CHAPTER-6

SUMMARY AND CONCLUSIONS

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Many new drug substances are only very slightly soluble or even practically insoluble. A substantial portion (40%) of these drugs fails full development, because of their poor and highly variable bioavailability. Upon peroral administration, very slightly water soluble or practically water-insoluble drugs have a limited and variable or erratic oral absorption. Further, the low solubility of such drugs limits their parenteral use. Low solubility of drug substances may result from hydrophobicity or high lattice energy. Highly hydrophobic drug substances possess insufficient capacity of molecular interactions with water, whereas molecules with high lattice energy resist to the weakening of the lattice upon molecular interactions with water. According to the law of Noyes-Whitney, low solubility yields a low concentration gradient towards the bulk of the solution and, thereby, a low dissolution rate. Therefore, absorption and bioavailability of orally administered drugs that possess good permeability, but low solubility, can be improved by increasing either the solubility or the surface area of the drug substance, both resulting in increased dissolution rates. And on the other hand, oral route is by far the most convenient one for drug administration. However, for oral administration, the low concentration gradient between the gut and blood vessel due to the poor solubility of the drug leads to a limited transport, consequently influencing the oral absorption. Among various approaches available for solubility and bioavailability improvement of such drugs, the formulation of nanosuspension and drug-cyclodextrin inclusion complex are very straight, effective and widely used techniques, which are having great impact on drug dissolution and absorption of drugs in the gastro intestinal tract.

The present study was aimed at the development of efficient nanosuspension and cyclodextrin inclusion complex for oral administration of Diacerein and Febuxostat to improve their bioavailability. Drug nanosuspensions were prepared by media milling technique and optimized by 3^3 full factorial designs while drug-cyclodextrin inclusion complexes were successfully prepared by slurry complexation method followed by freeze drying. The prepared formulation were characterized and subjected to in vitro/in vivo evaluations.

It was envisaged that drug nanosuspensions and drug-cyclodextrin inclusion complexes will improve solubility, dissolution rate and permeability which will ultimately increase absorption and hence bioavailability of selected poorly water soluble drugs.

6.1 Formulation and evaluation of DAR for bioavailability enhancement

6.1.1 DAR Nanosuspension

Results clearly indicated that the aim of the present study was fulfilled by developing an orally administrable and efficient nanosuspension of DAR with significantly improved bioavailability. In fact, this study has elaborated the role of media milling and P-407 in production of a potential nanosuspension. Moreover, data also revealed the importance of factorial design to understand the effect of various process variables on particle size and saturation solubility. Completely dried and fluffy powder was obtained by successful freeze drying of prepared DAR-NS with cryoprotectant, trehalose. The lyophilized powder was absolutely and easily re-dispersed in water. DSC and XRD studies supported each other that DAR lost its crystallinity after nanosizing leads to better dissolution properties and sustained stability of the drug during its shelf life. The SEM and TEM images of DAR-NS confirmed that the media milling process in presence of P-407 was effective in converting large aggregates of irregular shaped crystals of bulk DAR into submicron to nanometric sized particles which was in accordance with particle size obtained by DLS method. The dissolution profiles illustrated the superiority of DAR-NS over DAR-P and DAR-M in terms of % cumulative release of DAR, dissolution efficiency and mean dissolution time due to the nanosized particles of DAR in DAR-NS. The prepared DAR-NS was found physically and chemically stable over a time period of 6 months.

In vitro Cell Cytotoxicity Studies (MTT Assay) confirmed the biocompatibility of DAR-NS and explained that composition of nanosuspension did not contribute to toxicity of Caco2 cells. *In vitro* assessment of permeability using Caco-2 cell line model demonstrated that DAR-NS was successfully enhanced the permeability of DAR by 6.63 and 4.52 fold to DAR-P and DAR-M respectively. *In vivo* assessment demonstrated that DAR-NS exhibited better pharmacokinetic properties compared to DAR-P and DAR-M. The relative oral bioavailability of DAR in Albino rabbits resulted from DAR-NS was found 3.94 and 2.41 fold greater than DAR-P and DAR-M, respectively. Thus it can be inferred that media milling is efficient method for nanosizing of DAR with poloxamer 407 which further leads to improved dissolution properties and excellent oral bioavailability of DAR in DAR-NS.

6.1.2 DAR-Cyclodextrin inclusion complex

Another approach used for bioavailability enhancement of DAR was formulation of

drug-cyclodextrin inclusion complex. DAR-cyclodextrin inclusion complexes were successfully prepared by physical mixing, kneading and freeze drying methods. The results of phase solubility studies demonstrated that DAR formed more stable inclusion complex with HP-β-CD than those formed with β-CD, M-β-CD and γ-CD. Additionally the results of inclusion efficiency also stated that DAR:HP-β-CD inclusion complex in molar ratio of 1:2 was having superior solubilizing capacity and greater inclusion efficiency while others did not show satisfactory drug incorporation.

The results obtained by FTIR, DSC and XRD studies were in excellent agreement and confirmed the formation of true inclusion complex of DAR with HP-β-CD in 1:2 molar ratio by freeze drying method. Percentage assay of DAR in lyophilized inclusion complex of DAR:HP-β-CD in (1:2) molar ratio predicted the suitability of freeze drying method for production of inclusion complex. The prepared formulation was found physically and chemically stable at 5°C±3°C and at room temperature over the time period of 6 months. The freeze dried inclusion complex DAR:HP-β-CD in (1:2) molar showed a good performance in dissolution profile. The MTT assay of prepared formulation indicated the formation of a bio-tolerable inclusion complex. The P_{app} for Freeze dried inclusion complex was found 5.09 fold and 3.47 fold higher than the plain DAR and marketed formulation, respectively. The relative oral bioavailability of DAR in Albino rabbits resulted from Freeze dried inclusion complex was found 3.32 and 2.03 fold greater than plain DAR and marketed formulation, respectively.

