CHAPTER X
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The thesis describes factorial and formulation studies carried out to determine the effects of cyclodextrins and surfactant in enhancing the solubility, dissolution rate and bioavailability of efavirenz and ritonavir two BCS class II drugs. The thesis consists of 11 Chapters.

Objectives of the investigation are described in Chapter I. Efavirenz and ritonavir widely prescribed anti retroviral drugs belong to class II under BCS and exhibit low and variable oral bioavailability due to their poor aqueous solubility. They are practically in soluble in water and aqueous fluids. As such their oral absorption have dissolution rate limited and they requires enhancement in solubility and dissolution rate for increasing their oral bioavailability. Among the various approaches complexation with cyclodextrins has gained good acceptance in recent years in pharmaceutical industry for enhancing the solubility and dissolution rate of poorly soluble water drugs. Surfactants also have ability to increase the solubility of lipophilic water-insoluble drugs by micellar solubilization process. Though cyclodextrin complexation and use of surfactants for enhancing the solubility and dissolution rate of poorly water soluble drugs have been investigated individually, no reports are available on their combined use in enhancing the solubility and dissolution rate. In the present investigation cyclodextrins (βCD and HPβCD) and surfactant, Solutol HS15 were tried to enhance the solubility, dissolution rate and bioavailability of efavirenz and ritonavir.

The major objective is to evaluate the individual main effects and combined (interaction) effects of cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 on the solubility, dissolution rate and bioavailability of efavirenz and ritonavir in a
series of $2^2$ factorial experiments and also to evaluate the feasibility of formulating efavirenz and ritonavir tablets with enhanced dissolution rate and dissolution efficiency employing drug-CD-surfactant complex systems. The other objectives include: to evaluate the dissolution kinetics and characteristics of Drug-CD and Drug-CD-Surfactant inclusion complexes and tablets formulated employing them; to evaluate the compatibility of the selected drugs with cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 by FTIR and DSC studies; Pharmacokinetic evaluation of efavirenz-βCD and efavirenz-βCD-Solutol HS15 complexes in comparison to efavirenz pure drug with a view to evaluate their in vivo performance and to evaluate the stability of selected tablets formulated employing drug-CD-Solutol HS15 inclusion complexes.

Literature on dissolution rate, bioavailability and methods of enhancing dissolution rate and bioavailability is given in Chapter II. Literature on cyclodextrin complexation is reviewed in Chapter III. Drug profiles and past research work on drugs investigated is reviewed in Chapter IV. Analytical methods used in the study are described in Chapter V. U.V. Spectrophotometric methods were used for estimation of efavirenz and ritonavir in in vitro studies. A known HPLC method was used for the estimation of efavirenz in plasma samples.

Factorial studies on the effects of cyclodextrins and SolutolHS15 on the solubility and dissolution rate of efavirenz and ritonavir are described in Chapter VI. In the present study cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 were tried to enhance the solubility and dissolution rate of efavirenz and ritonavir two widely prescribed anti retroviral drugs. The individual main effects and combined (interaction) effects of cyclodextrins and surfactants on the solubility and
dissolution rate of efavirenz and ritonavir were evaluated in a series of $2^2$ factorial experiments. From the results obtained the following conclusions are drawn.

1. The solubility of efavirenz was markedly enhanced by cyclodextrins (βCD and HPβCD) and by surfactant Solutol HS15. A 4.35 and 30.37 fold increase in the solubility of efavirenz was observed respectively with βCD (5mM) and Solutol HS15 (2%) when used alone. A combination of βCD (5mM) and Solutol HS15 (2%) gave a 54.43 fold increase in the solubility of efavirenz.

2. A 1.28 and 30.37 fold increase in the solubility of efavirenz was observed respectively with HPβCD (5mM) and Solutol HS15 (2%). A combination of HPβCD (5mM) and Solutol HS15 (2%) gave a 14.76 fold increase in the solubility of efavirenz.

3. The individual main effects as well as combined effects of CDs (βCD and HPβCD) and surfactant Solutol HS15 in enhancing the solubility of efavirenz are highly significant ($P < 0.01$).

4. The order of increasing enhancement in the solubility of efavirenz observed with various CDs and Surfactants was Solutol HS15 > βCD > HPβCD.

5. Among all combinations, βCD-Solutol HS15 gave greater enhancement in the solubility of efavirenz (54.43 fold). The order of increasing enhancement in the solubility of efavirenz observed with various combinations was βCD-Solutol HS15 > HPβCD-Solutol HS15 > HPβCD.

