CHAPTER 2

LITERATURE REVIEW
2 Literature Review

The antiviral drugs are mainly used for the treatment in HIV infection. The Zidovudine and Nevirapine are Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV1). Both the drugs were selected for the present research work and proposed formulation was extended release (ER) matrix tablets. This chapter covers the literature review segment for Zidovudine and Nevirapine related formulation and drug profile.

2.1 Zidovudine

2.1.1 Patents

US patent no. 4917900 gives information for controlled release pharmaceutical formulation of Zidovudine and their use in the treatment of human retroviral infection such as AIDS. It also describes preparation method of controlled release spheroids with the use of nonionic polymer (alkyl ester of acrylic and methacrylic acid) in combination with ethyl cellulose.\textsuperscript{56}

US patent no. 20050175694A1 provides the information about the formulation of modified release tablet of Zidovudine and Lamivudine in combination and also alone, with use of polymer i.e. HPMC, guar gum and sodium alginate. The composition also contains water soluble diluents; the quantities of the hydrophilic polymers, the calcium salt and water soluble are such that the therapeutically effective active ingredients are released at a rate suitable for once daily administration of the pharmaceutical composition. The tablets are coated with a water soluble polymeric film coat.\textsuperscript{57}

EP patent no. 1501521B1 gives information of long acting composition of Zidovudine and Lamivudine with the use of different hydrophilic polymer for once a daily extended release tablets.\textsuperscript{58}

EP patent no. EP0550714B1 provides information for recovering Zidovudine from mixture of chemicals. This patent describes method of manufacturing of drug substance.\textsuperscript{59}
2.1.2 Publish Literature Review

Himansu B. S. *et al.* describe the design and characterization of oral sustained release matrix tablet of Zidovudine in order to improve efficacy and better patient compliance. The matrix tablets were prepared by wet granulation method using various proportions of hydrophilic polymers like Sodium CMC, HPMC (E.LV-15), Eudragit L155 & Xanthan gum alone or in combination with hydrophobic polymer i.e. ethyl cellulose. The *in vitro* drug release studies were performed using USP type II apparatus (rotary paddle type). The release kinetics was analyzed using Zero order, First order, Higuchi and Hixson Crowell. Compatibility of drug with various formulation excipients was also studied.\(^{60}\)

Margret C. *et al.* describe the formulation of sustained release matrix tablets of Zidovudine by using different drug polymer ratio. They have mainly used Kollidon SR, hydroxypropyl methylcellulose K15M, K100M for the preparation of matrix tablets by the wet granulation technology and evaluated the *in vitro* release up to 12 hrs.\(^{61}\)

Kar R.K. *et al.* give information about the preparation of Zidovudine matrix tablets with direct compression method by using various proportions of hydrophilic polymer i.e. Eudragit RS100 and RL100 alone or in combination with hydrophobic polymer ethyl cellulose. The dissolution study revealed that either Eudragit RS100 or RL100 10%, 20% w/w of tablet preparations were able to sustain the drug release up to 9 hours, but 30%, 40% as well as ethyl cellulose combination with 20% and 25% w/w of Eudragit RS100 and RL100 were able to sustain the drug release for 12 hours.\(^{62}\)

Raju P.N. *et al.* describe how the matrix tablets of Zidovudine were prepared by using Eudragit L 100, polyethylene oxide and carbopol 971P. The granules were prepared by different techniques i.e. direct compression, wet granulation by using water and isopropyl alcohol. The study gives idea about the feasibility of granulation process for the preparation of matrix tablets and their relation with *in vivo* drug release profile.\(^{63}\)
Jucimary V. S. et al. describe the study of different Zidovudine formulations containing polymers (both cellulosic and acrylic), in order to evaluate the influence of the compression force on the antiviral release from the matrix tablets. The formulation consists of Eudragit® RLPO, Eudragit® RSPO, HPMC as release controlling agent. The effect of the compression force on the drug release was analyzed and a statistically significant difference was observed (P < 0.05). Using lower compression forces leads to slightly better release profiles, i.e., profiles close to an ideal Higuchi kinetics for a total release of drug in a 12 hrs. period.\textsuperscript{64}

Kuksal A. et al. describe the preparation and characterization of ER matrix tablets of Zidovudine using hydrophilic Eudragit® RLPO and RSPO alone or their combination with hydrophobic ethyl cellulose. Release kinetics was evaluated by using United States Pharmacopeia (USP)-22 type I dissolution apparatus. Scanning electron microscopy was used to visualize the effect of dissolution medium on matrix tablet surface. Furthermore, they compared \textit{in vitro} and \textit{in vivo} of newly formulated ER tablets with conventional marketed tablet.\textsuperscript{65}

