CHAPTER – 7

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS
7.1 SUMMARY

The therapeutic efficacy of a drug product intended to be administered by the oral route depends on its absorption by the gastro-intestinal tract. It is well established that dissolution is frequently the rate-limiting step in the gastrointestinal absorption of a drug from a solid dosage form. Poorly soluble drugs have been shown to be unpredictable and are slowly absorbed as compared with drugs with higher solubility. Consequently, these drugs present great challenges to further development into bioavailable dosage forms. Hence it is important to enhance the aqueous solubility, dissolution rate and bioavailability of these drugs from its oral solid dosage forms. Solid dispersion technique and cyclodextrin complexation have been used as effective methods to improve the dissolution properties and bioavailability of poorly water-soluble drugs. This study has demonstrated the possibility of markedly improving the dissolution performance of NVP by solid dispersion technique and complexation with cyclodextrins.

NVP has been used in the treatment of HIV infections. The major drawback in the therapeutic application and efficacy of NVP as oral dosage forms was its very low aqueous solubility because of its hydrophobic nature. The drug belongs to BCS class II and absorption (rate) of class-II drugs can be enhanced by accelerating the dissolution. Therefore, a favourable formulation which can enhance
solubility and dissolution rate of this model drug may help effectively in the therapeutic area of HIV prevention and treatment. Thus, studies were carried out to improve the solubility and hence dissolution rate, efficiency and bioavailability of poorly soluble drug NVP through solid dispersion technique using dextran fraction as a novel carrier and also inclusion complexation with βCD and its derivative HPβCD.

The brief introduction about HIV and AIDS therapy and its current limitations were given in Chapter 1. The currently marketed anti-HIV drugs and strategy for the treatment of HIV infection were highlighted. Furthermore, in this chapter introduction on drug solubility, dissolution rate and various approaches to improve the solubility, particularly on solid dispersion technology and inclusion complexation was elaborated. In addition, this chapter discussed general information on SDs as well as inclusion complexation with CDs and reviews the application of both techniques in drug delivery and pharmaceutical formulations.

The aims, study objectives and then plan of investigations of the present study were discussed in Chapter 2.

Chapter 3 dealt with the literature review which included an overview on anti-HIV agents and outlined their drawbacks responsible for poor or low bioavailability. Literature survey related to past research work on solid dispersion technology using various carriers
and also inclusion complexation with CDs was also included in this chapter. Chapter 3 further, discussed about past research work on antiretroviral SDs and antiretroviral inclusion complexation.

Chapter 4 discussed about materials used, analytical and experimental methods employed in the present investigation. The first part of this chapter provided drug profile and then excipient profile. Then later part of this chapter described in detail about method of preparation of solid dispersions of NVP with DEX and solid binary systems with CDs. SDs of NVP were prepared using a DEX fraction of low molecular weight in two different ratio of drug and carrier (3:1 and 1:1 w/w) by three methods i.e., kneading, microwave irradiation and freeze drying method. Solid binary systems of NVP with βCD and HPβCD (1:1M and 1:2M) were prepared by four methods such as kneading, solvent evaporation, microwave irradiation and freeze drying methods. The preceding part of this chapter dealt with the methods for characterization of SDs and also solid binary systems in solution and solid state. Characterization in solution state was made by drug content uniformity, phase solubility studies, saturation solubility studies, $^1$HNMR spectroscopic studies and in vitro dissolution studies. Characterization in solid state was done by various analytical techniques such as FT-IR, XRD, DSC and SEM studies.

Chapter 5 summarized all the research results of SDs as well as solid binary systems obtained according to our plan of investigations.
as described in chapter 4. This chapter provided two sections for ease of presentation. Discussion in section 5.2 dealt with the results of SDs and section 5.3 dealt with the results of solid binary systems.

All the research results of SDs and solid binary systems of NVP have been discussed in detail in Chapter 6. The first section of this chapter i.e., 6.1 discussed about research results of SDs whereas, the later section, 6.2 is committed to discuss about the results of solid binary systems of NVP with both CDs studied.