6. The solubility of ritonavir was also markedly enhanced by cyclodextrins (βCD and HPβCD) and by surfactant Solutol HS15. A 1.57 and 21.72 fold increase in the solubility of ritonavir was observed respectively with βCD (5mM) and Solutol HS15 (2%) when used alone. A combination of βCD
(5mM) and Solutol HS15 (2%) gave a 28.97 fold increase in the solubility of ritonavir.

7. A 1.47 and 21.72 fold increase in the solubility of ritonavir was observed respectively with HPβCD (5mM) and Solutol HS15 (2%). A combination of HPβCD (5mM) and Solutol HS15 (2%) gave a 22.31 fold increase in the solubility of ritonavir.

8. The order of increasing enhancement in solubility of ritonavir observed with various CDs and Surfactant was Solutol HS15> βCD >HPβCD.

9. Among all the combinations, βCD-Solutol HS15 exhibited greater enhancement in the aqueous solubility (28.97fold) of ritonavir. The order of increasing enhancement observed with various combinations was βCD-Solutol HS15> HPβCD-Solutol HS15.

10. The individual main effects as well as combined effects of CDs (βCD and HPβCD) and surfactant Solutol HS15 in enhancing the solubility of ritonavir are highly significant (P < 0.01).

To evaluate the individual main and combined effects of cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 on the dissolution rate of efavirenz and ritonavir solid inclusion complexes of Drug-CD-Surfactant were prepared in each case as per $2^2$ factorial design and were evaluated. From the results obtained the following conclusions are drawn.

11. Drug-CD and Drug-CD-Surfactant complexes gave rapid and higher dissolution of efavirenz and ritonavir when compared to the corresponding pure drug.

12. Drug Dissolution from all CD-Surfactant complexes prepared followed first order kinetics.
13. The dissolution rates \( (K_1) \) and Dissolution efficiency \( (DE_{15}) \) values were several times higher in the case of CD-Surfactant complexes when compared to those of pure drug with both efavirenz and ritonavir.

14. Among individual effects, CDs (βCD and HPβCD) gave higher enhancement in the \( K_1 \) and \( DE_{15} \) of efavirenz than the surfactant Solutol HS15. The order of increasing enhancement in \( K_1 \) and \( DE_{15} \) observed with various CDs and surfactants was \( βCD > HPβCD > Solutol \) HS15. βCD gave highest increase in \( DE_{15} \) (6.44 fold) and \( K_1 \) (5.50 fold) of efavirenz.

15. The results of ANOVA indicated that all individual and combined effects in enhancing the dissolution rate \( (K_1) \) of efavirenz were highly significant \( (P < 0.01) \).

16. Among the combined effects, βCD-Solutol HS15 gave highest enhancement in dissolution rate \( (K_1) \) of efavirenz (5.95 fold).

17. Among the individual effects, CDs (βCD and HPβCD) gave higher enhancement in the \( K_1 \) and \( DE_{15} \) of ritonavir than the surfactant Solutol HS15. The order of increasing enhancement in dissolution rate \( (K_1) \) observed with various CDs and surfactants was \( βCD > HPβCD > Solutol \) HS15.

18. The results of ANOVA indicated that all individual and combined effects in enhancing the dissolution rate \( (K_1) \) of ritonavir were highly significant \( (P < 0.01) \).

19. Among the combined effects, βCD-Solutol HS15 gave highest enhancement in dissolution rate \( (K_1) \) of ritonavir (8.66 fold).

20. The compatibility of the selected drugs (Efavirenz and Ritonavir) with βCD and surfactant Solutol HS15 used in the study was evaluated by FTIR and
DSC studies. FTIR spectra and DSC thermograms indicated no chemical interaction between efavirenz, ritonavir, βCD and surfactant, Solutol HS 15.

Factorial studies carried out on the formulation and evaluation of efavirenz and ritonavir tablets employing cyclodextrins and Solutol HS15 are described in Chapter VII. The feasibility of formulating the Drug-CD-Solutol HS15 complex systems into compressed tablets with enhanced dissolution rate was investigated. The individual main and combined (interaction) effects of CDs and Solutol HS15 on the dissolution rate of (i) Efavirenz and (ii) Ritonavir from tablet formulations was investigated in a series of $2^2$ – factorial experiments. Tablets of (i) Efavirenz (100 mg) and (ii) Ritonavir (100 mg) were formulated employing selected combinations of CD (βCD and HPβCD) and Solutol HS15 in each case as per a $2^2$ factorial design. The tablets were prepared by wet granulation method and were evaluated. From the results obtained the following conclusions are drawn.