Emeje M. et al. describe formulation of oral sustained release matrix tablets of Zidovudine of different types, proportions and blends of carbopol 71G (C71) and a plant gum obtained from Abelmoschus esculentus (AEG). The effect of various formulation factors like polymer proportion, polymer type and pH of the dissolution medium on the \textit{in vitro} release of the drug was studied using the half change technique, in 900 ml of dissolution medium at 100 rpm.\textsuperscript{66}

Yadav A.S. et al. describe the formulation rational of manufacturing of ER Zidovudine tablet by using guar gum as rate controlling polymer and to evaluate drug release parameters as per various release kinetic models. The tablets were prepared by wet granulation method.\textsuperscript{67}
2.1.3 Drug Profile

2.1.3.1 Physicochemical Properties of Zidovudine

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{10}H_{13}N_{5}O_{4}</td>
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<tr>
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<td>267.2</td>
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<tr>
<td>Chemical name</td>
<td>3-Azido-3-deoxythymidine</td>
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<tr>
<td>Appearance</td>
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<tr>
<td>CAS No.</td>
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<td>Polymorphism</td>
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<tr>
<td>Solubility</td>
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</tr>
<tr>
<td>pKa</td>
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<tr>
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<tr>
<td>Optical rotation</td>
<td>[\alpha]2D 5, +99° (c = 0.5 in water)</td>
</tr>
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</table>

2.1.3.2 Pharmacological Properties of Zidovudine

Mode of action:
Zidovudine is an antiviral agent, which is highly active in vitro against retroviruses including the Human Immunodeficiency Virus (HIV). Zidovudine is phosphorylated in both infected and uninfected cells to the monophosphate (MP) derivative by cellular thymidine kinase. Subsequent phosphorylation of Zidovudine-MP to the diphosphate (DP) and then the triphosphate (TP) derivative is catalyzed by cellular thymidylate kinase and non-specific kinases respectively. Zidovudine-TP acts as an inhibitor of and substrate for the viral reverse transcriptase. The formation of further proviral DNA is blocked by incorporation of Zidovudine-MP into the chain and subsequent chain termination.
Competition by Zidovudine-TP for HIV reverse transcriptase is approximately 100-fold greater than for cellular DNA polymerase alpha.

**Absorption:**
Zidovudine is well absorbed from the gut and at all dose levels studied; the bioavailability was 60-70%. From a bioequivalence study, steady-state mean (CV %) C[ss]max, C[ss]min and AUC[ss] values in 16 patients receiving Zidovudine 300 mg tablets twice daily were 8.57 (54%) microM (2.29 μg/ml), 0.08 (96%) microM (0.02 μg/ml) and 8.39 (40%) h .microM (2.24h.μg/ml) respectively.

**Distribution:**
From studies with intravenous Zidovudine, the mean terminal plasma half-life was 1.1 hours, the mean total body clearance was 27.1 ml/min/kg and the apparent volume of distribution was 1.6 lit/kg. In adults, the average cerebrospinal fluid/plasma Zidovudine concentration ratio 2 to 4 hours after dosing was found to be approximately 0.5. Data indicate that Zidovudine crosses the placenta and is found in amniotic fluid and foetal blood. Zidovudine has also been detected in semen and milk. Plasma protein binding is relatively low (34-38%) and drug interactions involving binding site displacement are not anticipated.

**Metabolism:**
Zidovudine is primarily eliminated by hepatic conjugation to an inactive glucuronidated metabolite. The 5’glucuronide of Zidovudine is the major metabolite in both plasma and urine, accounting for approximately 50-80% of the administered dose eliminated by renal excretion. 3’-amino-3’- deoxythymidine (AMT) has been identified as a metabolite of Zidovudine following intravenous dosing.

**Excretion:**
Renal clearance of Zidovudine greatly exceeds creatinine clearance indicating that significant tubular secretion takes place.
Adverse Effects

Most commonly reported adverse reactions in adult HIV-1 clinical studies were hematologic toxicity, including neutropenia and anemia & symptomatic myopathy, lactic acidosis and severe hepatomegaly with steatosis. Hepatic decompensation in patients co-infected with HIV-1 and hepatitis C.

