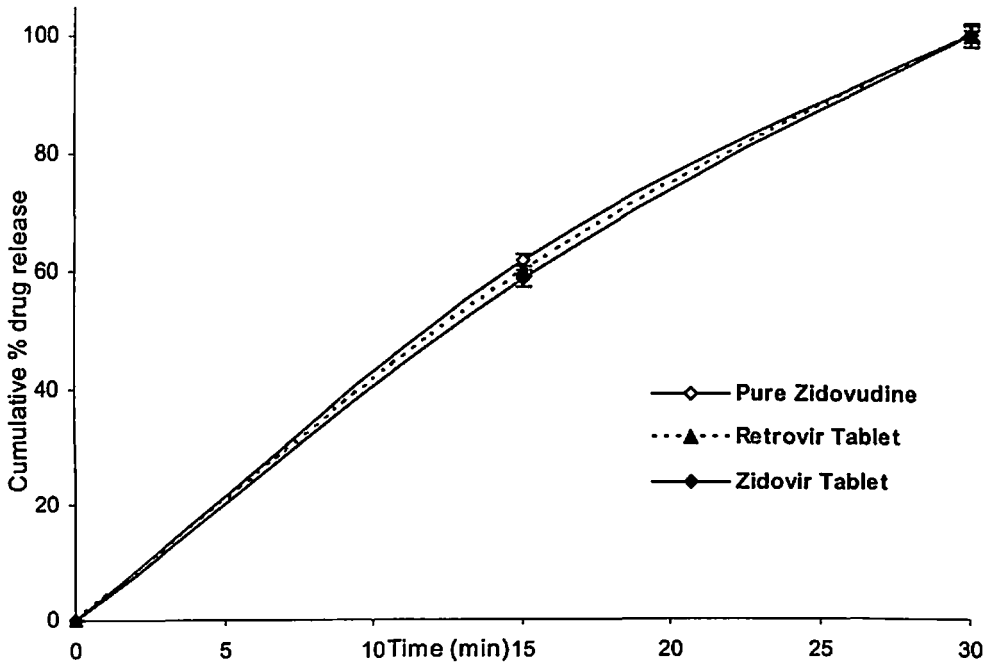

Figure 5.1: Comparative release profile of pure zidovudine (300 mg) and commercial tablets of zidovudine (Retrovir® tablets and Zidovir tablets). Each data point represents the mean of 6 tablets from three batches with SD

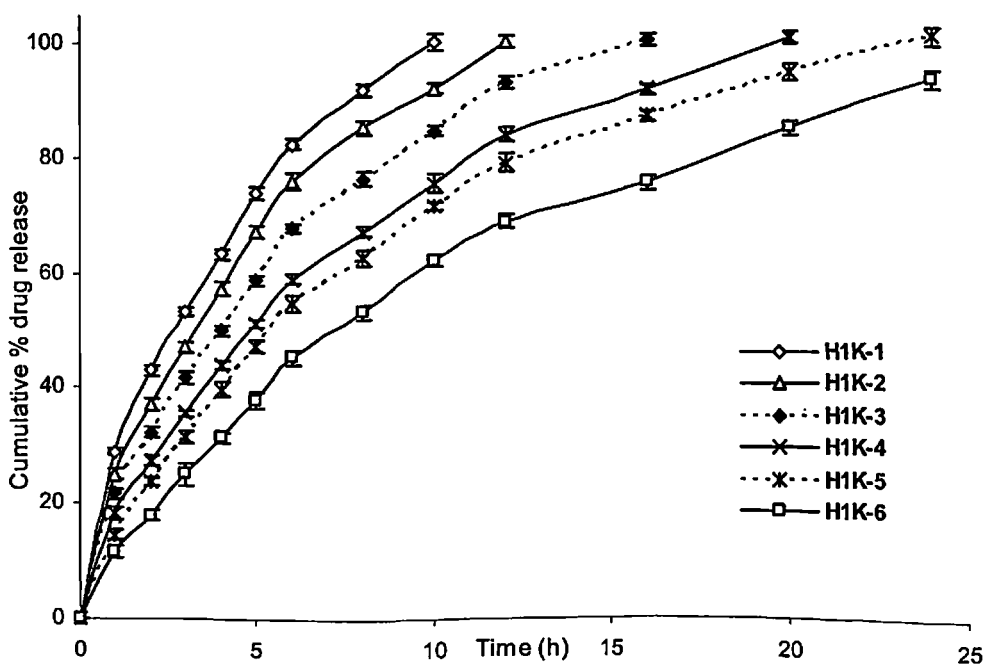

Figure 5.2: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of HPMC 1000 cPs. Each data point represents the mean of 6 tablets from three batches with SD

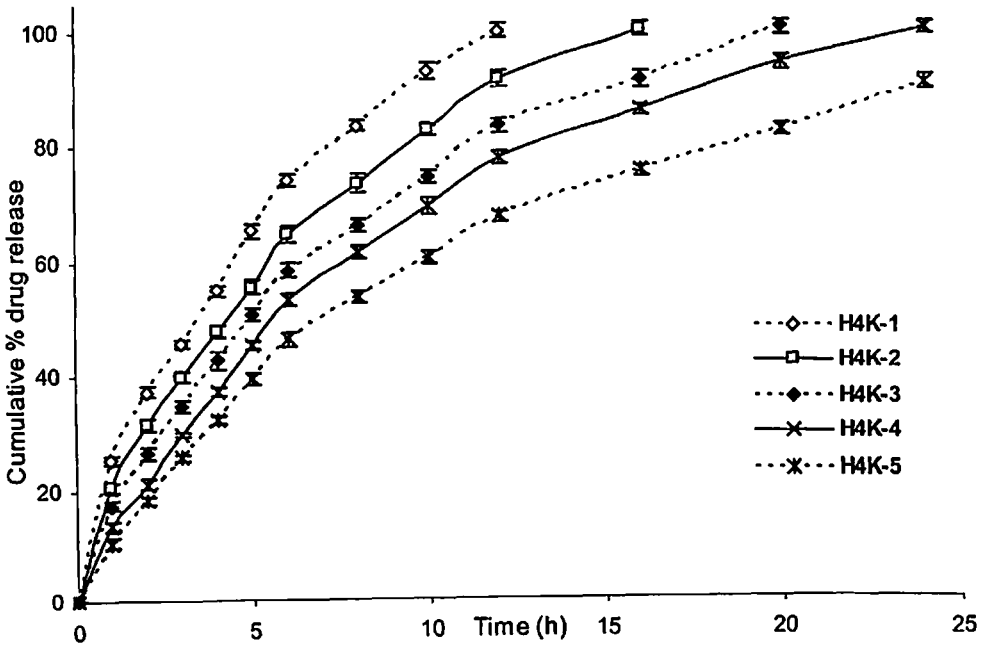

Figure 5.3: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of HPMC 4000 cPs. Each data point represents the mean of 6 tablets from three batches with SD

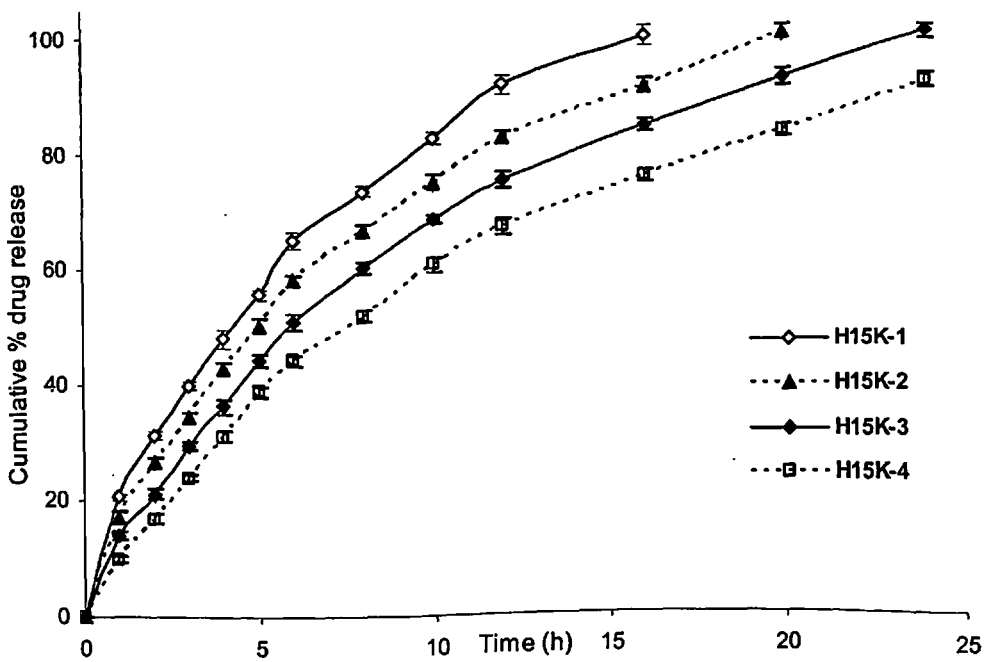

Figure 5.4: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of HPMC 15000 cPs. Each data point represents the mean of 6 tablets from three batches with SD

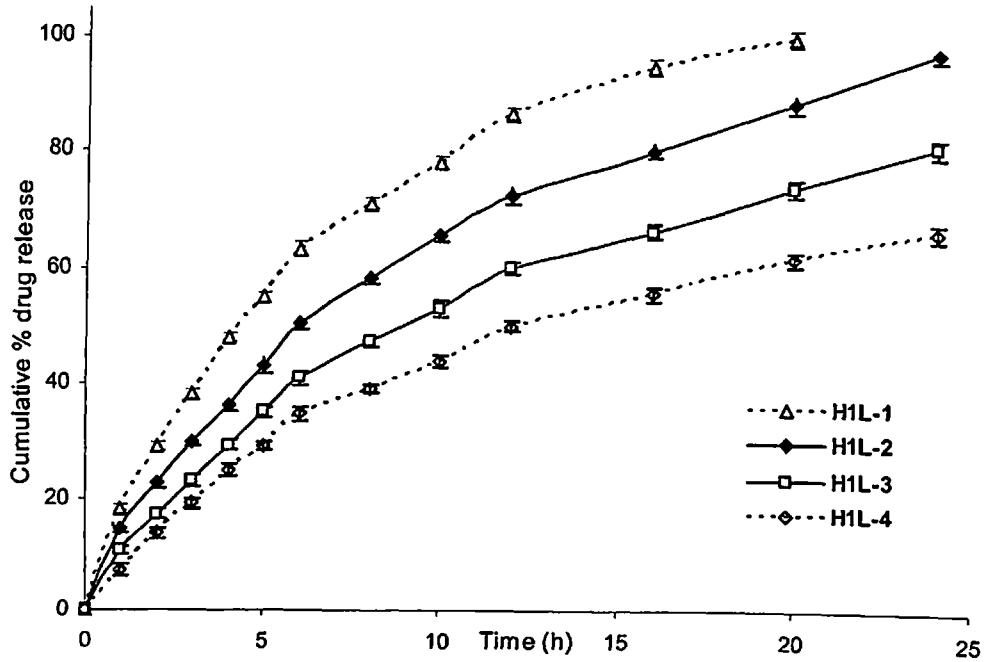

Figure 5.5: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of HPMC 100000 cPs. Each data point represents the mean of 6 tablets from three batches with SD

