CHAPTER 5

RESULT AND DISCUSSIONS
5.1 Characterization of Drug and Excipients

5.1.1 Characterization of Drug
From the section 4.3.1, it was confirmed that Zidovudine is complying the tests like of assay, infrared spectroscopy, polymorphic identity, dynamic vapor sorption.

5.1.2 Assay
The assay of Zidovudine was carried out by HPLC method which was found to be 99.70% w/w within the USP34 limit of 90-110% of label amount. The chromatogram of assay preparation by HPLC test is shown in figure 4.1.

5.1.3 Microscopy
From the microscopy study, it was concluded that drug sample appeared crystalline shaped, some longer shape particles were seen under cross-polarized light (Figure 4.2).

5.1.4 Infrared Spectrum
The study of the FTIR spectra of Zidovudine demonstrated that the characteristic absorption peaks for the carbonyl group at 1685 cm\(^{-1}\) and azido group 117 stretching at 2082. Both the test and standard spectra are matching and are given in figure 4.3.

5.1.5 Powder X-Ray Diffractometry
From the powder X-ray diffractometry (XRD), it was concluded that API does not exist in any polymorphic form (Figures 4.4 and 4.5).

5.1.6 Differential Scanning Calorimetry
The DSC thermogram of API was recorded and thermogram showed endotherm at 121.99°C with onset temperature at 111.09°C similar to that reported in literature (Figure 4.6).
5.1.7 Water Vapor Sorption Analysis

Dynamic vapor sorption studies were conducted at different humidity conditions at 25°C and API was found to be non-hygroscopic in nature and result is given in figure 4.7.

5.1.8 Stress Stability of Zidovudine

The results of stress stability of Zidovudine are recorded in table 4.3. All the impurities at stress and super stress conditions were found to be within acceptable limit and as per ICH limit.

5.1.9 Characterization of Excipients

All the excipients used in present research work like HPMC K4M premium CR, HPMC K15M premium CR, microcrystalline cellulose (Avicel PH101 and PH102), magnesium stearate and opadry white (Y-1-7000) were analyzed as per USP 34 and the certificates of analysis of all excipients are given in the appendix section.

5.2 Characterization of Innovators (Reference) Drug Product

From the physical characterization of innovator drug product (Retrovir®), it can be concluded that tablets packed in HDPE bottles are in 30s count and closed with a child resistant closure. Dissolution profile of innovator drug product was performed in OGD media i.e. water and is represented graphically in figure 4.8. More than 80% drug was released in 15 mins. The other excipients in innovator tablets are magnesium stearate, microcrystalline cellulose, polyethylene glycol, sodium starch glycolate, titanium dioxide, hypromellose.

5.3 API-Excipient Compatibility

The compatibility of API alone and their combination with other excipients was studied. The results for API-excipient compatibility study are recorded in table 4.7. In accordance with the stress data of API, a highest level of unknown impurity was observed at 50°C moist at 4 weeks condition. In API and HPMC combination, all the known and unknown impurities at all three conditions were found to be within limit. In API & microcrystalline cellulose combination,
highest level of impurity C was observed at 4 weeks 50°C moist condition. Also, with magnesium stearate, highest level of impurity was observed at 4 weeks at 40°C/75% RH. From the data of compatibility, it was concluded that all excipients are compatible with Zidovudine.

5.4 Formulation Development

5.4.1 Initial Concept
The purpose was to develop controlled release formulation of Zidovudine. Formulation was kept similar to immediate release (IR) tablets except controlled release excipient i.e. HPMC. Initially both granulation approaches were tried. Initial trial batches ZID/01 and ZID/02 were taken with dry granulation process. Various processing problems like flow of lubricated blend through hopper not proper and less hardness were observed with dry granulation. In wet granulation method, initially non aqueous granulation with isopropyl alcohol (IPA) was tried, but it was observed that consumption of the IPA to achieve desired granulation was very high and also residual solvent content of final coated tablet showed presence above residual solvent limit. Hence, next batch ZID/06 was granulated with aqueous solvent. This batch showed faster release i.e. more than 80% drug was released within 14 hrs. containing 16% polymers in tablet. Hence, it was decided to use 20-25% of polymer level for further development.