1. Drug-CD and Drug-CD-Solutol HS15 complex systems could be formulated into compressed tablets by wet granulation method.

2. All the tablets prepared employing drug-CD and drug-CD-Solutol HS15 complex systems were of good quality fulfilling the official (I.P) standards with regard to hardness, friability, disintegration time and drug content.

3. Drug dissolution from the tablets formulated employing drug-CD and drug-CD-Solutol HS15 complexes followed first order kinetics with both efavirenz and ritonavir.

4. Tablets formulated employing CDs and Solutol HS15 gave relatively higher rates of dissolution ($K_1$) and dissolution efficiency ($DE_{30}$) values when compared to those of efavirenz plain tablets with both efavirenz and ritonavir.

5. The order of increasing dissolution rate ($K_1$) observed with various tablets was $E1$ (plain) < $E2$ (βCD) = $E6$ (HPβCD) < $E3$ (Solutol HS15) < $E8$ (

6. Efavirenz tablet formulations E4 and E8, which are formulated employing βCD-Solutol HS15 and HPβCD-Solutol HS15 respectively, gave much higher dissolution rates when compared to plain tablets, E1. A 42.5 and 34.2 fold increase in $K_1$ was observed respectively with formulations E4 and E8 when compared to formulation E1 (plain tablets).

7. The dissolution efficiency ( $DE_{30}$ ) was also increased from 4.56% for formulation E1 to 41.54 % and 36.59 % respectively for formulations E4 and E8.

8. Ritonavir tablet formulations R4 and R8, which are formulated employing βCD-Solutol HS15 and HPβCD-Solutol HS15 respectively, gave much higher dissolution rates when compared to plain tablets, R1. A 21.35 and 16.85 fold increase in $K_1$ was observed respectively with formulations R4 and R8 when compared to formulation R1 (plain tablets).

9. The dissolution efficiency ( $DE_{30}$ ) was also increased from 7.29% for Formulation R1 (plain tablets) to 43.32 % and 39.36  % respectively for formulations R4 and R8.

10. ANOVA indicated that the individual main effects of βCD, HPβCD and Solutol HS15 and their combined effects in enhancing the dissolution rate ($K_1$) of efavirenz and ritonavir tablets were highly significant ($P<0.01$).

11. βCD and Solutol HS15 alone gave an enhancement of 9.7 and 12.5 fold in the dissolution rate ($K_1$) of efavirenz tablets respectively. Whereas in combination βCD-Solutol HS15 gave 42.5 fold increase in the dissolution rate.
12. HPβCD and Solutol HS15 alone gave an enhancement of 9.7 fold and 12.5 fold in the dissolution rate (K\textsubscript{1}) of efavirenz tablets respectively. Whereas HPβCD and Solutol HS15 in combination gave a 34.2 fold increase in the dissolution rate.

13. βCD and Solutol HS15 alone gave an enhancement of 4.75 and 6.10 fold in the dissolution rate (K\textsubscript{1}) of ritonavir tablets respectively. Whereas in combination βCD-Solutol HS15 gave 21.35 fold increase in the dissolution rate.

14. HPβCD and Solutol HS15 alone gave an enhancement of 4.85 fold and 6.10 fold in the dissolution rate (K\textsubscript{1}) of ritonavir tablets respectively. Whereas in combination HPβCD and Solutol HS15 gave a 16.85 fold increase in the dissolution rate.

Pharmacokinetic evaluation was done on efavirenz -βCD and efavirenz -βCD-Solutol HS15 complexes in comparison to efavirenz pure drug with a view to evaluate their \textit{in vivo} performance. These \textit{in vivo} studies are described in Chapter VIII. From the results obtained the following are the conclusions drawn.

1. Efavirenz was found to be absorbed slowly when given orally and a peak plasma concentration (C\textsubscript{max}) of 11.35±0.7µg/ml was observed at 4.0 h after administration. The absorption rate constant (K\textsubscript{a}) was found to be 0.4859 h\textsuperscript{-1}.

2. All the pharmacokinetic parameters (Table 8.2) namely C\textsubscript{max}, T\textsubscript{max}, K\textsubscript{a} and (AUC)\textsubscript{0-∞} indicated rapid and higher absorption and bioavailability of efavirenz when administered as CD complexes.