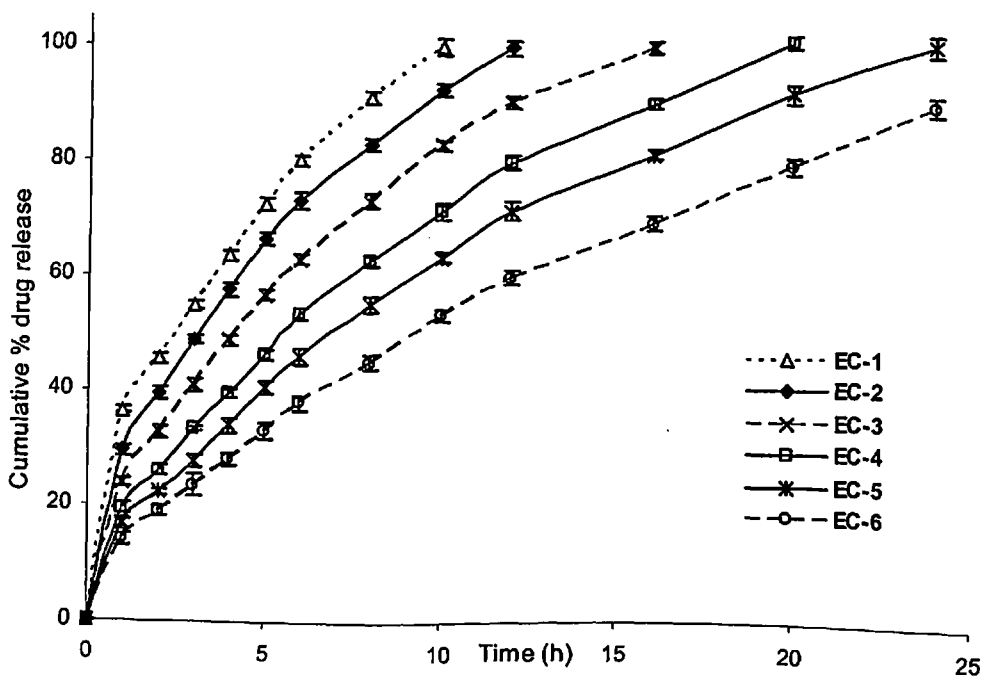

Figure 5.6: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of EC. Each data point represents the mean of 6 tablets from three batches with SD

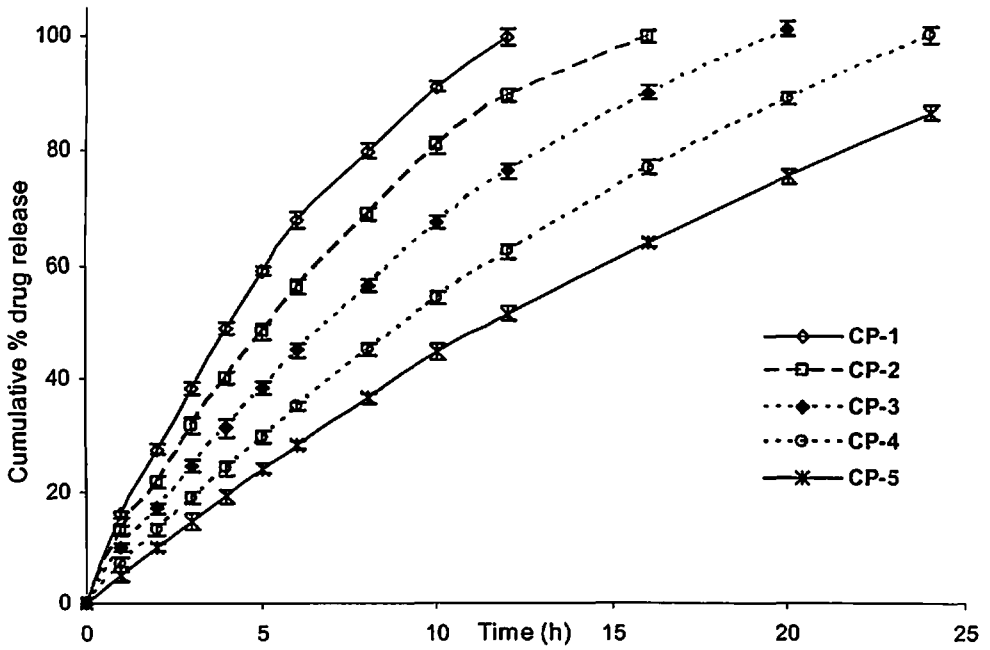

Figure 5.7: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of CP. Each data point represents the mean of 6 tablets from three batches with SD

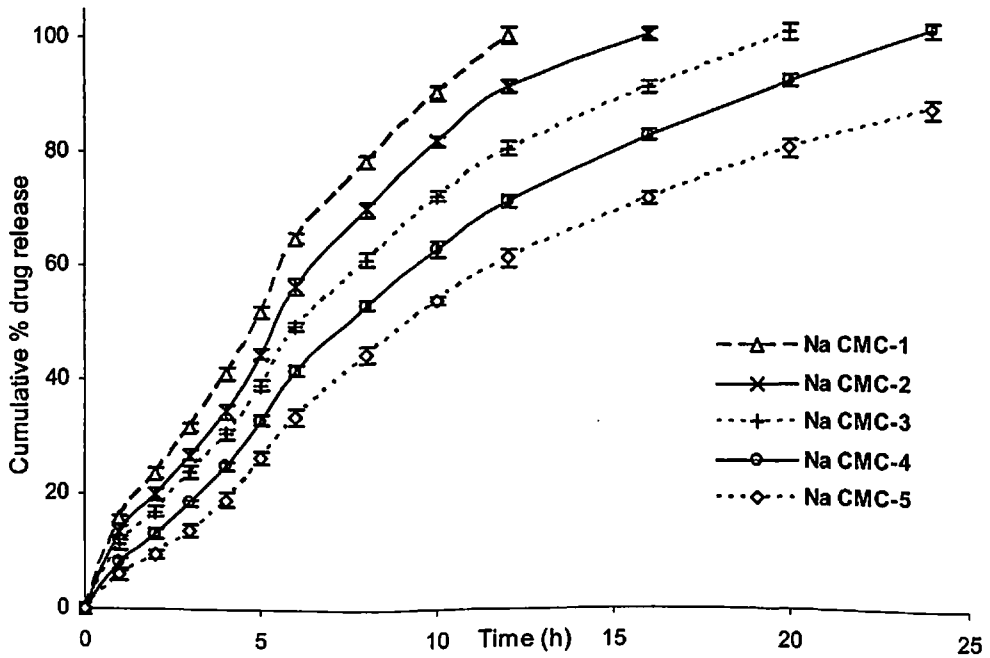

Figure 5.8: Comparative release profile of zidovudine from controlled release matrix tablets prepared using different proportions of Na CMC. Each data point represents the mean of 6 tablets from three batches with SD

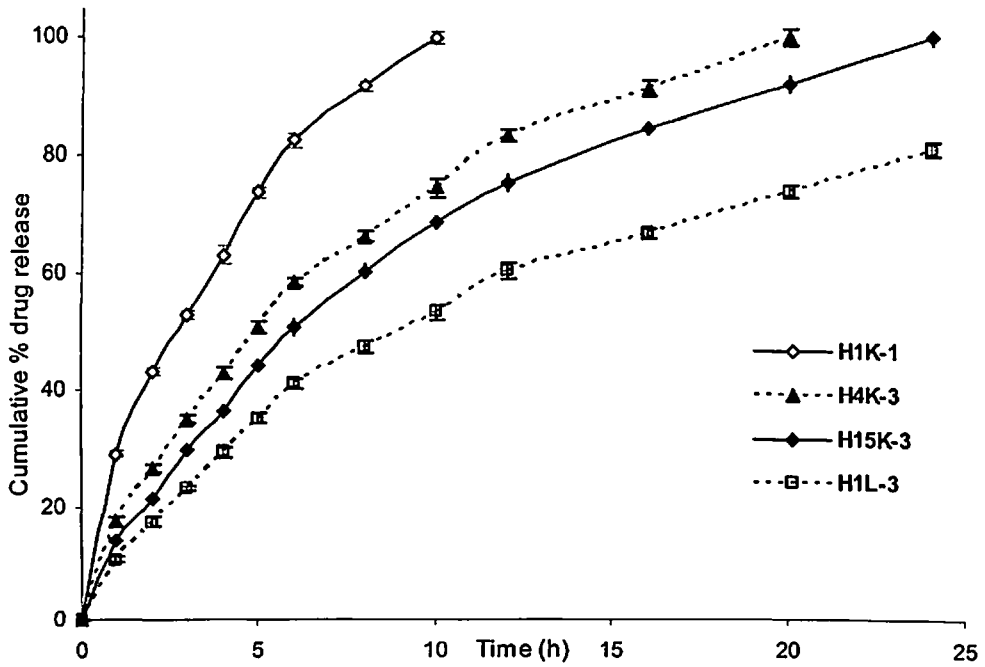

Figure 5.9: Comparative release profile of zidovudine from matrix tablets prepared using different viscosity grades of HPMC at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

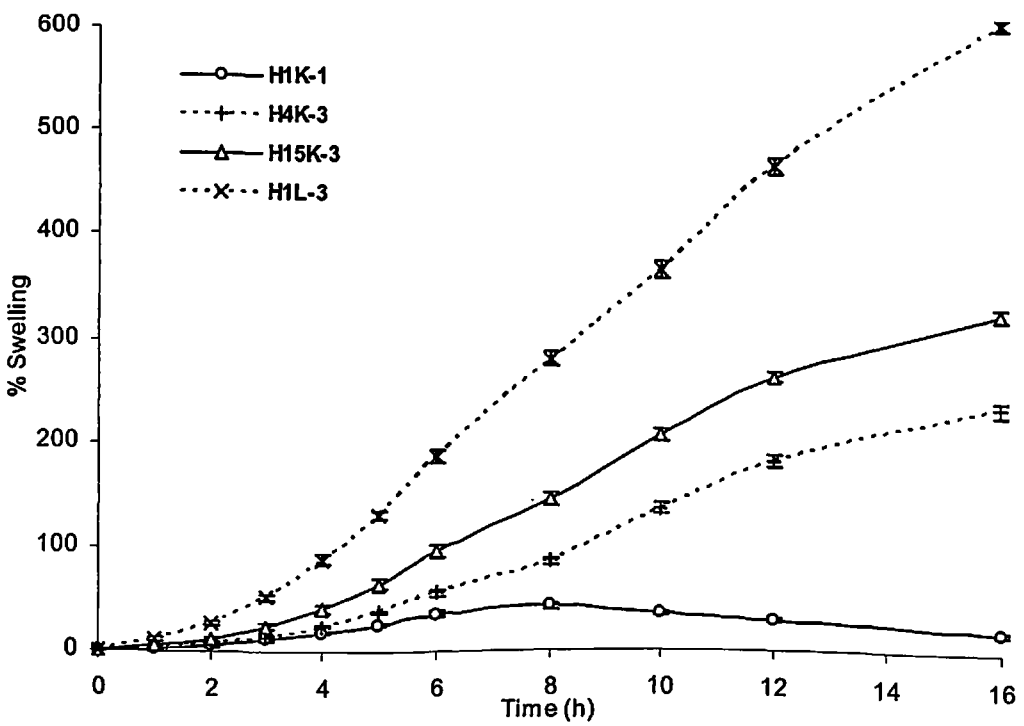

Figure 5.10: Swelling behavior of controlled release matrix tablets of zidovudine prepared using different viscosity grades of HPMC at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