2.2 Nevirapine

2.2.1 Patents

US patent no. 5366972 gives information for the preparation of novel 5, 11-dihydro-6H-dipyrido [3,2-b:2,3-e][1,4] diazepine i.e. Nevirapine and use of these compounds in the prevention or treatment of HIV infection.73

US patent no. 8212025B2 describes the improved process for the preparation of Nevirapine with good quality and purity. It also describes various methods for the production of commercial scale drug substance.74

US patent no. 20100278918 provides information about pharmaceutical composition for the preparation of Nevirapine ER tablets and its method of manufacturing. The rate controlling polymer used for the preparation of ER tablet is HPMC. It also gives information about in vivo bio-availability of different strengths of Nevirapine and dependent and independent claims.75

US patent no. 6680383B1 gives information about manufacturing process of Nevirapine. It also describes the concentration of intermediates for the preparation of final drug substance. Patent claims the method of manufacturing of drug substance.76

2.2.2 Publish Literature Review

Iris Usach et al. describe the bioavailability of Nevirapine in rats after oral and subcutaneous administration, in vivo absorption from gastrointestinal segments and effect of bile on its absorption from duodenum. It also provides the information about the method of analysis and statistical conclusion of the study.77
Kappelhoff B.S. et al. give information about pharmacokinetics of Nevirapine once-daily versus twice-daily dosing in the 2NN study. They also describe in vivo performance of once-daily Nevirapine. They give the details of the in vivo study design. The daily exposure to Nevirapine (AUC\(_{24h}\)) was similar for the 400 mg once-daily and the 200 mg twice-daily dosing regimens. The C\(_{\text{min}}\) of Nevirapine is lower and the C\(_{\text{max}}\) of Nevirapine is higher for the once-daily regimen as compared to the twice-daily regimen.\(^{78}\)

Sreeraj M. et al. describe the in vitro and in vivo correlation for Nevirapine extended release tablets. The pharmacokinetics of extended release formulations were assessed in a parallel group study with healthy volunteers and compared with corresponding in vitro dissolution data obtained using a USP apparatus type 1. The deconvolution of the in vivo concentration time data was performed using the UIR to estimate an in vivo drug release profile. A linear model with a time-scaling factor clarified the relationship between in vitro and in vivo data.\(^{79}\)

Michael J. L. et al. have evaluated the single dose pharmacokinetics and bioavailability of Nevirapine in healthy volunteers.\(^{80}\)

### 2.2.3 Drug Profile\(^{68,69,70,81-84}\)

#### 2.2.3.1 Physicochemical Properties of Nevirapine

- **Molecular formula**: \(\text{C}_{15}\text{H}_{14}\text{N}_{4}\text{O}\)
- **Molecular weight**: 266.3
- **Chemical name**: 11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one; (anhydrous)

![Chemical Structure of Nevirapine](image)
2.2.3.2 Pharmacological Properties of Nevirapine

Mechanism of Action

Nevirapine is a non-nucleoside reverse transcriptase inhibitor of HIV-1. Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of Nevirapine does not compete with template or nucleoside triphosphates. HIV-2 RT and eukaryotic DNA polymerases (such as human DNA polymerases α, β, γ, or δ) are not inhibited by Nevirapine.

Antiviral Activity

The antiviral activity of Nevirapine has been measured in a variety of cell lines including peripheral blood mononuclear cells, monocyte-derived macrophages and lymphoblastoid cell lines. In an assay using human embryonic kidney 293 cells, the median EC50 value (50% inhibitory concentration) of Nevirapine was 90 nM against a panel of 2923 isolates of HIV-1 that were primarily (93%) clade B clinical isolates from the United States. The 99th percentile EC50 value was 470 nM in this trial. The median EC50 value was 63 nM (range 14-302 nM, n=29) against clinical isolates of HIV-1 clades A, B, C, D, F, G, H.
and circulating recombinant forms CRF01_AE, CRF02_AG and CRF12_BF. Nevirapine had no antiviral activity in cell culture against group O HIV-1 isolates (n=3) or HIV-2 isolates (n=3) replicating in cord blood mononuclear cells. Nevirapine in combination with efavirenz exhibited strong antagonistic anti-HIV-1 activity in cell culture and was additive to antagonistic with the protease inhibitor ritonavir or the fusion inhibitor enfuvirtide. Nevirapine exhibited additive to synergistic anti-HIV-1 activity in combination with the protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir and the NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zidovudine. The anti-HIV-1 activity of Nevirapine was antagonized by the anti-HBV drug adefovir and by the anti-HCV drug ribavirin in cell culture.