All SDs prepared were found free flowing. The percentage drug content and yield were estimated to confirm that there was no degradation of drug and expected amount of drug was present in obtained product. Low standard deviation (SD) and coefficient of variation (CV) values in the drug content of SDs indicated uniform drug distribution in all prepared batches.

Solubility studies of pure NVP in increasing weight fractions of DEX demonstrated the relative affinity of the drug for the carrier. The saturation solubility studies of SDs confirmed that solubility of NVP from all SDs was method dependent and increase in enhancement of solubility was found in the following rank order in both ratio studied,

\[ \text{FD} > \text{MW} > \text{KM} > \text{PM} > \text{Pure NVP} \]

\(^1\text{H} \) NMR spectroscopic studies of NVP alone, DEX alone, NVP:DEX PMs and SDs were presented to gain insight into interactions between
drug and DEX. It was evident from the $^1$H NMR studies that the upfield shifting of freeze dried product demonstrated its superiority over other preparations.

FT-IR spectra of all SDs prepared by different methods were compared with the corresponding PMs as well as drug alone. The FT-IR studies of SDs suggested the possibility of intermolecular hydrogen bonding between amide group of NVP and the hydroxyl group of the carrier dextran. DSC thermogram of NVP was compared with the NVP:DEX PMs and SDs prepared by different methods. SDs prepared by all methods at both ratio exhibited little shift of NVP endothermic peak with significant change in the heat flow. This change in heat flow indicated the change in a crystallinity of drug and attesting a decrease in crystallinity or partial change in crystal form of drug in SDs. These results were justified by FT-IR and XRD studies. In powder XRD studies, pure drug peak at 21.16° (2θ) was used for calculating RDC of values of SDs prepared by all methods. All SDs exhibited lower RDC values than the respective PMs indicated decrease in crystallinity. However a marked decrease in the crystalline character was observed in freeze dried product as reflected by the lowest RDC values compared to other SDs prepared at 3:1 and 1:1 ratio. These observations were further justified by the results obtained by DSC studies. Morphological features of the NVP:DEX SDs prepared by different methods were examined by SEM analysis. A change in the original morphology, size and shape of both NVP and DEX particles
were observed in SDs, being the morphology influenced by the preparation method adopted. The SEM pictures of all SDs revealed the formation of large masses of undifferentiated particles, which were different from those of raw materials.

All SDs and their corresponding PMs were tested for dissolution properties and compared with the pure NVP to assess the benefits of preparing SDs. *In vitro* dissolution studies of pure NVP, PMs and its SDs were carried out in 900 ml of 0.1N HCl using a USPXXIV type 2-dissolution rate test apparatus by the powder dispersed method (powder samples were spread over the dissolution medium). The release of NVP from PMs and SDs was measured spectrophotometrically at 314 nm. The *in vitro* results of pure drug, PMs and its SDs were computed by using dissolution software PCP DISSO V.3.0. The results of the dissolution rate studies indicated higher dissolution rate of NVP from SDs when compared to NVP itself and the corresponding PMs. The $DE_{30}$ and $DE_{60}$ values of the SDs prepared by kneading, microwave and freeze drying methods were relatively high when compared with the values from the PMs and NVP alone. Further, it was evident that the drug dissolution was higher in 1:1 drug to carrier ratio compared to 3:1 ratio. Overall the rank order of improvement in dissolution properties of NVP in both ratios with different methods was found in the following rank order,

\[
FD > MW > KM > PMs > Pure \text{ NVP}
\]
The release pattern in NVP, PMs and all SDs were found to be first order (best fit model) compared to Hixson-Crowell’s cube root model. Significant differences in the means of DE$_{30}$ values of NVP, NVP:DEX PMs and SDs were tested at 95% confidence with One-way ANOVA using Dunnett multiple comparison test. The DE$_{30}$ values of NVP:DEX PMs and all SDs prepared by different methods were significantly higher (P < 0.01) when compared to the DE$_{30}$ values of NVP alone.