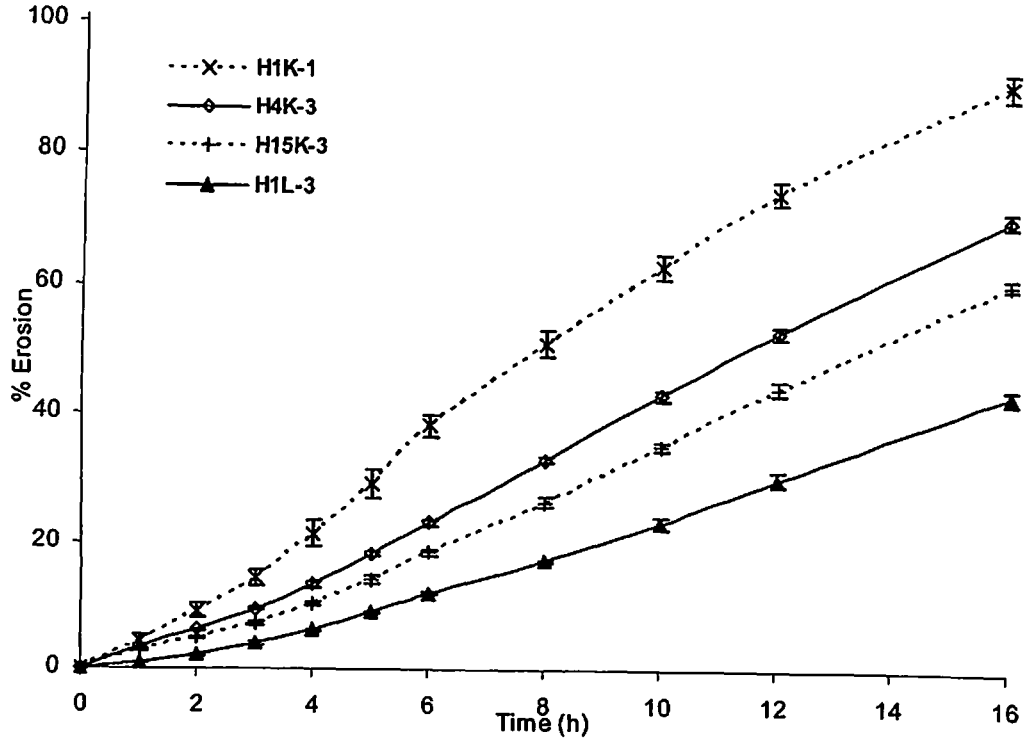

Figure 5.11: Eroding behavior of controlled release matrix tablets of zidovudine prepared using different viscosity grades of HPMC at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

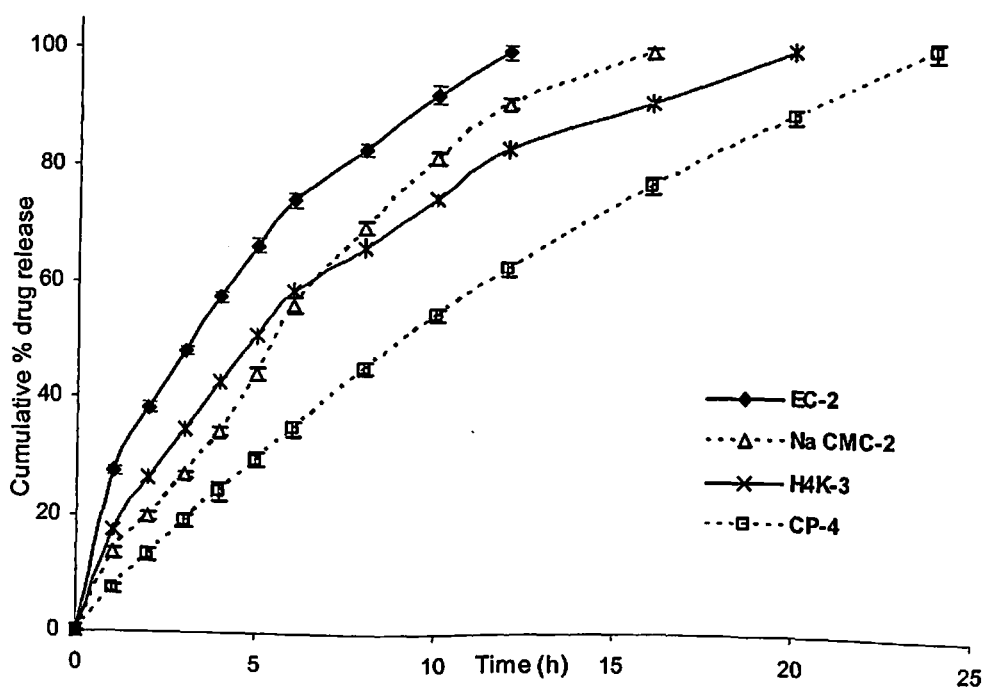

Figure 5.12: Comparative release profile of zidovudine from matrix tablets prepared using different types of polymers (EC, Na CMC, HPMC 4000 cPs and CP) separately at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

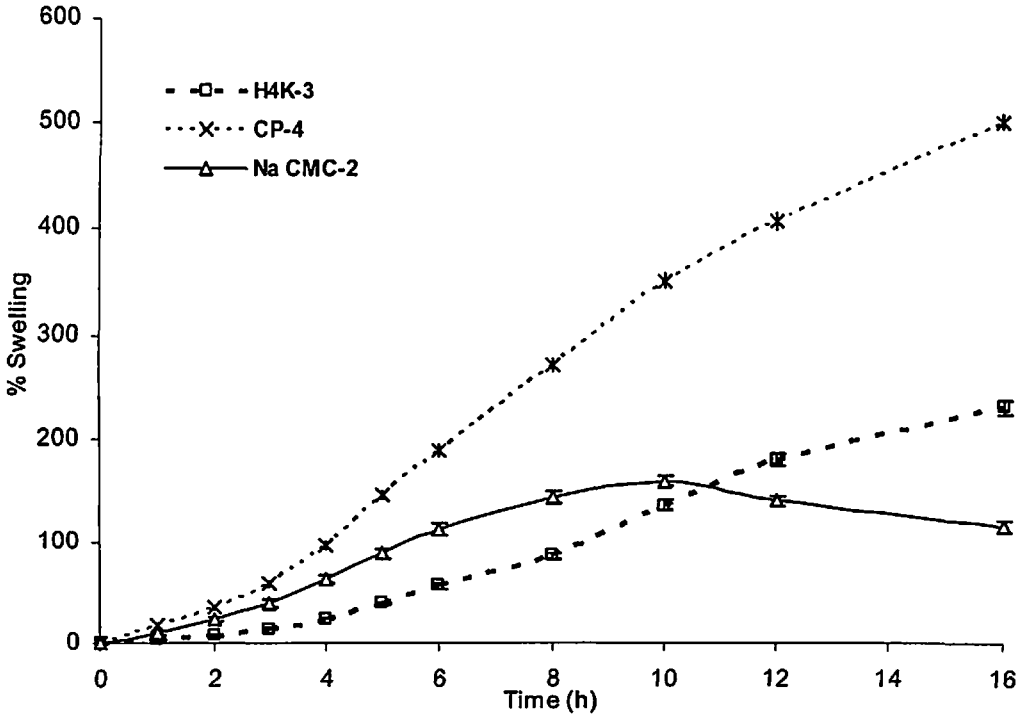

Figure 5.13: Swelling behavior of controlled release matrix tablets of zidovudine prepared using different types of polymers (HPMC 4000 cPs, CP and Na CMC) separately at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

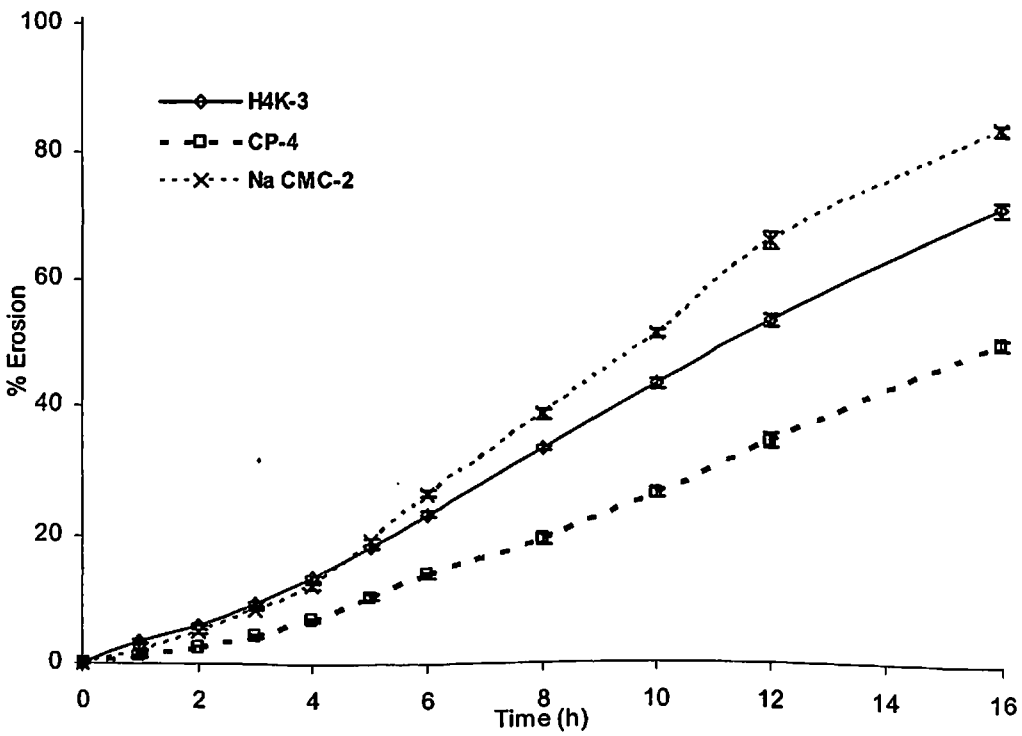

Figure 5.14: Eroding behavior of controlled release matrix tablets of zidovudine prepared using different types of polymers (HPMC 4000 cPs, CP and Na CMC) separately at 20% w/w of drug weight. Each data point represents the mean of 6 tablets from three batches with SD

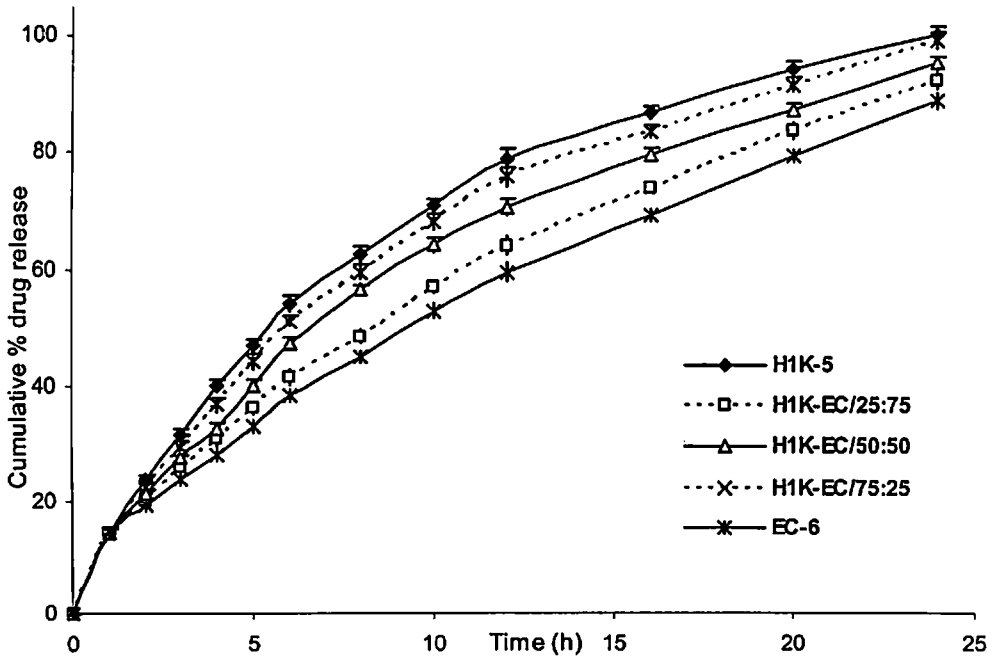

Figure 5.15: Comparative release profile of zidovudine from controlled release matrix tablets prepared using HPMC 1000 cPs and EC alone and in various combinations. Each data point represents the mean of 6 tablets from three batches with SD

