Summary and Conclusions
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The enhancement of oral bioavailability of poorly water soluble drugs remains one of the most challenging aspects of drug development. Although there are many approaches available for improving dissolution rate and oral bioavailability, each method is having its own limitations. Utilization of cyclodextrins attracted the attention of many researchers for the enhancement of the dissolution rate.

βCDs are receiving more and more attention both in pharmaceutical formulations and drug carrier systems. Many of the βCDs are being used as carriers which increase the dissolution rate of the poorly soluble drugs due to their hydrophilic nature associated with complex formation and increasing wettability. Although many earlier studies on CDs were reported for enhancement of solubility and dissolution rate, very few comparative studies are reported. Similar is the case with methods of preparation of drug-CD complexes. Hence in the present investigation an attempt was made to compare the applicability of different CD derivatives βCD, HPβCD, RMβCD and SBE7βCD and influence of different methods of preparation in the development of complexes for improving dissolution and oral bioavailability.

In the present research work, two poorly soluble HIV-protease inhibitors, saquinavir (SQV) and ritonavir (RTV) were selected. The absorption of these drugs is dissolution rate limited and hence it is
proposed to prepare inclusion complexes with different CDs using different preparation methods to identify the best suitable CD derivative and ideal method in enhancing dissolution rate and oral bioavailability.

Physical mixing (PM), kneading technique (KN), co evaporation (COE), spray drying (SD) and freeze drying (FD) were used for the preparation of inclusion complexes of saquinavir using the selected βCD, HPβCD, RMβCD and SBE7βCD.

Based on the results obtained with SQV, physical mixing (PM), co evaporation (COE), spray drying (SD) and freeze drying (FD) were used for the preparation of inclusion complexes of ritonavir using HPβCD and RMβCD.

Phase solubility studies were performed for both the drugs using CDs indicated good enhancement in solubility. Phase solubility diagrams obtained were of $A_L$ type confirming complexation occurred at 1:1 molar ratio. Hence 1:1 molar ratio was fixed for preparation of complexes for both the drugs.

Drug-CD complexes were prepared by using the selected methods and the prepared complexes after drying were found to be fine free flowing powders. Low % CV values in the percent drug content ensured drug content uniformity in each batch and reproducibility of the method of preparation. 10-15% loss in drug was observed in co evaporation, spray drying and freeze drying, whereas no such loss was observed with physical mixing and kneading.
The dissolution studies were carried out on the prepared complexes of saquinavir and ritonavir in pH 6.8 phosphate buffer and in 0.1N HCl respectively. All complexes showed higher dissolution compared to pure drug. Freeze dried complexes showed higher dissolution for both drugs among different methods used for preparation of complexes. SBE7βCD improved good enhancement in dissolution for saquinavir compared to other CDs.

Different dissolution parameters like \( DE_{60}, DP_{30}, DE_{10}, T_{50} \) and MDT for both drugs were calculated. The calculated dissolution parameters were subjected for statistical analysis using one way analysis of variance test (ANOVA). The differences between complexes prepared by various methods were compared by using Tukey multiple comparison test. ANOVA test gave significant difference between the different methods and different CDs. Tukey multiple comparison test showed significant difference between pure drug and complexes prepared by different methods.

Complete drug release was observed in 60 min for saquinavir with SBE7βCD prepared by freeze drying technique. Between HPβCD and RMβCD used for ritonavir complexation, RMβCD complexes prepared by freeze drying showed rapid release of drug within 10 minutes where as pure ritonavir showed very less release and these two were considered as optimized drug-CD complexes and subjected for further studies.
Drug-CD interaction studies were carried out for the freeze dried complexes by FTIR, DSC, XRD and $^1$H-NMR and compared the spectra with respective pure drugs, CDs and physical mixture of drug-CDs in 1:1 molar ratio. SEM studies were done for pure drugs and their freeze dried complexes to study the morphological characters. The FTIR spectra of complexes showed substantial decrease in intensity and broadening compared to that of pure drugs alone indicating the interaction of drug and cyclodextrin and confirmed the formation of complex. DSC studies indicated the disappearance of characteristic endothermic peak of both the drugs in freeze dried complexes confirming the formation of complex. In XRD studies, SQV and RTV exhibited characteristic diffraction pattern, whereas in the case of inclusion complexes with all the CDs, there was considerable decrease in the intensity of the sharp peaks in the diffractograms of freeze dried complexes indicating the reduced crystallinity of the drug in all the complexes. This could be due to partial conversion of the drug to amorphous form from crystalline states. NMR studies proved complexation of drugs showing strong interaction between drug and CDs, which may be due to complex formation. SEM studies showed irregular shape with bulky particles, spherical shaped particles in case of spray dried complexes and elongated particles in case of freeze dried complexes. The change of particle’s shape and disappearance of original structure of drugs in freeze dried complexes indicated existence of single phase with loss of crystallinity of drugs which matched with XRD results.
Optimized complexes and pure drugs were filled into capsules to find out the suitability of converting into a dosage form. The capsules were subjected to uniformity of weight test and dissolution studies. All capsules were uniform in weight and showed similar dissolution profiles compared to the powdered complexes.

Stability studies were conducted on prepared capsules as per ICH guidelines. No visible physical changes were observed in all the formulations withdrawn from the stability chambers. The average drug content in all the formulations was found to be similar compared to initial sample. The drug release profiles of all the formulations did not change significantly after storage at 25±2°C/60±5%RH and 40±2°C/75±5%RH for a period of six months. The release profiles were found similar for saquinavir after calculating $f_1$ and $f_2$. The dissolution profiles of ritonavir remained unaltered.

Pharmacokinetic studies were conducted in healthy male Wistar rats showed marked increase in the AUC of saquinavir and ritonavir when both the drugs were administered orally in combination with cyclodextrins. Significant enhancement in bioavailability of both drugs was observed with optimized CD complexes compared to pure drugs. The pharmacokinetic parameters like $t_{\%}$, $T_{\text{max}}$ were in the range of the reported values. The absorption profile of SQV from oral SQV suspension was highly irregular and variable due to its poor solubility. This variation was not seen with SQV-SBE7βCD complex due to improvement in solubility.
The present study clearly indicated the superiority of SBE7βCD and RMβCD in improving the solubility and dissolution of poorly soluble drugs like SQV and RTV. Among the methods of preparation freeze drying technique was found to be more useful compared to other methods.

**Significant contributions of the present investigation**

1. For the first time, comparative studies with different CDs like SBE7βCD and RMβCD for improving bioavailability of BCS class II drugs, saquinavir and ritonavir were carried out.
2. Simultaneously studied the effect of method of preparation and influence of CD on enhancement of solubility and dissolution.
3. Quantities of CDs were minimized in the preparation of drug-CD complexes with the help of phase solubility studies.
4. With the help of FTIR, DSC, 1H-NMR, XRD and SEM studies formation of inclusion complexes was established.
5. Freeze drying method was found to be useful in improving the solubility and dissolution of both the drugs and the in vivo studies confirmed the improvement in oral bioavailability of these drugs.

Further studies on these complexes may lead to reduction of the dose of these drugs due to improved bioavailability.