The section 6.2 discussed the results of solid binary systems of NVP with βCD and HPβCD. All solid binary systems prepared were found to be free flowing. The drug content estimations of all preparations confirmed that there was no degradation of the drug and expected amount of drug present in the obtained product. Low standard deviation (SD) and coefficient of variation (CV) values in the drug content of solid binary systems indicated uniform drug distribution in all prepared batches.

The phase solubility studies of NVP with βCD and HPβCD were carried out in 0.1N HCl according to Higuchi and Connors. The phase solubility profiles were classified as A$_{L}$-type, which indicated the formation of 1:1 stoichiometric complex with respect to βCD and HPβCD concentrations. The apparent stability constant was much greater with HPβCD compared to βCD.

The saturation solubility studies of solid binary systems confirmed that NVP solubility from all solid binary systems were method
dependent as well as type of CDs. The increase in enhancement of solubility was found in the following rank order,

\[
\text{HP\textbeta}CD > \beta CD \\
FD > MW > KM > PM > \text{Pure NVP}
\]

The \textsuperscript{1}H NMR spectroscopic studies were carried out for NVP solid binary systems with both CDs. The \textsuperscript{1}H NMR spectra of solid binary systems were compared with the spectra of individual components. In the presence of NVP, both H-3 and H-5 inner portion of \beta CD and HP\textbeta CD underwent a consistent downfield shift, which demonstrated a clear involvement of the hydrogen atoms in host-guest interactions. The insertion of NVP into \beta CD and HP\textbeta CD cavities was clearly demonstrated by changes in proton chemical shift values of NVP and as well as CDs protons in NVP:\beta CD and NVP:HP\textbeta CD solid binary systems.

FT-IR spectra of all solid binary systems prepared by different methods were compared with the corresponding PMs as well as drug alone. In the spectra of solid binary systems of NVP with \beta CD prepared by kneading and solvent evaporation method, N-H streching of amide group of the drug was shifted towards higher wavelength. In case of microwave irradiation and freeze drying method, N-H of drug and OH of \beta CD overlapped and resulted in broadening of bands in the region of 3000-3500 cm\textsuperscript{-1}. Further, C=O streching of drug in all solid binary systems shifted to higher wavelength. Similar results were observed in case of solid binary systems of NVP with HP\textbeta CD. The shift
in peaks associated with N-H and C=O groups of drug indicated the interaction with βCD and HPβCD through hydrogen bonding, which could result in its inclusion into the hydrophobic cavity of the cyclodextrin.

The DSC thermograms of NVP were compared with the solid binary systems of NVP with both CDs prepared by kneading, solvent evaporation, microwave irradiation and freeze drying methods. DSC thermograms indicated gradual reduction in NVP endothermic peak intensity as well as melting enthalpy (ΔH) values. This decrease in melting enthalpy (ΔH) values in solid binary systems with both the CDs indicated partial to moderate interaction with respect to the method and CD suggested decrease in crystallinity. These results were further justified by XRD studies.

The powder XRD studies of NVP solid binary systems with both CDs revealed that the crystallinity of NVP was significantly reduced, evidenced by marked decrease in intensity of peaks. The peak intensities of 1:2M binary systems were lower than the corresponding 1:1M binary systems in both cases. From the RDC values, it was observed that there was decrement in crystallinity in all 1:1M and 1:2M solid binary systems and found in the order of FD > MW > SE > KM > PM. The XRD results of all solid binary systems suggested that no alteration in the crystal structure of NVP, but the crystallinity being modified and reduced to a considerable extent. FT-IR and DSC
studies also supported the same hypothesis, which was confirmed by the XRD results.

Morphological features of the NVP solid binary systems with both the CDs were examined by scanning electron microscopy. Original morphology of NVP as well as βCD disappeared in all solid binary systems prepared by kneading, solvent evaporation, microwave irradiation and freeze drying methods and tiny aggregates of small irregular pieces were present. Further, a change in the size and shape of HPβCD and drug particles were observed in all solid binary systems. All SEM pictures of solid binary systems revealed the interaction between drug and CDs in the solid state and were in accordance with the results obtained from FT-IR, XRD and DSC studies.