Absorption and Bioavailability
Nevirapine is readily absorbed (greater than 90%) after oral administration in healthy volunteers and in adults with HIV-1 infection. Absolute bioavailability in 12 healthy adults following single-dose administration was 93 ± 9% (mean ± SD) for a 50 mg tablet and 91 ± 8% for an oral solution. Peak plasma Nevirapine concentrations of 2 ± 0.4 mcg/mL (7.5 µM) were attained by 4 hours following a single 200 mg dose. Following multiple doses, Nevirapine peak concentrations appear to increase linearly in the dose range of 200 to 400 mg/day. Steady-state trough Nevirapine concentrations of 4.5 ± 1.9 mcg/mL (17 ± 7 µM), (n=242) were attained at 400 mg per day. Nevirapine tablets and suspension have shown to be comparably bioavailable and interchangeable at doses up to 200 mg. When Viramune (200 mg) was administered to 24 healthy adults (12 female, 12 male), with either a high-fat breakfast (857 kcal, 50 g fat, 53% of calories from fat) or antacid (Maalox® 30 mL), the extent of Nevirapine absorption (AUC) was comparable to that observed under fasting conditions. In a separate trial in HIV-1 infected subjects (n=6), Nevirapine steady-state systemic exposure (AUCτ) was not significantly altered by didanosine, which is formulated with an alkaline buffering agent. Nevirapine may be administered with or without food, antacid or didanosine.
Distribution

Nevirapine is highly lipophilic and is essentially non-ionized at physiologic pH. Following intravenous administration to healthy adults, the apparent volume of distribution (Vdss) of Nevirapine was 1.21 ± 0.09 L/kg, suggesting that Nevirapine is widely distributed in humans. Nevirapine readily crosses the placenta and is also found in breast milk. Nevirapine is about 60% bound to plasma proteins in the plasma concentration range of 1-10 mcg/mL. Nevirapine concentrations in human cerebrospinal fluid (n=6) were 45% (±5%) of the concentrations in plasma; this ratio is approximately equal to the fraction not bound to plasma protein.

Metabolism and Elimination

*In vivo* trials in humans and *in vitro* studies with human liver microsomes have shown that Nevirapine is extensively bio transformed via cytochrome P450 (oxidative) metabolism to several hydroxylated metabolites. *In vitro* studies with human liver microsomes suggest that oxidative metabolism of Nevirapine is mediated primarily by cytochrome P450 (CYP) isozymes from the CYP3A and CYP2B6 families, although other isozymes may have a secondary role. In a mass balance/excretion trial eight healthy male volunteers were dosed to steady state with Nevirapine 200 mg given twice daily followed by a single 50 mg dose of $^{14}$C-Nevirapine. Approximately 91.4 ± 10.5% of the radiolabeled dose was recovered, with urine (81.3 ± 11.1%) representing the primary route of excretion compared to feces (10.1 ± 1.5%). Greater than 80% of the radioactivity in urine was made up of glucuronide conjugates of hydroxylated metabolites. Thus, cytochrome P450 metabolism, glucuronide conjugation and urinary excretion of glucuronidated metabolites represent the primary route of Nevirapine biotransformation and elimination in humans. Only a small fraction (less than 5%) of the radioactivity in urine (representing less than 3% of the total dose) was made up of parent compound; therefore, renal excretion plays a minor role in elimination of the parent compound.

Nevirapine is an inducer of hepatic cytochrome P450 (CYP) metabolic enzymes 3A and 2B6. Nevirapine induces CYP3A and CYP2B6 by approximately 20-25%, as indicated by erythromycin breath test results and urine metabolites. Autoinduction of CYP3A and
CYP2B6 mediated metabolism leads to an approximately 1.5-2 fold increase in the apparent oral clearance of Nevirapine as treatment continues from a single dose to two to four weeks of dosing with 200-400 mg/day. Autoinduction also results in a corresponding decrease in the terminal phase half-life of Nevirapine in plasma, from approximately 45 hours (single dose) to approximately 25-30 hours following multiple dosing with 200-400 mg/day.

**Biotransformation**

In humans, Nevirapine is extensively metabolised via the oxidative cytochrome P450 to several hydroxylated metabolites, which subsequently are glucuronide. *In vitro* studies with human liver microsomes suggested that the metabolic pathway was primarily mediated by cytochrome P450 isozymes from the CYP3A family and to a lesser degree CYP2B6.

**Adverse effects**

Serious adverse effects can occur with Nevirapine. The most serious side effects have included hepatitis, hepatic failure, Stevens-Johnson syndrome, toxic epidermal necrolysis and hypersensitivity reactions. Hepatitis/hepatic failure may be isolated or associated with signs of hypersensitivity which may include severe rash or rash accompanied by fever, general malaise, fatigue, muscle or joint aches, blisters, oral lesions, conjunctivitis, facial edema, eosinophilia, granulocytopenia, lymphadenopathy or renal dysfunction.