3. The absorption rate constant (K\textsubscript{a}) was found to be 1.7583 h\textsuperscript{-1} 2.3918 h\textsuperscript{-1} respectively with Efavirenz - βCD (1:2) and Efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes, whereas in the case of efavirenzK\textsubscript{a} was only 0.4859 h\textsuperscript{-1}.
4. A 3.62, and 4.92 fold increase in the $K_a$ was observed respectively with efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes when compared to efavirenz pure drug.

5. (AUC)₀^∞ (extent of absorption) was also much higher in the case of CD complexes when compared to efavirenz pure drug. (AUC)₀^∞ was increased from 111.50 µg.h/ml for efavirenz pure drug to 174.35 and 207.27 µg.h/ml for efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes respectively.

6. A 1.56 and 1.85 fold increase in (AUC)₀^∞ was observed respectively with efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes when compared to efavirenz pure drug.

7. The biological half-life ($t_{1/2}$) was found to be 4.77, 3.91 and 4.58 h respectively following the oral administration of efavirenz, and its CD complexes, efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) indicating that the elimination characteristics of efavirenz have not changed when it was administered as CD complexes.

8. βCD has markedly enhanced both the rate ($K_a$) and extent (AUC) of absorption (i.e. bioavailability) of efavirenz. Addition of Solutol HS15 has further enhanced both the rate of absorption and extent of absorption of efavirenz from efavirenz – βCD- Solutol HS15 (1:2:0.05) complex.

Stability studies carried out are described in Chapter IX. The stability of tablets formulated employing Drug-CD-Solutol HS15 complex was evaluated as per ICH guidelines. Drug content and dissolution profiles of the tablets remained unaltered after storage for 3 months at $40^\circ \pm 2^\circ$ C and $75 \pm 5$ % RH. The fast dissolution
characteristics of the tablets remained unaltered during the storage period with both efavirenz and ritonavir.

**Innovativeness of the Research Work:**

The combined use of cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 is an innovative and novel approach for enhancing the solubility, dissolution rate and bioavailability of poorly soluble drugs. Solutol HS15, a non ionic surfactant has not been studied alone or in combination with cyclodextrins for enhancing the solubility, dissolution rate and bioavailability of BCS class II drugs. A systematic investigation in the present work revealed that the individual main effects as well as combined effects of CDs (βCD and HPβCD) and surfactant, Solutol HS15 in enhancing the solubility and dissolution rate ($K_1$) of efavirenz and ritonavir are highly significant ($P < 0.01$). Combination of Solutol HS15 with CDs (βCD and HPβCD) resulted in a much higher enhancement in the solubility and dissolution rate ($K_1$) of efavirenz and ritonavir than is possible with CDs and Solutol HS15 alone. βCD- Solutol HS15 gave 54.43 and 28.97 fold increase in the solubility of efavirenz and ritonavir respectively. βCD- Solutol HS15 also gave a 5.95 and 3.81 fold increase in the dissolution rate ($K_1$) of solid inclusion complex of efavirenz and ritonavir respectively. FTIR studies indicated no physical and chemical interaction between the selected drugs and CDs and Solutol HS15.

Drug-CD-Solutol HS15 inclusion complexes could be formulated in to tablets by wet granulation method and the resulting tablets also exhibited rapid and higher dissolution rate ($K_1$) and dissolution efficiency ($DE_{30}$) values when compared to plain tablets and tablets containing CDs alone. Efavirenz tablets formulated employing drug- βCD- Solutol HS15 gave a 42.5 fold increase in the dissolution rate of efavirenz when compared to plain tablets. Ritonavir tablets formulated employing drug-βCD-
Solutol HS15 gave 21.35 fold increase in the dissolution rate of ritonavir when compared to plain tablets. In the *in vivo* pharmacokinetic evaluation, efavirenz -βCD-Solutol HS15 inclusion complex exhibited a 4.92 fold increase in the absorption rate ($K_a$) and 1.85 fold increase in the (AUC) $\infty$ when compared to efavirenz pure drug. Tablets formulated employing drug-CD-Solutol HS15 inclusion complexes were also quite stable with regard to fast dissolution rate characteristics during stability studies.

**Hence a combination of cyclodextrins (βCD and HPβCD) and solutol HS15 is recommended for enhancing the solubility, dissolution rate and bioavailability of efavirenz and ritonavir and for formulation of their tablets with fast dissolution characteristics.**