The in vitro dissolution rate tests were used to characterize the inclusion complexation between drug and cyclodextrin. In vitro dissolution studies of pure NVP, PMs and its solid binary systems were carried out in 900 ml of 0.1N HCl using a USPXXIV type 2-dissolution rate test apparatus by the powder dispersed amount method (powder samples were spread over the dissolution medium). The release of NVP from PMs and solid binary systems were measured spectrophotometrically at 314 nm. The dissolution rate of NVP was significantly improved after preparing its solid binary systems with both CDs when compared to NVP alone. Higher dissolution rates and dissolution efficiency of NVP from all solid binary systems with both
CDs was in accordance with the good stability constant for complexation between NVP and CDs obtained from phase solubility studies.

The DE\textsubscript{30} and DE\textsubscript{60} values of the solid binary systems prepared by different methods were relatively high when compared with the values from the PMs and NVP alone. Comparatively, the increment in drug dissolution from the HP\(\beta\)CD solid binary systems was higher than the corresponding ones with the \(\beta\)CD. Overall the rank order of dissolution efficiency of various solid binary systems with \(\beta\)CD and HP\(\beta\)CD prepared by different methods were found method dependent as well as type of CD and was in the following rank order,

\[ \text{HP\(\beta\)CD} > \text{\(\beta\)CD} \]

\[ \text{FD} > \text{MW} > \text{SE} > \text{KM} > \text{PM} > \text{Pure NVP} \]

Further, the DE\textsubscript{30} and DE\textsubscript{60} values of the solid binary systems with both CDs prepared by freeze drying method were higher than those prepared by other method. These results were supported by other parameters such as XRD, DSC and FTIR studies. Dissolution profiles of all solid binary systems were analysed according to Hixson-Crowell’s cube root law and first order kinetics. The release of drug from the preparations followed predominantly first order kinetics compared to Hixson-Crowell’s cube root law according to correlation coefficient (r) values.
Significant differences in the means of $\text{DE}_{30}$ values of NVP, PMs and solid binary systems with $\beta$CD and HP$\beta$CD were tested at 95% confidence with One-way ANOVA using Dunnett multiple comparison test. The $\text{DE}_{30}$ values of all solid binary systems with both CDs at 1:1 ratio prepared by different methods were significantly higher ($P < 0.01$) compared to the $\text{DE}_{30}$ values of corresponding PMs and NVP alone. Whereas, the $\text{DE}_{30}$ values of PMs and solid binary systems with both CDs at 1:2 ratio prepared by different methods were significantly higher ($P < 0.01$) compared to the $\text{DE}_{30}$ values of NVP alone.
7.2 CONCLUSIONS

Solid dispersions of NVP with DEX and solid binary systems of NVP with βCD and HPβCD were successfully prepared and evaluated. The following conclusions were drawn from the current investigations.

- All SDs and solid binary systems prepared were found free flowing and low standard deviation (SD) and coefficient of variation (CV) values in drug content estimation indicated uniform drug distribution in all prepared batches.

- Solubility studies of pure NVP in increasing weight fractions of DEX fraction demonstrated the relative affinity of the drug for the carrier. The saturation solubility studies of SDs and solid binary systems prepared by all methods showed enhancement of solubility as compared to pure drug alone.

- The phase solubility studies of NVP with βCD and HPβCD indicated the formation of 1:1 stoichiometric complex with respect to βCD and HPβCD concentrations. Solubility enhancement was much greater with HPβCD, compared to βCD.

- Changes in proton chemical shift values of NVP in SDs as well as solid binary systems demonstrated the interaction between drug and carriers. Further, it was evident from the $^1$H NMR studies that the upfield shifting of freeze dried product demonstrated its superiority over other preparations.