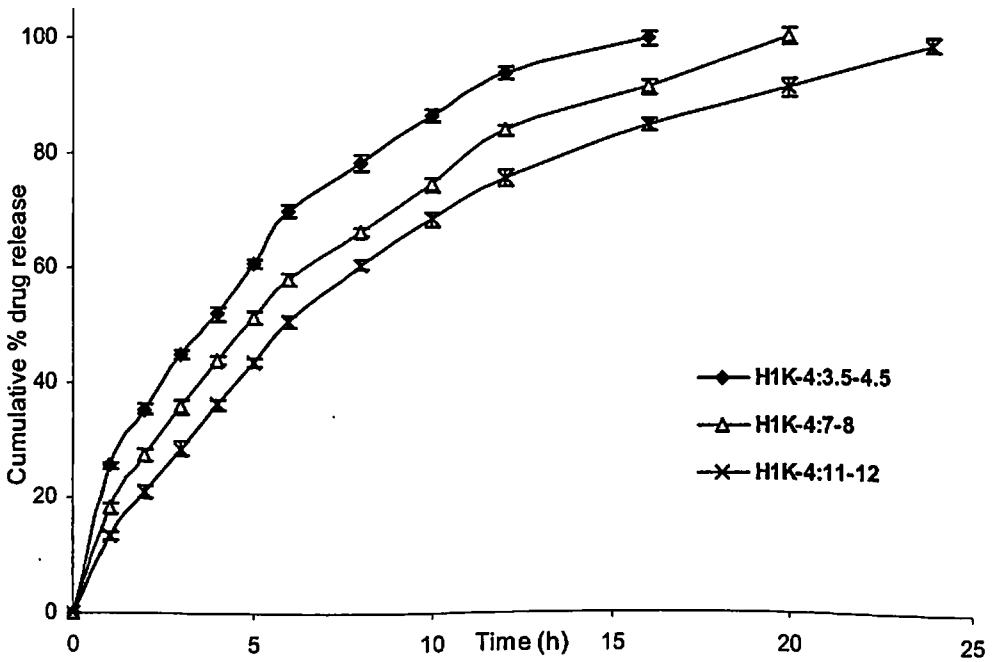

Figure 5.16: Comparative release profile of zidovudine from matrix tablets prepared using 80% w/w of HPMC 1000 cPs with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

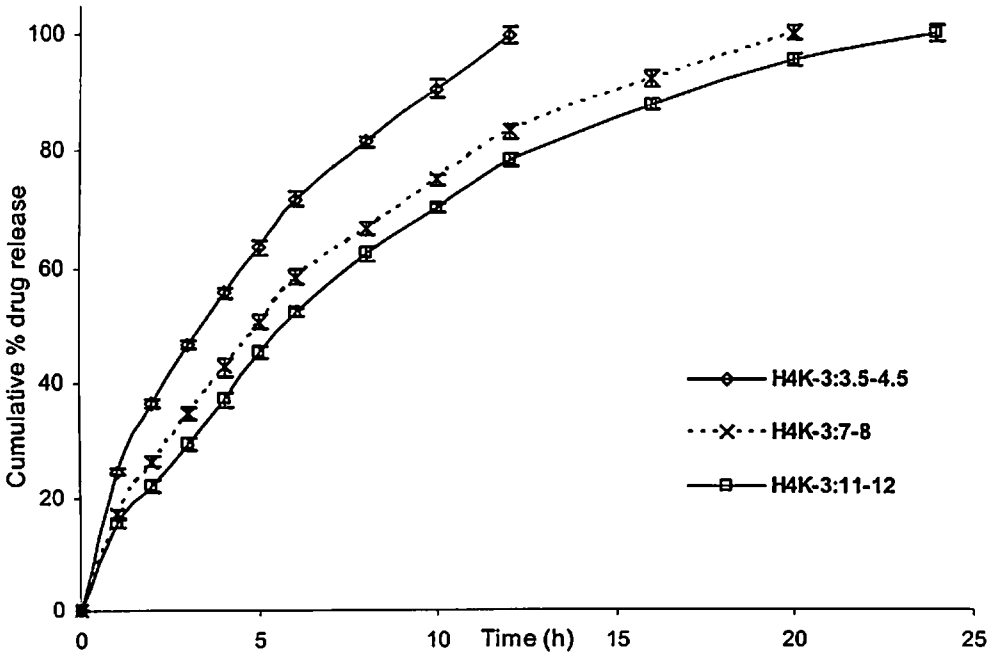

Figure 5.17: Comparative release profile of zidovudine from matrix tablets prepared using 20% w/w of HPMC 4000 cPs with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

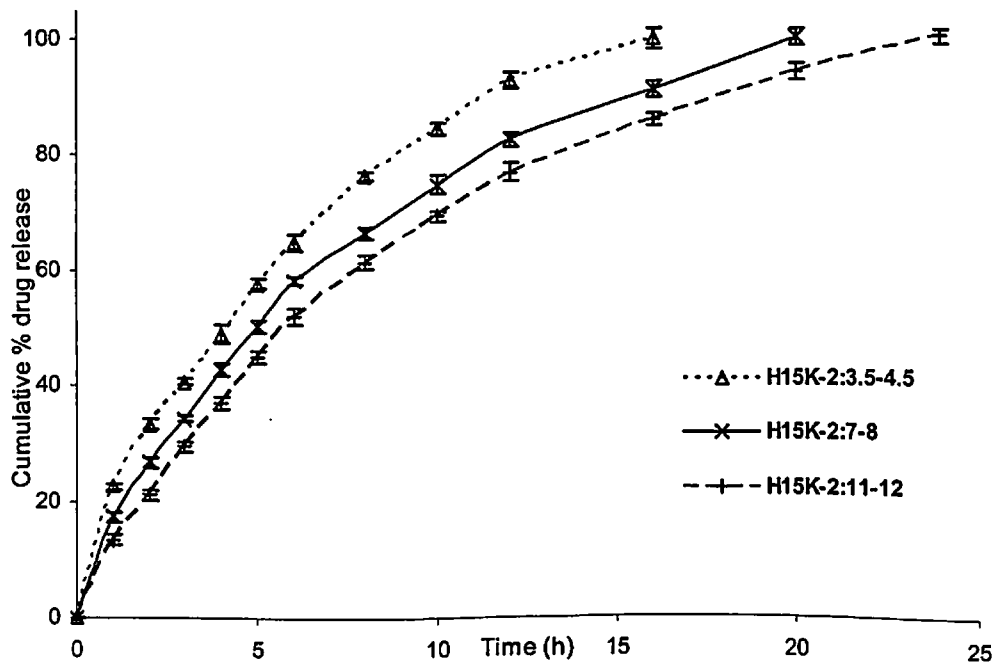

Figure 5.18: Comparative release profile of zidovudine from matrix tablets prepared using 10% w/w of HPMC 15000 cPs with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

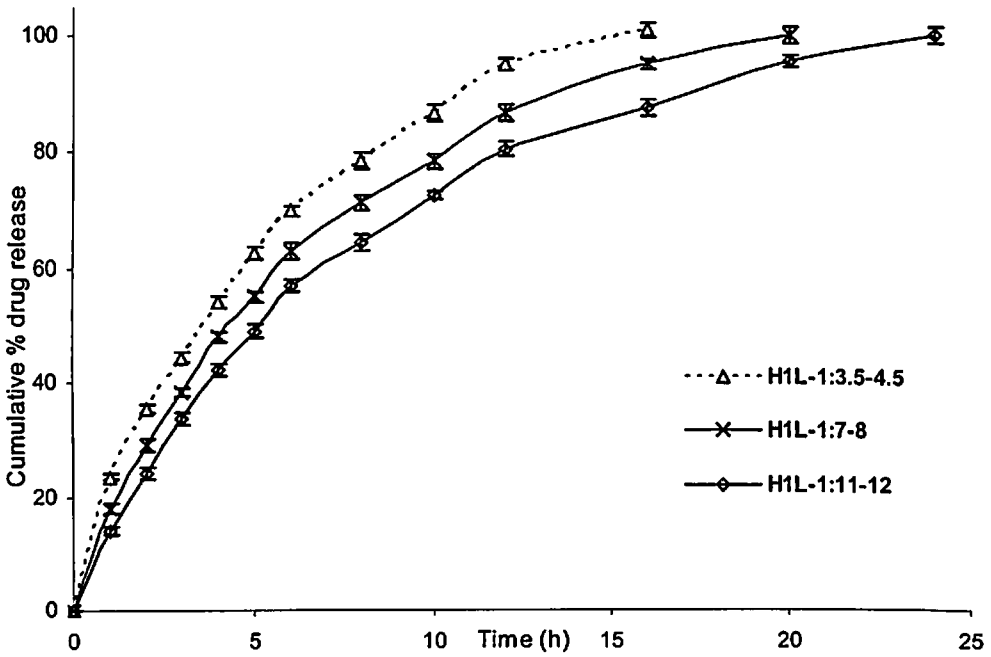

Figure 5.19: Comparative release profile of zidovudine from matrix tablets prepared using 5% w/w of HPMC 100000 cPs with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

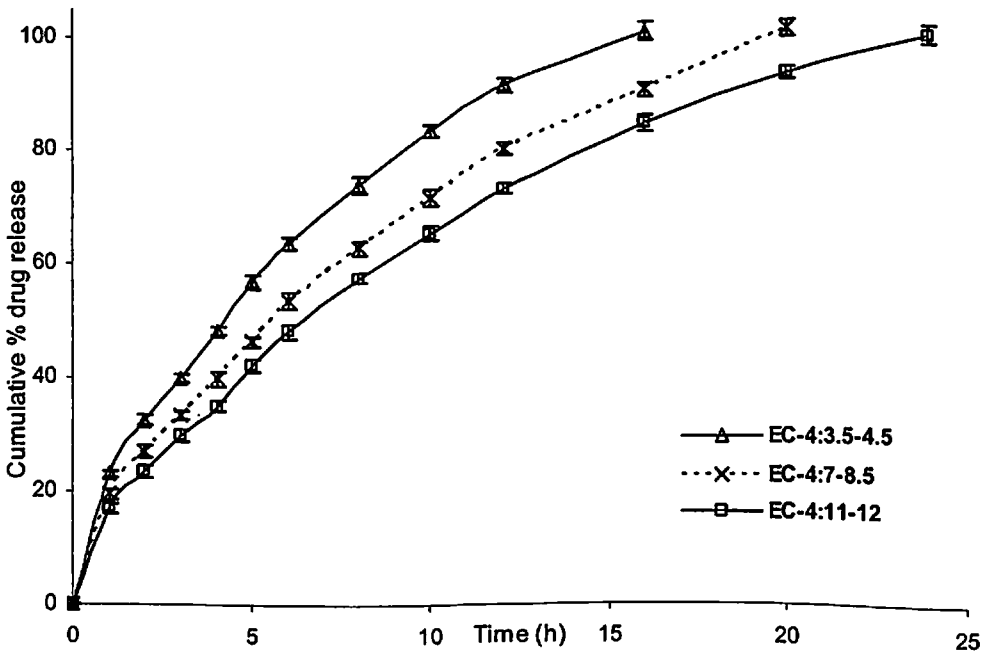

Figure 5.20: Comparative release profile of zidovudine from matrix tablets prepared using 60% w/w of EC with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