5.4.2 Design Batches of Zidovudine ER Matrix Tablets
The different design of experiment was designed to evaluate the effect of different grades of HPMC as matrix former for Zidovudine 300 mg tablets and details are given in table 4.10. The dissolution release profiles of these batches are given in figure 4.9. The batch ZID/07 was manufactured with 25% polymers ratio (i.e. HPMC K15M and HPMC K100M). With the use of these high viscosity grade polymers during dissolution; erosion of tablets was observed. The dissolution profile of batch ZID/08 with 20% of polymers ratio (HPMC K15M & K100M), drug release was slow and after 16 hrs. less than 45% drug was released as shown in figure 4.9. This was due to the use of both higher viscosity grade polymers i.e. HPMC K15M and HPMC K100M. The batches ZID/08 & ZID/09 manufactured with higher grade of polymers showed slow release profile. The dissolution profile of batch ZID/10 and ZID/11 with use of 20% and 25% ratio of HPMC K4M & K15M respectively was comparable but still batch ZID/11 was considered for in-vivo drug study on animal.
5.4.3 *In-vivo* Drug Release Study

The release patterns of Zidovudine from immediate release (IR) marketed tablet and from different designed batches of formulated matrix tablet are illustrated in figures 4.8 and 4.9. The immediate release formulation showed complete drug release dissolution (99.5%) within 30 mins in OGD media i.e. water. Tablets containing release modifiers exhibited slow release of Zidovudine as compared to conventional formulation. Zidovudine has pH-independent solubility and is absorbed uniformly throughout the GIT. The successful sustained release formulation must show pH-independent release. The commercially available conventional tablet produces initial high plasma concentration owing to absence of release modifiers, which may cause unwanted toxic effects like bone marrow depression that sometimes leads to withdrawal of drug therapy; hence it is essential to maintain the plasma level within the therapeutic index for eliminating these toxic effects.

The release rate kinetic data for all the models i.e. for immediate release tablet (MF 0066) and matrix tablet batch (ZID/11) for *in-vivo* studies is shown in table 4.11. Drug release data of conventional tablet was fitted in first order equation ($r^2 = 0.936$), while drug release data of batch ZID/11 matrix tablets showed good fit into the Higuchi equation ($r^2 = 0.991$). Tablets of batches ZID/11 showed high linearity with Korsemeyer equation ($r^2 = 0.999$) indicating combined effect of diffusion and erosion mechanisms for controlled drug release.

Plasma concentration and pharmacokinetic parameters after oral administration of formulated matrix tablets and conventional tablets are summarized in table 4.12 and figure 4.11. Sustained blood level of drug was evident after oral administration of the conventional formulation. Although, plasma concentration-time profile was characterized by significantly ($P < 0.05$) higher plasma concentration after 1.46 hours of administration, and then the plasma concentration declined rapidly. The formulated matrix tablets (batch ZID/11) showed significantly lower $C_{max}$ than conventional tablet ($P < 0.05$) and required significantly more time to reach $C_{max}$ ($T_{max} 4.3 \pm 0.2$ hours) as compared to conventional tablets ($T_{max} 1.45 \pm 0.2$ hours). However, these tablets maintained constant plasma concentration for more than 20 hours. Due to low therapeutic index (0.4-4.0 μmol/L), Zidovudine is known to exhibit dose-dependent side effects resulting in withdrawal of therapy. The smooth and extended absorption phase coupled with maintenance of plasma concentration for longer duration after administration in matrix tablets suggests reduced chance of dose-dependent side effects of Zidovudine.
A good correlation between the dissolution profiles and bioavailability was observed and illustrated in figure 4.10. The \textit{in vitro} & \textit{in vivo} (IVIVC) correlation was determined by plotting a graph showing the fraction of drug absorbed versus the fraction of drug released \textit{in vitro}. A high value of correlation coefficient (\(r^2 = 0.9082\)) suggested good correlation between \textit{in vitro-in vivo} (IVIVC). Based on the IVIVC correlation data of conventional tablets versus extended release tablet, batch ZID/11 gave more sustained plasma concentration for more than 20 hours as compared to the conventional tablet. This can be expected to reduce the frequency of administration and decrease the dose dependent side effects associated with repeated administration of conventional tablets. So, formulation of batch ZID/11 was considered as optimum formulation and further development was performed on this formulation.

5.4.4 Manufacturing Process optimization

Based on the \textit{in vivo} result and IVIVC correlation of batch ZID/11 with conventional formulation; manufacturing process was optimized on the final composition as given in table 4.13. The different process parameters like dry mixing time, granulation kneading time, water quantity, blending time, compression and coating parameters were optimized on different batches.

5.4.4.1 Optimization of Dry Mixing Time

Blend uniformity samples were withdrawn at 5 mins, 7 mins and 10 mins of dry mixing time and results are recorded in table 4.14 and represented graphically in figure 4.12. Based on data, 7 mins dry mixing time could be considered as optimum as % RSD is minimum, individual values are within a narrow range and near to 100%.