**2.2.4 Innovator Drug Product**

**Summary of pack insert**

The reference drug product for Nevirapine 400 mg extended release (ER) tablet is manufactured and marketed by Boehringer Ingelheim and FDA approval was obtained in the year 2011. This section summarizes the literature information available for the innovator product.
Composition

The innovator product contains the following inactive ingredients:

**Core tablets**: lactose monohydrate, hypromellose, iron oxide and magnesium stearate.

**Formulation and Manufacturing Process**

The manufacturing process for Nevirapine ER tablets is a conventional high shear wet granulation process, followed by fluid bed drying, milling, final blending and compression into tablets using a rotary tablet press.(Ref: EMEA).

**Pack Profile**

Available in HDPE bottles of 30s’ count, a child resistant closure (CRC).

**Storage**

Following are the storage instructions from the pack insert.

Store at 25°C (77°F), excursions permitted to 15-30°C (59-86°F) (see USP controlled room temperature).

**Indications and Usage**

For the treatment of HIV-1 infection in adults.

**Dosage and Administration**

Once daily taken orally with or without food.

**2.3 Quality by Design (QbD)**

Naseem A. C. *et al.* reported the application of quality by design (QbD) approach to the development of dispersible tablets. Critical material and process parameters are linked to the critical quality attributes of the product. Variability is reduced by product and process understanding which translates into quality improvement, risk reduction and productivity enhancement. The risk management approach further leads to better understanding of the risks, ways to mitigate them and control strategy is proposed commensurate with the level of the risk. Design space in combination with pharmaceutical quality management system provides for flexible regulatory approaches with opportunity for continuous
improvement that benefits patient and manufacturer alike. The current study through a QbD paradigm for a better patient compliance and product quality is incorporated. The quality target product profile, Initial risk analysis led to the identification of the critical quality attributes. Physicochemical characterization and compatibility studies of the drug with commonly used excipients were performed. Experiments were designed with focus on critical material and process attributes. Design space was identified and risk factors for all the possible failure modes were below critical levels after the implementation of control strategy. Compliance to the design space provides an opportunity to release batches in real time. In conclusion, QbD tools together with risk and quality management tools provided an effective and efficient paradigm to build the quality into dispersible tablet.85

Jun H. et al. reported how a combination of experimental design, optimization and multivariate techniques was integrated into the process development of a drug product to facilitate an in-depth process understanding and offer opportunities for developing control strategies to ensure product quality. A process DOE was used to evaluate effects of the design factors on manufacturability and final product CQAs and establish design space to ensure desired CQAs. Two types of analyses were performed to extract maximal information, DOE effect & response surface analysis and multivariate analysis (PCA and PLS). The DOE effect analysis was used to evaluate the interactions and effects of three design factors (water amount, wet massing time and lubrication time), on response variables (blend flow, compressibility and tablet dissolution). The design space was established by the combined use of DOE, optimization and multivariate analysis to ensure desired CQAs. Multivariate analysis of all variables from the DOE batches was conducted to study relationships between the variables and to evaluate the impact of material attributes/process parameters on manufacturability and final product CQAs. The integrated multivariate approach exemplifies application of QbD principles and tools to drug product and process development.86
Ales B. et al. reported quality-by-design (QbD) principle, including process analytical technology, is becoming the principal idea in drug development and manufacturing. The implementation of QbD into product development and manufacturing requires larger resources, both human and financial; however, large-scale production can be established in a more cost-effective manner and with improved product quality. The objective of the present work was to study the influence of particle size distribution in powder mixture for tableting and the settings of the compression parameters on the tablet quality described by the capping coefficient, standard deviations of mass and crushing strength of compressed tablets. Fuzzy models were used for modeling of the effects of the particle size distribution and the tableting machine settings on the tablet quality. The results showed that the application of mathematical models, based on the contemporary routinely measured quantities can significantly improve the trial-and-error procedures.\(^8\)

Mattias A. et al. reported use of multivariate methods in a screening design with several responses of a tablet formulation. The aim was to get a predictive model by using as few experiments as possible. Six designed factors and one uncontrolled factor were initially screened in a fractional factorial design assuming a linear model. The results were analyzed by partial least squares (PLS). A tablet formulation of desired tablet strength and a fast drug release profile was obtained with a high drug: filler ratio and high amount of disintegrant. If these variables were set at proper levels, it was possible to avoid addition of a surfactant despite its significant effect on the in vitro drug release profile.\(^8\)