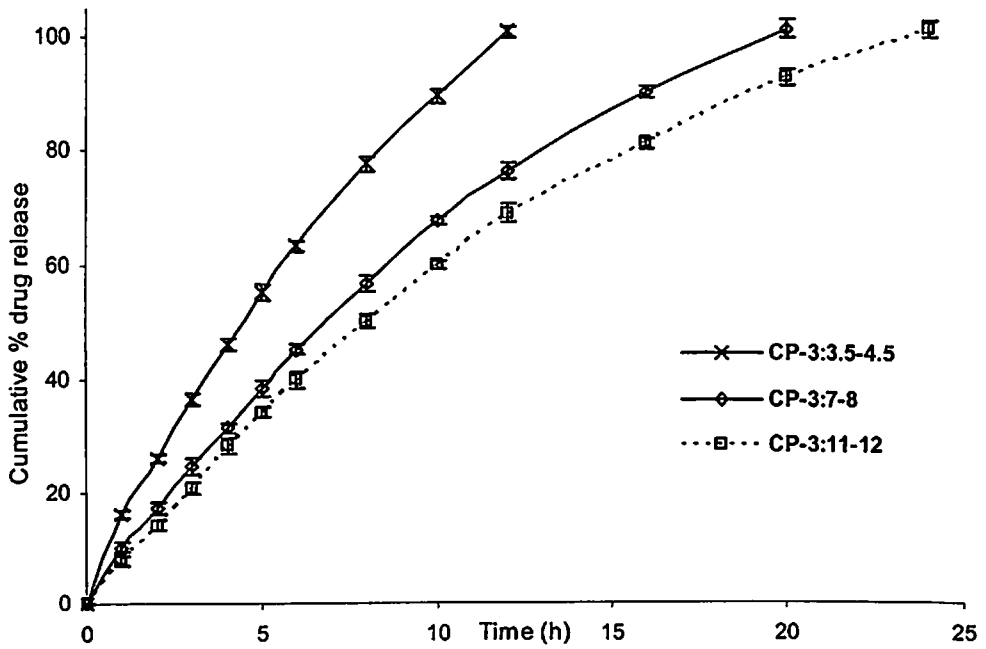

Figure 5.21: Comparative release profile of zidovudine from matrix tablets prepared using 15% w/w of CP with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

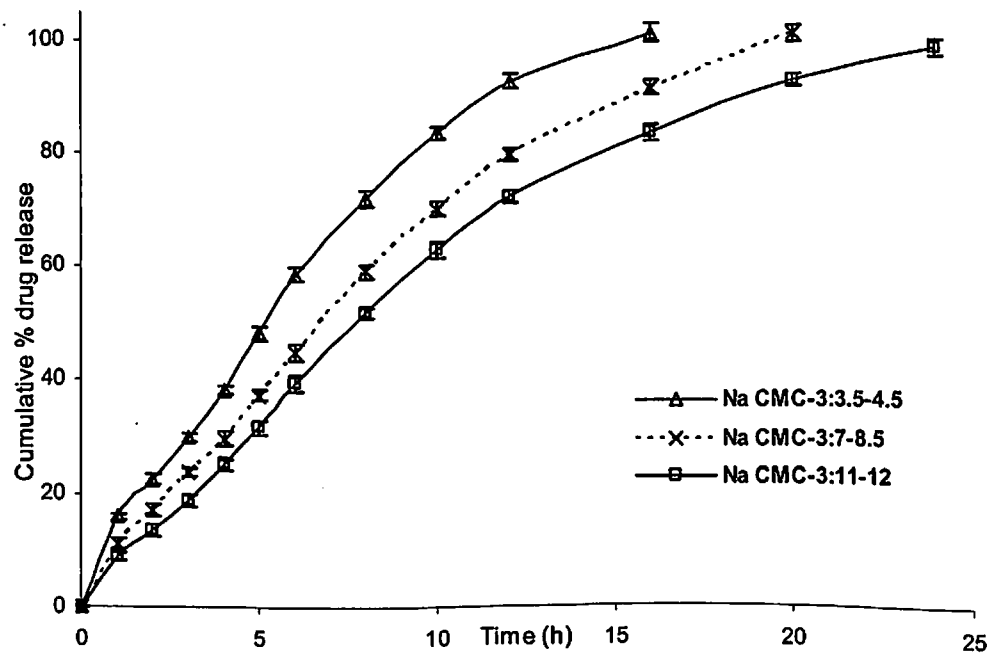

Figure 5.22: Comparative release profile of zidovudine from matrix tablets prepared using 40% w/w of Na CMC with different compression forces. Each data point represents the mean of 6 tablets from three batches with SD

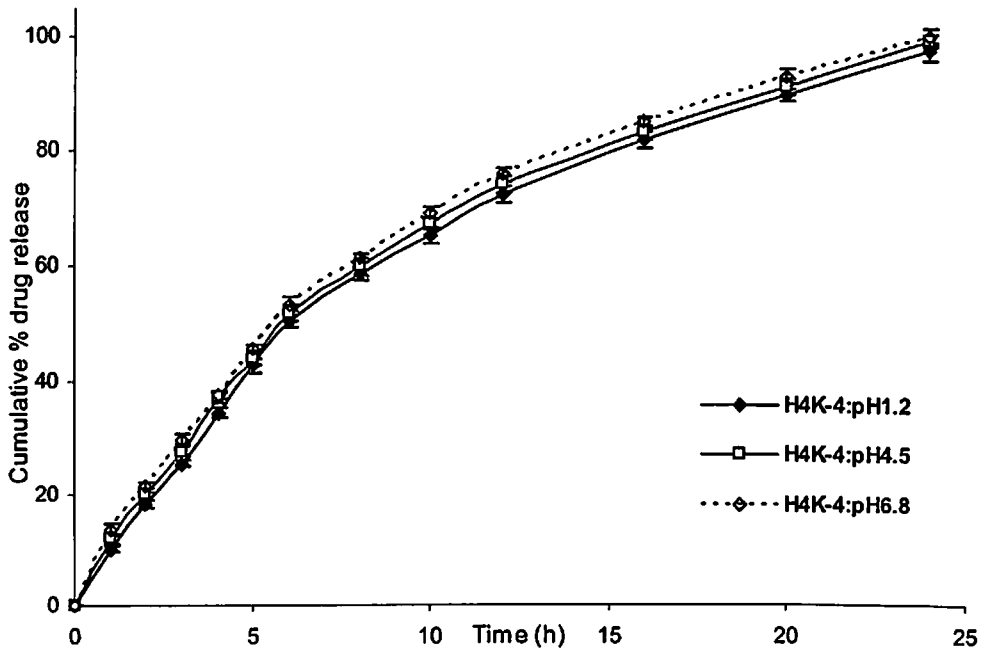

Figure 5.23: Comparative release profile of zidovudine from matrix tablets prepared using 40% w/w of HPMC 4000 cPs in different dissolution media (pH 1.2, 4.5 and 6.8). Each data point represents the mean of 6 tablets from three batches with SD

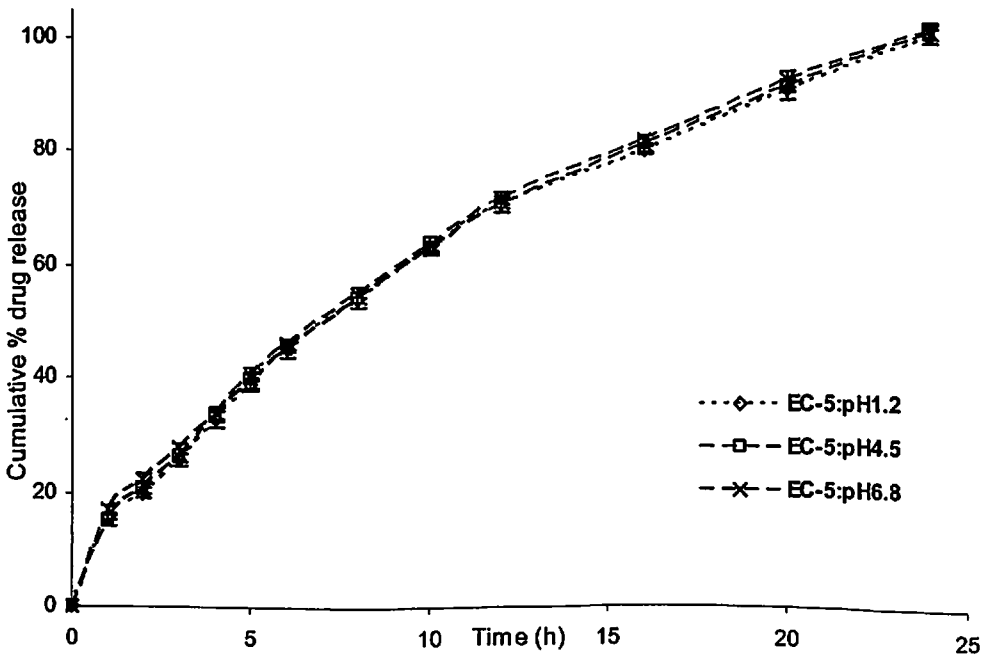

Figure 5.24: Comparative release profile of zidovudine from matrix tablets prepared using 80% w/w of EC in different dissolution media (pH 1.2, 4.5 and 6.8). Each data point represents the mean of 6 tablets from three batches with SD

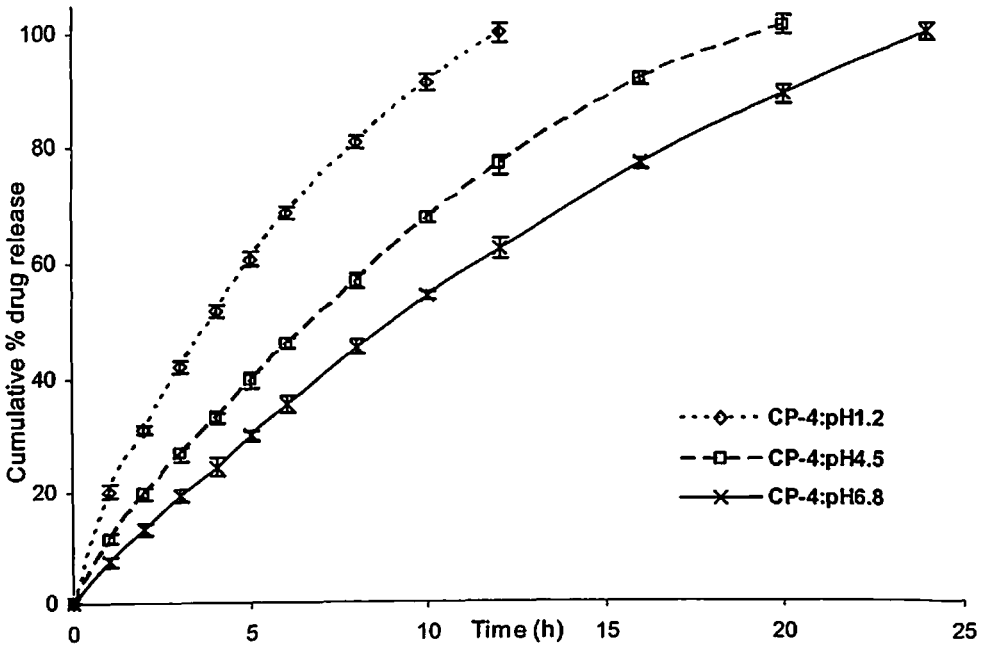

Figure 5.25: Comparative release profile of zidovudine from matrix tablets prepared using 20% w/w of CP in different dissolution media (pH 1.2, 4.5 and 6.8). Each data point represents the mean of 6 tablets from three batches with SD