- The FT-IR studies of SDs with DEX fraction and solid binary systems with βCD and HPβCD suggested the possibility of
intermolecular hydrogen bonding between amide group of NVP and the hydroxyl group of the carriers.

- DSC studies of all SDs and solid binary systems (with both CDs) demonstrated the decrease in melting enthalpy ($\Delta H$) values which indicated decrease in crystallinity or partial change in crystal form of the drug.

- The XRD results of all SDs and solid binary systems suggested that no alteration in the crystal structure of NVP, but the crystallinity being modified and reduced to a considerable extent. Further, lower RDC values of SDs and solid binary systems compared to respective PMs indicated decrease in NVP crystallinity.

- All SEM pictures of SDs and solid binary systems revealed the interaction between the drug and carriers in solid state and were in accordance with the results obtained from FT-IR, XRD and DSC studies.

- The dissolution rate of NVP was significantly improved after preparing SDs with DEX fraction and solid binary systems with $\beta$CD as well as HP$\beta$CD compared to drug alone.

- The DE$_{30}$ and DE$_{60}$ values of the SDs prepared by kneading, microwave and freeze drying methods were relatively high compared to the values from the PMs and NVP alone.

- Drug dissolution in SDs was higher in 1:1 drug to carrier ratio compared to 3:1 ratio. Overall rank order of enhancement in dissolution properties of NVP in both ratios with different methods were found in the following rank order,
The DE_{30} and DE_{60} values of the solid binary systems prepared by kneeling, solvent evaporation, microwave and freeze drying methods were relatively high compared to the values from the PMs and NVP alone.

Overall rank order of dissolution efficiency of various solid binary systems with βCD and HPβCD prepared by different methods were found method dependent as well as type of CD and was in the following rank order,

$$\text{HPβCD} > \beta\text{CD}$$

The DE_{30} and DE_{60} values of SDs with DEX and solid binary systems with both CDs prepared by freeze drying method were higher than those prepared by other methods.

The release pattern in NVP, PMs, SDs and solid binary systems were found to be first order and the best fit model was first-order and then followed Hixson-Crowell’s cube root model.

One-way ANOVA was used to analyse significant differences in the means of DE_{30} values of NVP, NVP:DEX PMs and SDs. The DE_{30} values of PMs and all SDs prepared by different methods were significantly higher (P < 0.01) compared to the DE_{30} values of NVP alone. Similarly, the DE_{30} values of all solid binary systems prepared with βCD and HPβCD by different methods were
significantly higher \((P < 0.01)\) compared to the \(\text{DE}_{30}\) values of corresponding PMs and NVP alone.

- On the basis of the results, it could be concluded that the type of CD employed and also formulation method adopted to prepare solid binary systems have significant impact on NVP dissolution properties.

- The overall study demonstrated the feasibility of preparing SDs of NVP with low molecular weight DEX fraction as a novel carrier and also solid binary systems with βCD and HPβCD to improve the solubility and dissolution rate of the model drug.
7.3 RECOMMENDATIONS

The purpose of this research was to develop a suitable drug delivery system which could increase the solubility and dissolution rate of poorly soluble antiretroviral drug NVP. The hypothesis of this research is successful through the preparation of solid dispersions (SDs) of NVP with low molecular weight dextran (DEX) as a novel carrier and also solid binary systems with βCD and HPβCD. In our study, all three carriers i.e., dextran, βCD and HPβCD were able to enhance dissolution efficiency of the model drug NVP, which could minimize variable dissolution rates with increase in oral bioavailability. Hence, the study strongly recommends that developed SDs of NVP with dextran and solid binary systems with both CDs can be further utilised for the formulation of suitable dosage form such as tablets. Further, the drastic increase in the aqueous solubility of NVP afforded by the solid dispersion and complexation approach will enable new formulation technologies to be tested in the development of new more effective anti-HIV products. Furthermore, the in vitro and in vivo RT inhibition activity (anti-HIV activity) of NVP in animal models needs to be evaluated in the formulated state.