5.4.4.2 Optimization of Kneading Time

In the wet granulation process, the kneading time of the formulation was very important parameter. Under granulation or over granulation was possible due to changes in kneading time during granulation. To evaluate impact on drug product’s quality like physical attributes, dissolution, manufacturing process and physical properties of granules; batches ZID/16 and ZID/18 were prepared with optimum and high kneading time. The observations for different batches are recorded in table 4.15. Batch with increase in kneading time from 4 mins 30 sec to 7
mins 10 sec showed increase in % fines below #60 sieve; indicating increase in finer fraction of blend due to over kneading and also harder granules were seen. Kneading time has significant impact on physical properties of granules. For the sizing of higher kneading, batch (ZID/18) will take long time as compared to optimum batches and previous design batches. In the compression; slight pinhole was observed because of higher proportion of coarser granules in final lubricated blend. This appearance issue (pinhole) was resolved during film coating as batch size of this formulation with higher kneading is less i.e.1000 units, but at higher scale it may have significant impact on appearance and may not be resolved with film coating. Dissolution profile of high kneading batch was slow as compared with optimum batch as shown in figure 4.13 because of harder granules formation. Significant impact was seen on drug product’s quality like physical attributes, dissolution of this process parameter. Based on this study and formula composition details, it is recommended to keep minimum kneading time for wet mass.

5.4.4.3 Optimization of Inlet Drying Temperature
Two batches having higher inlet temperature (ZID/20) and lower inlet (ZID/21) temperature during drying were formulated to see the effects on physical characteristics of granules and degradation of product at stability. The process results are summarized in table 4.16 and stability results are recorded in table 4.17. With the increase in the inlet temperature, there was no significant change in physical parameters but the unknown impurity (0.20%) level after 3 months at 40°C/75% RH was out of the limits. So, based on the formula composition, excipients compatibility details and stress stability data; it was advisable not to dry product at higher inlet temperature. Target inlet temperature of 60°C ±5°C (55°C - 65°C) during drying should be kept.

5.4.4.4 Impact of LOD of Dried Granules on Compression Parameters
In the batch ZID/23B with 0.81% LOD, slight pinhole was observed due to very low moisture but batch ZID/23A with 1.71% LOD compression parameters was found to be satisfactory. So, based on the formula composition and available LOD data of various batches, LOD range was kept between 1.5-2.0% as a precautionary measure.
5.4.4.5 Optimization of Lubrication Time
For the optimization of lubrication time, blend uniformity data was generated on batch ZID/28 at 3 mins and 5 mins lubrication time to see the effect on assay & blend uniformity (BU) of the formulation. Based on this, it can be concluded that lubrication time would not have critical impact on product quality like assay and CU. Based on the available data, 3 mins could be considered as optimum blending time as % RSD is minimum as compared to 5 mins lubrication time.

5.4.4.6 Optimization of Compression Parameters
The batch ZID/31 was taken at a higher scale to see the process feasibility and different compression parameters like compression machine speed, compression run times and hardness range. The results of each parameter are recorded in tables 6.20 to 6.22 respectively. The observations drawn are given below.

- Based on the available data of compression; it is advisable to keep the compression machine speed range at 30-60 rpm, but target speed for compression is 40 rpm. Compression parameters were found to be within acceptable limits at different speed range of machine.
- There was no significant effect at different stages of compression run i.e. start, middle and end of compression on the physical parameters and content uniformity.
- Dissolution profile was found to be satisfactory at different hardness ranges i.e. low, optimum and high hardness as shown in figure 4.14.

5.4.4.7 Optimization of Film Coating Parameters
For the optimization of film coating parameters, three different batches i.e. ZID/35 (Optimum spray rate and optimum temperature), ZID/39A (High spray rate and low exhaust temperature) and ZID/39A (Low spray rate and high exhaust temperature) were taken to see the effect on the appearance and impurity of drug substance. The aqueous coating was performed with opadry white (Y-1-7000) 15% solution to achieve desired appearance. The results of coating parameters are summarized in table 4.23. There is no impact on physical characteristics of tablet except LOD is high (2.90%) in batch ZID/39 with high spray rate and as compared to other two batches. The impurity profile was similar in all three batches and within the limit.
5.4.4.8 Final Composition and Process

Based on the lab scale and process optimization batches, final composition and manufacturing process for Zidovudine ER matrix tablets was arrived and is given in table 4.24. Process flow chart for manufacturing process is given in figure 4.15.

5.4.4.9 Scale up of Optimized Formulation

The scale up batch ZID/SCA/01 of Zidovudine ER matrix tablets 300 mg was prepared at 1X to 5X scale and all final process parameters for validation batches are given in table 4.25. All the physical parameters were found to be satisfactory and the analytical results were within the acceptable limit. *In vitro* (figure 4.17) study showed that dissolution profile of scale up batch was similar with batch ZID/11 (*in vivo* batch) and \( f_2 \) value was found to be 71. Stability studies carried out at an accelerated condition show that developed formulation is stable as shown in table 4.27. From the multimedia dissolution of scale up batch, it was observed that drug release was slow in pH 4.5 acetate buffer as compared to pH 1.2 and pH 6.8 buffers.