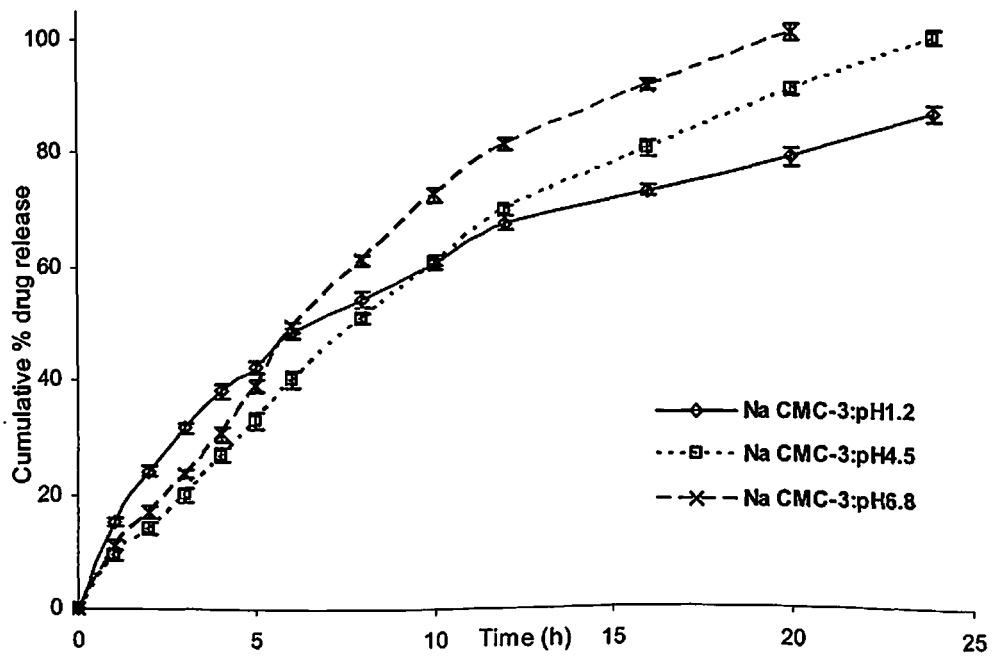

Figure 5.26: Comparative release profile of zidovudine from matrix tablets prepared using 40% w/w of Na CMC in different dissolution media (pH 1.2, 4.5 and 6.8). Each data point represents the mean of 6 tablets from three batches with SD

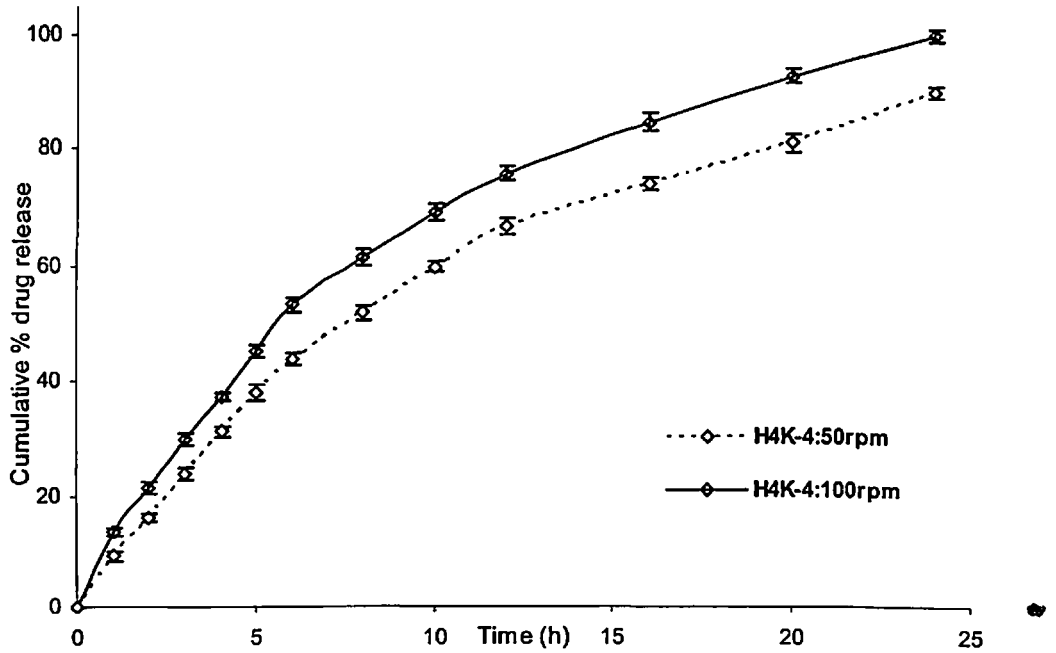

Figure 5.27: Comparative release profile showing the effect of agitation speed on release of zidovudine from matrix tablets prepared using 40% w/w of HPMC 4000 cPs. Each data point represents the mean of 6 tablets from three batches with SD

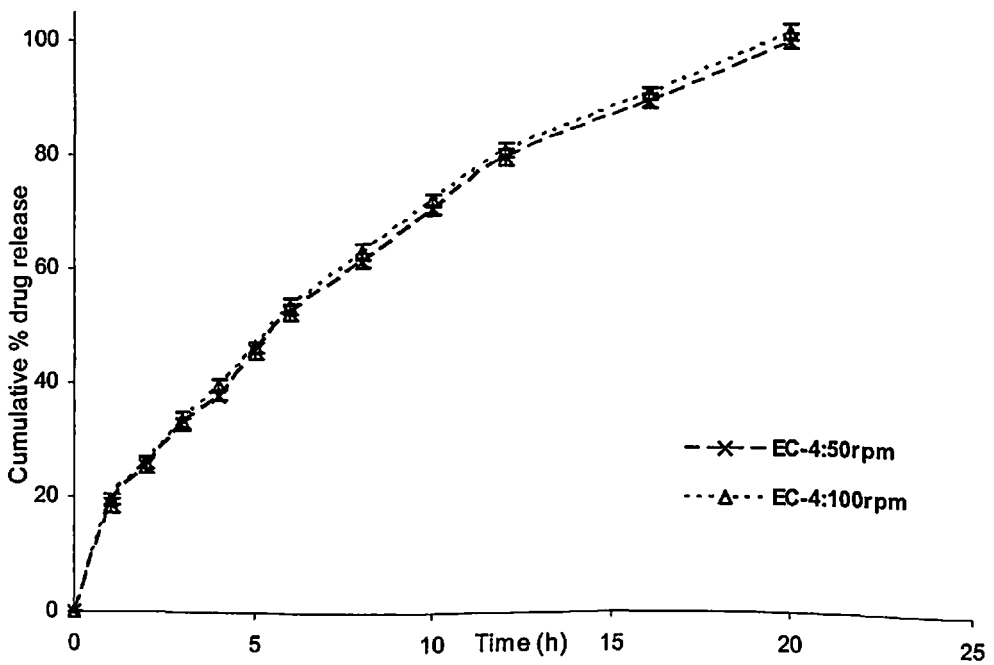

Figure 5.28: Comparative release profile showing the effect of agitation speed on release of zidovudine from matrix tablets prepared using 60% w/w of EC. Each data point represents the mean of 6 tablets from three batches with SD

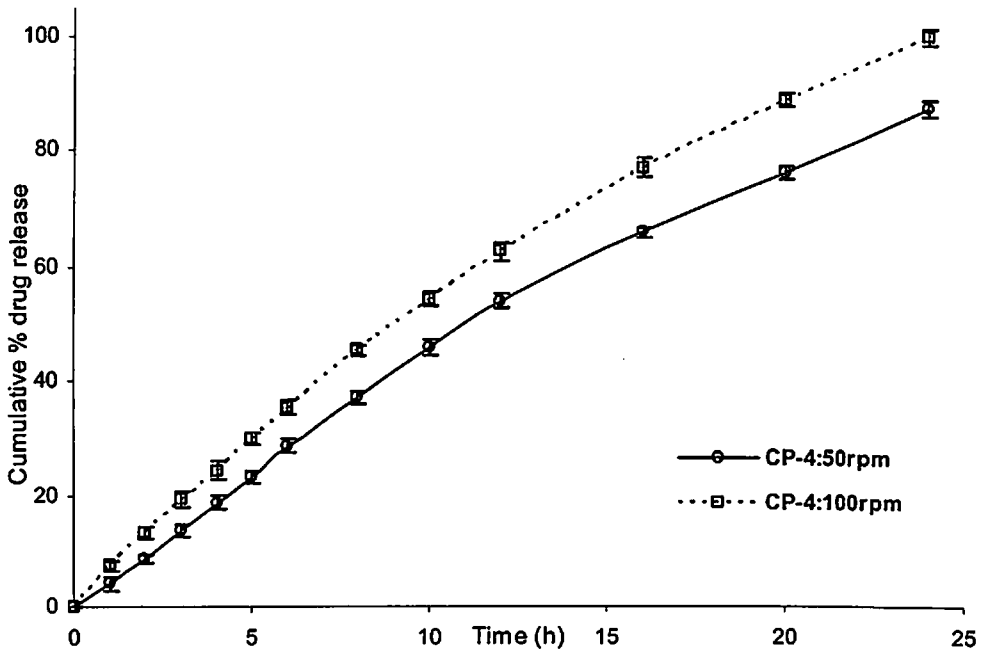

Figure 5.29: Comparative release profile showing the effect of agitation speed on release of zidovudine from matrix tablets prepared using 20% w/w of CP. Each data point represents the mean of 6 tablets from three batches with SD

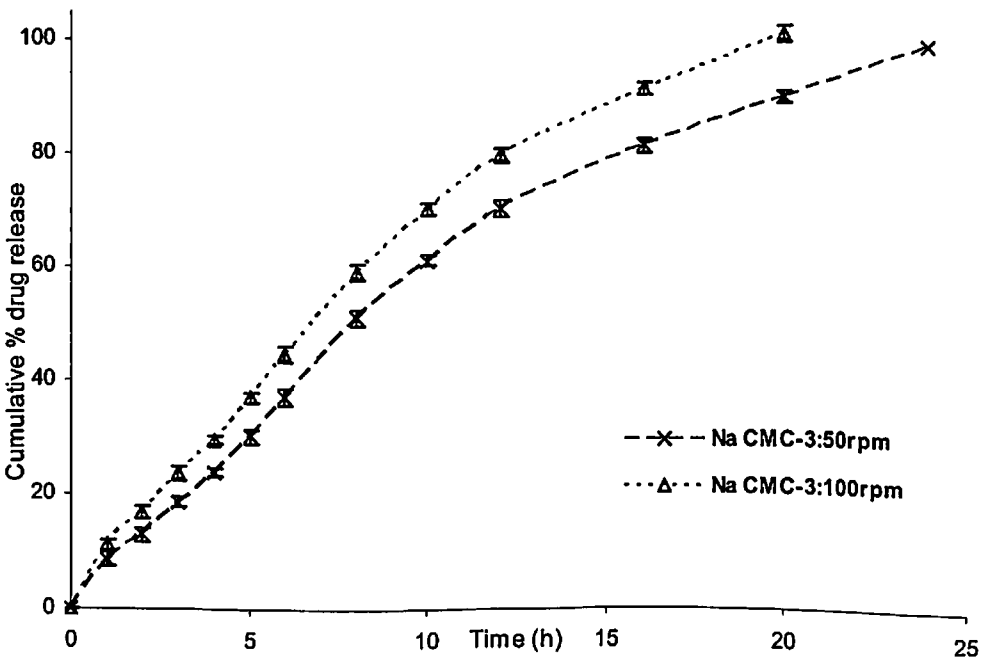

Figure 5.30: Comparative release profile showing the effect of agitation speed on release of zidovudine from matrix tablets prepared using 40% w/w of Na CMC. Each data point represents the mean of 6 tablets from three batches with SD

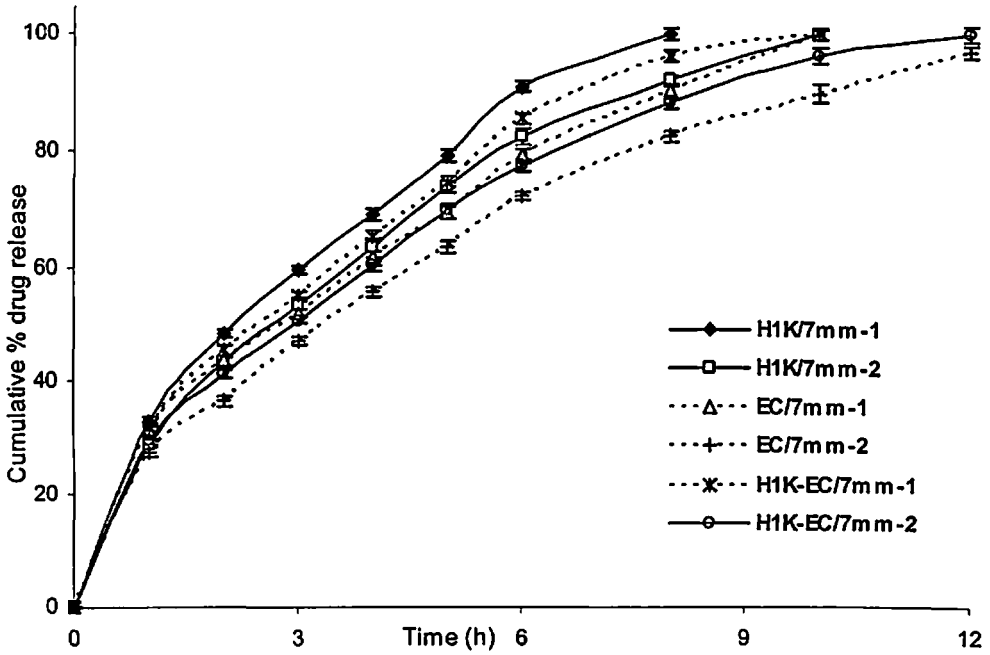

Figure 5.31: Comparative release profile of zidovudine from selected controlled release matrix tablets prepared using HPMC 1000 cPs and EC alone and in various combinations and compressed using 7 mm diameter punches. Each data point represents the mean of 6 tablets from three batches with SD

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Chapter 6

In vivo Bioavailability Studies

6.1. Introduction

In vivo evaluation of any newly developed delivery systems of a drug in appropriate animal models or human subjects is very essential in order to ascertain the pharmacokinetic behavior and expected clinical efficacy of the developed delivery systems. Oral controlled release delivery systems are mostly developed to maintain uniform drug levels for extended period of time; to reduce dose, side effects and frequency of dosing; to improve bioavailability and to improve patient compliance particularly for drugs required to be administered in multiple doses. So, the in vivo evaluation of oral controlled release delivery systems should demonstrate these benefits compared to the conventional delivery systems administered through the same route. Several animal models like rabbits (Dunn and Hollister, 1995; Diana et al., 1999; Atul et al., 2006; Nworu et al., 2008), dogs (Acosta et al., 1996; Abu-Izza et al., 1997), guinea pigs (Qurrat et al., 2003), monkeys (Ayers et al., 1996; Rao et al., 2008) have been used and reported for carrying out the pharmacokinetic and bioavailability studies of oral delivery systems.

In this chapter, in vivo studies of oral controlled release (CR) matrix tablets of zidovudine (ZDV), designed under the project, have been presented. Oral bioavailability and pharmacokinetics of selected CR matrix tablets of ZDV were studied in New Zealand white rabbits. CR matrix tablets containing 100 mg of ZDV were selected on the basis of in vitro studies mentioned in chapter 5. Serum drug concentrations versus time profiles and pharmacokinetic parameters of developed CR matrix tablets were compared with that of immediate release (IR) tablets administered through oral route.

6.2. Materials

CR matrix tablets and IR tablets of ZDV manufactured in the laboratory were used. All other chemicals and reagents used were same as those mentioned in chapter 3 and chapter 4.

6.3. Equipments/Instruments

Analytical equipments and other processing equipments used in the in vivo studies were same as those mentioned in chapter 3 and chapter 5.

6.4. In vivo Pharmacokinetics Study in Rabbits

6.4.1. Animal model

New Zealand white male rabbits weighing between 2.0 to 2.5 kg were provided by the central Animal Facility of Birla Institute of technology and Science, Pilani. Prior approval was obtained from Institutional Animal Ethics Committee for carrying out the study (Protocol approval no. IAEC/RES/10/2). The study was conducted as per the guidelines given by the Institutional Animal Ethics Committee and under the guidance of a registered veterinarian. Animals were housed in standard cages in light controlled room at 25 ± 2 °C and 50 ± 5% RH. Animals were issued and acclimatized 6 days prior to the actual experimentation. Animals were kept on standard pellet diet (Hindustan Lever Ltd., India) and water ad libitum during the period of acclimatization. Animals were kept on fasting 6 h prior to the actual start of the experimentation. Food and water was not given till 4 h after the start of the study.

6.4.2. Preparation of formulations

IR tablets containing 100 mg of ZDV, compressed using 7 mm diameter punches, were manufactured by wet granulation process according to the procedure given in chapter 5, except that no polymer was added in the tablets. Fresh batches of CR tablets (100 mg of ZDV) containing 20% HPMC (H1K/7mm-1) and 20% EC (EC/7mm-1) alone and combination of HPMC 1000 cPs and EC at 10/10 proportion (H1K-EC/7mm-1) compressed using 7 mm diameter punches were prepared prior to animal experimentation. The complete composition of the above mentioned formulations is given Table 5.1d. Physical characteristics, drug content and in vitro release profiles of the prepared formulations were determined according to the methods given in chapter 5.

6.4.3. Dosing

The oral administration of tablets (IR or selected CR matrix tablets) was done by opening the mouth of rabbits using a specially designed restrainer and placing the tablet carefully behind the tongue to ensure proper administration of the tablet. Small volume (1 to 2 ml) of distilled water was ingested through the mouth with the help of syringe to make sure that the tablet was swallowed down into the stomach.

6.4.4. Blood sample collection and processing

Blood sample were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 16 h post dosing of each tablet using a 21 G needle in clean and dry centrifuge tubes. Blood samples were also collected from all the rabbits just before the administration of the tablets. The collected blood was harvested for 45 min at room temperature and centrifuged at 2000 rpm for 20 min. The clear supernatant serum layer was collected and stored at -20 °C until analysis.

6.4.5. Sample analysis

Frozen samples were thawed by keeping the sealed tubes at room temperature (25 ± 2 °C) for at least 60 min and analyzed using bioanalytical method mentioned in chapter 3. The serum drug concentrations at various time points of the study were determined.

6.4.6. Data analysis

The serum drug concentration versus time data obtained for different tablets during the study was subjected to non-compartmental analysis using WinNonlin Standard edition, Version 2.1 software (WinNonlin Scientific Consultants, USA) to obtain various pharmacokinetic parameters like C_{max} (Maximum serum concentration), T_{max} (Time taken to reach maximum concentration), $AUC_{(0-\infty)}$ (Area under the serum drug concentration versus time curve from zero time to infinite time) , $AUMC_{(0-\infty)}$ (Area under the first moment curve from zero time to infinite time) and MRT (Mean residence time). The elimination half-life was determined based on the last two time points in the elimination phase obtained for each tablet. Relative bioavailability (F_r) values for the CR matrix tablets were determined as the ratio of $AUC_{(0-\infty)}$ of CR matrix tablet to the $AUC_{(0-\infty)}$ of IR tablet.

6.5. Results and Discussion

The serum concentration versus time profiles of ZDV following administration of single dose of IR tablet (100 mg of ZDV) and selected CR matrix tablets are given in Figure 6.1. Various pharmacokinetic parameters were determined by treating the serum drug concentration versus time data for each tablet in non-compartmental model. The summary of pharmacokinetic parameters obtained is presented in Table 6.1.

The absorption of ZDV from IR was found to be rapid with high C_{max} (1.154 ± 0.095 µg/ml) value reaching at 1 h post administration of the tablet. But the serum

concentrations of ZDV were not sustained and the concentrations declined rapidly, reaching very low concentrations of 0.121± 0.009 μg/ml at 3 h post administration (Figure 6.1).

However, CR tablets showed low but extended serum drug concentration profiles. The C_{max} values of H1K/7mm-1, EC/7mm-1 and H1K-EC/7mm-1 were found to be 0.862 ± 0.074, 0.795 ± 0.064 and 0.829 ± 0.068 μg/ml respectively. The C_{max} values obtained for CR matrix tablets were found to be significantly ($P < 0.5$) lower than that produced by IR tablets. The T_{max} value for all the CR matrix tablets was found to be 3 h. The rates of absorption of the drug from the CR matrix tablets were lesser compared to IR tablets and thus produced lesser C_{max} values with higher T_{max}. However, the serum drug concentrations were better controlled and extended to more than 12 h. Even at 12 h, the serum drug concentrations were found to be 0.416 ± 0.031, 0.574 ± 0.038 and 0.481 ± 0.034 μg/ml in case of H1K/7mm-1, EC/7mm-1 and H1K-EC/7mm-1 formulations respectively, which were much higher than 0.173 ± 0.013 μg/ml found in case of IR tablets at 2 h. The $AUC_{(0-\infty)}$ and MRT values of CR matrix tablets were found to be significantly ($P < 0.5$) higher than that of the IR tablets. The MRT values of H1K/7mm-1, EC/7mm-1 and H1K-EC/7mm-1 were found to be 7.18 ± 0.45, 7.91 ± 0.50 and 7.47 ± 0.48 respectively, indicating a significant ($P < 0.5$) increase in the residence time of ZDV in the serum by the administration of CR tablets than compared to IR tablets (MRT = 2.33 ± 0.16). The relative bioavailability (F_r) values of H1K/7mm-1, EC/7mm-1 and H1K-EC/7mm-1 were found to be 4.30, 4.49 and 4.41 respectively, indicating a significant ($P < 0.5$) increase in the bioavailability of ZDV from the developed CR matrix tablets than compared to IR tablets.

All the selected CR tablets showed better serum drug concentration profile compared to IR tablet and seem to be therapeutically more useful.

6.6. Conclusions

The in vivo pharmacokinetic studies of the selected CR matrix tablets of ZDV (H1K/7mm-1, EC/7mm-1 and H1K-EC/7mm-1) in rabbits were found to show a extended serum drug concentration with probable increase in the bioavailability of the drug compared to IR tablets. The serum drug concentration levels were controlled and extended for longer duration thereby reducing the chances of dose-dependent side effects of ZDV. The results from the in vivo studies have also demonstrated the chances for reduction of dose and frequency of administration, improvement in the patient's compliance and adherence (which is very vital in the treatment of HIV/AIDS, because of the chances of development of

resistance if therapy is discontinued) to multi-dose anti-HIV therapy. Designed CR tablets may be useful for extended and better therapy as alternate to multi-dose administration.

The smooth and extended absorption phase coupled with maintenance of serum drug concentrations for longer duration after the administration of CR matrix tablets would definitely help in the reduction of dose-dependent side effects associated with ZDV due to its low therapeutic index.

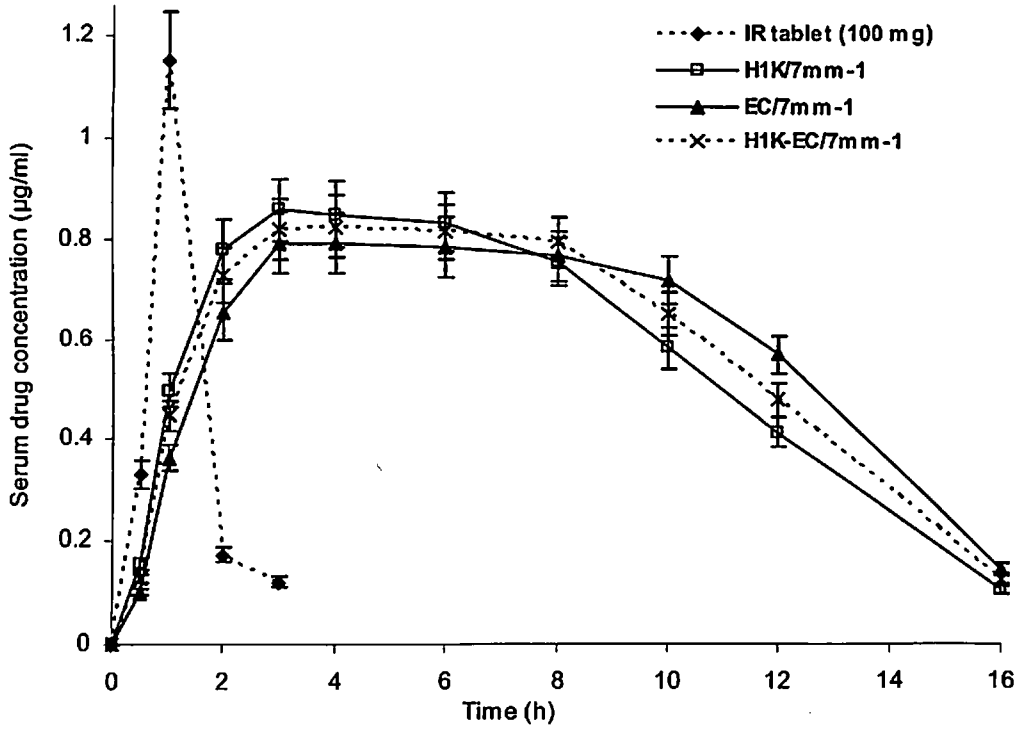

Figure 6.1: Comparative in vivo absorption profile of zidovudine obtained following the oral administration of single dose of immediate release (IR) tablet of zidovudine and some selected CR matrix tablets of zidovudine containing 100 mg of drug separately in rabbits. Each data point represents the mean of three independent determinations with SD

Table 6.1: Summary of pharmacokinetic parameters obtained following the oral administration of single dose of immediate release (IR) tablet of zidovudine and some selected CR matrix tablets of zidovudine containing 100 mg of drug separately in rabbits

Pharmacokinetic parameter	**IR tablet**	**H1K/7mm-1**	**EC/7mm-1**	**H1K-EC/7mm-1**
C_{max}[a] (μg/ml)	1.154 ± 0.095	0.862 ± 0.074	0.795 ± 0.064	0.829 ± 0.068
T_{max}[b] (h)	1.0	3.0	3.0	3.0
$AUC_{(0-\infty)}$[c] (μg.h/ml)	2.175 ± 0.089	9.362 ± 0.251	9.782 ± 0.314	9.587 ± 0.287
$AUMC_{(0-\infty)}$[d] (μg.h^2/ml)	5.09 ± 0.39	67.19 ± 3.67	77.35 ± 5.08	71.64 ± 4.52
MRT[e] (h)	2.33 ± 0.16	7.18 ± 0.45	7.91 ± 0.50	7.47 ± 0.48
Elimination half-life[f] (h)	1.61 ± 0.11	4.97 ± 0.24	5.48 ± 0.35	5.17 ± 0.31
F_r[g]	-	4.30	4.49	4.41

[a] Maximum serum concentration
[b] Time taken to reach maximum concentration
[c] Area under the serum drug concentration versus time curve from zero time to infinite time
[d] Area under the first moment curve from zero time to infinite time
[e] Mean residence time
[f] Elimination half-life calculated using the formula: 0.693 x MRT
[g] Relative bioavailability with respect to IR tablet

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Chapter 7

Conclusions

7.1. Conclusions

The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery systems of existing drugs molecules in order to maximize the effectiveness of their therapeutic action and patent protection and eventually to reduce the health care costs. As oral controlled release drug delivery systems have become very popular in pharmaceutical industry, this project was aimed at designing controlled release (CR) matrix tablets of an anti-HIV drug zidovudine (ZDV).

Matrix embedded CR tablets of ZDV designed during this study were found to possess good physical characteristics indicating that wet granulation technique was suitable for manufacturing good quality tablets using different polymers. The designed formulations were found to be stable for at least 2 years when stored at controlled room temperature conditions; indicating that the excipients, process and packaging materials used were compatible with the drug. Preformulation studies have indicated that ZDV has pH independent solubility and good liquid state stability in all the pH conditions.

Release of the drug from matrix embedded CR tablets was affected by the polymer type and polymer proportion. Increase in compression force was found to decrease the release rate from the designed formulations irrespective of the polymer used. Formulations prepared using HPMC and EC formulations showed pH independent drug release, while formulations prepared using CP and Na CMC showed pH dependent drug release. Agitation speed was found to effect the release rate from formulations prepared using swellable hydrophilic polymers (like HPMC, CP and Na CMC) but no significant effect was observed on formulations prepared using hydrophobic polymer like EC. The drug release was chiefly dependent on diffusion of drug through the matrix in case of EC formulations, while drug diffusion as well as polymer relaxation had an influence on drug release mechanism in case of HPMC formulations. In case of CP and Na CMC formulations, polymer relaxation had a dominating influence on drug release mechanism than drug diffusion.

The in vivo pharmacokinetic studies of the selected CR matrix tablets of ZDV in rabbits were found to show a significant extension of serum ZDV concentrations and probable increase in the bioavailability of the drug than compared to immediate release tablets. The serum drug concentration levels were controlled and extended for longer duration thereby reducing the chances of dose-dependent side effects of ZDV.

UV Spectrophotometric and HPLC methods were developed in-house and validated for the analysis of various samples involved in the study. The developed analytical methods

were found to be accurate, precise, sensitive and suitable for the estimation of ZDV in variety of samples. The HPLC methods for the estimation of ZDV in stability and biosamples were found to be highly sensitive and were successfully used for the stability and in vivo studies.

The designed CR matrix tablets of ZDV are promising for commercialization and have demonstrated the chances for reduction of dose and frequency of administration, improvement in the patient's compliance and adherence (which is very vital in the treatment of HIV/AIDS, because of the chances of development of resistance if therapy is discontinued) to multi-dose anti-HIV therapy. The tablet manufacturing method was relatively simple and can be easily adopted in industries on a commercial scale.

However, further studies of the developed delivery systems have to be carried out in human subjects to establish the clinical effectiveness of the designed formulations. CR tablets of the drug can be prepared using other techniques like polymer coating in order to evaluate which technique would require lesser drug to polymer ratio for achieving desired release characters and with lesser cost.

Appendix

List of Publications and Presentations

Full Length Papers Published in Peer Reviewed Journals

1. Punna, R.R., Udaya, K.K., Saha, R.N., 2008. Controlled release matrix tablets of zidovudine: Effect of formulation variables on the in vitro drug release kinetics. AAPS PharmSciTech., 9, 302-313.
2. Punna, R.R., Sindhura, G., Saha, R.N., 2008. Design and in vitro evaluation of zidovudine oral controlled release tablets prepared using hydroxypropyl methylcellulose. Chem. Pharm. Bull., 56, 518-524.

Full Length Papers Published in Peer Reviewed Journals (out side the thesis work, but related to the topic)

1. Punna, R.R., Sindhura, G., Saha, R.N., 2007. Design and study of lamivudine oral controlled release tablets. AAPS PharmSciTech., 8, article 101.
2. Punna, R.R., Sajeev, C., Saha, R.N., 2006. Design and in vitro evaluation of oral controlled release formulations of celecoxib using optimization techniques. Yakugaku Zasshi, 126, 505-514.

Abstracts Published in Peer Reviewed Journals

1. Punna, R.R., Udaya, K.K., Saha, R.N., 2006. Design and evaluation of oral controlled release tablet formulations of zidovudine prepared using Ethyl cellulose and Carbopol 971P. The AAPS Journal, 8(S2), Abstract W4164.
2. Punna, R.R., Amit, M., Saha, R.N., 2004. Design of oral controlled release formulations of zidovudine using hydroxypropyl methylcellulose based tablets. The AAPS Journal, 6(4), Abstract T2145.
3. Punna, R.R., Amit, M., Saha, R.N., 2004. A new rapid, simple and validated UV spectrophotometric method for estimation of zidovudine in bulk, formulations and dissolution samples. The AAPS Journal, 6(4), Abstract T3054.

Paper Presentations at International and National Conferences

1. Design and evaluation of oral controlled release tablet formulations of zidovudine prepared using ethylcellulose and Carbopol 971P. 2006 AAPS Annual Meeting and Exposition, 2006, San Antonio, Texas, USA.

2. Design of oral controlled release formulations of zidovudine using hydroxypropyl methylcellulose based tablets. 2004 AAPS Annual Meeting and Exposition, 2004, Baltimore, Maryland, USA.
3. A new rapid, simple and validated UV spectrophotometric method for estimation of zidovudine in bulk, formulations and dissolution samples. 2004 AAPS Annual Meeting and Exposition, 2004, Baltimore, Maryland, USA.

Biography of Dr. Ranendra N. Saha

Dr. Ranendra N. Saha is Professor of Pharmacy and Dean, Faculty Division III and Educational Development Division, Birla Institute of Technology and Science, Pilani. He obtained his B. Pharm and M. Pharm (Pharmaceutics) degrees from Jadavpur University, Kolkata and Ph.D. from Birla Institute of Technology and Science, Pilani. He has more than 28 years of teaching and research experience and guided several doctoral, M. Pharm and B. Pharm students. He has many publications in reputed international and national journals and presented papers in international and national conferences in India and abroad. He has successfully completed several government and industry sponsored projects. Dr. Saha has developed commercial products for industries and transferred technologies of production to industries and filed patents. He is an expert member to various committees of UGC and other agencies and selection committee member of CSIR laboratories and several universities and colleges. He is also member of Board of Studies of several universities and colleges and visiting Professor to few universities.

Biography of Punna Rao Ravi

Mr. Punna Rao Ravi has completed his bachelor degree in Pharmacy (B. Pharm) from Birla Institute of Technology and Science, Pilani, in the year 2000 and Post graduation (M. Pharm) from Birla Institute of Technology and Science, Pilani, in 2002. He has been working as a faculty at BITS, Pilani since 2000 and continuing his Ph.D. work. He has published research articles in well renowned journals and presented papers in national and international conferences.