The obtained results justified the selection of cyclodextrin, molar ratio and method of preparation for the formulation of efficient and stable inclusion complex of DAR with cyclodextrin. The outcome was supported by FTIR, DSC and XRD studies which further lead to enhanced dissolution properties, low cytotoxicity and improved bioavailability of DAR in inclusion complex with HP-β-CD.

6.2 Formulation and evaluation of Febuxostat for bioavailability enhancement

6.2.1 FBX Nanosuspension

The purpose of this study was to develop an orally administrable nanosuspension of poor water soluble drug, “FBX” with enhanced bioavailability and results clearly indicated the fulfilment of the aim. This research illustrated that an efficient nanosuspension loaded with high amount of drug could be perfectly prepared by media milling technique in presence of P-188. Study also revealed that how statistical designing played an important role in optimization and development of a formulation.

FBX nanosuspension was prepared by media milling technique and optimized using 3^3 full factorial designs. Efficient particle size (149.6±7.3nm) with low PDI (<0.2) of lyophilized FBX-NS was achieved using suitable excipients that provide physical stabilization and improved saturation solubility of water insoluble FBX in FBX-NS. Lyophilization of FBX-NS with trehalose produced a completely dried and fluffy powder with good redispersibility in water. DSC and XRD studies revealed that crystallinity of FBX in FBX-NS is reduced significantly after nanonization leads to better dissolution properties and sustained stability of the drug during its shelf life compared to pure FBX. TEM & SEM images confirmed that the media milling process in presence of poloxamer 188 was effective in converting large aggregates of irregular shaped crystals of bulk FBX into submicron to nanometric range with relatively narrow size distribution. Drug content and dissolution profile of FBX-NS again proved the suitability of method for particle size reduction. In short, characterization result revealed the effectiveness of FBX-NS in terms of increased saturation solubility, better dissolution properties, drug content and sustained stability during its shelf life compared to pure FBX. The prepared FBX-NS was found physically and chemically stable over a time period of 6 months. *In vitro* cell line studies confirmed the reduced cytotoxicity and enhanced permeability of formulation. The relative oral bioavailability of FBX from FBX-NS in Albino rabbits resulted from NS was found 2.59 and 1.93 fold greater than FBX-P and FBX-M, respectively. Thus it can be summarized that nanosuspension of FBX with poloxamer 188 produced by wet media milling technique has confirmed its efficiency in sense of increased saturation solubility, improved dissolution rate, enhanced permeability and bioavailability of poorly water soluble drug.

6.2.2 FBX-Cyclodextrin inclusion complex

This section of project was designed to improve bioavailability of FBX by developing a stable and orally administrable drug:cyclodextrin inclusion complex with better solubility, dissolution and bio-tolerability. FBX:Cyclodextrin inclusion complexes were prepared with β-CD, HP-β-CD, M-β-CD and γ-CD in 1:1, 1:2 and 1:3 molar ratios. The physical mixing, kneading method and freeze drying method were opted for preparation of inclusion complexes. Phase solubility study in liquid state and inclusion efficiency estimation of prepared solid complexes were performed to finalize the best suitable CD and molar ratio. Phase solubility study provides the stability constant for drug-cyclodextrin inclusion complex as well as it also present the insight into stoichiometry

of the complex at equilibrium. It indicated that FBX was uniformly distributed in FBX:HP-β-CD inclusion complex at all molar ratios while others did not show satisfactory drug incorporation. Results also showed that there are minor differences in the inclusion efficiencies of physical mixtures, kneaded mixture and freeze dried inclusion complex of FBX:HP-β-CD at all the three molar ratios, respectively which described that FBX:HP-β-CD in the molar ratio of 1:1 is sufficient to produce an efficient inclusion complex. The characterization results obtained by FTIR, DSC and XRD studies were in excellent agreement and confirmed the formation of true and stable inclusion complex of FBX with HP-β-CD in 1:1 molar ratio by freeze drying method. Percentage FBX content in lyophilized inclusion complex of FBX:HP-β-CD in (1:1) molar ratio was found to be 99.84±1.28%, indicating the suitability of freeze drying method for peoduction of inclusion complex. Stability studies concluded that inclusion complex of FBX with HP-β-CD in (1:1) molar ratio was stable for a period of 6 months and indicating its suitability for storage at 5°C±3°C and at room temperature.

The dissolution of FBX from inclusion complex with HP-β-CD in (1:1) molar ratio prepared by freeze drying method was found higher than the pure FBX and marketed formulation of FBX in phosphate buffer pH-6.8, acetate buffer pH-4.5, 0.1N HCl and water. The lyophilized inclusion complex of FBX showed significantly enhanced and highest dissolution rate in phosphate buffer pH-6.8 among all the selected dissolution mediums. In vitro cytotoxicity study using Caco-2 cell lines concluded that prepared freeze dried inclusion complex was safe and bio-tolerable. In-vitro permeability assessment of freeze dried inclusion complex of FBX:HP-β-CD::(1:1)M, plain FBX and marketed formulation was performed and found results were very much satisfacory and matching with the aim of the project.

In vivo assessment demonstrated that freeze dried inclusion complex of FBX:HP-β-CD::(1:1)M exhibited better pharmacokinetic properties compared to plain FBX and commercial formulation. The relative oral bioavailability of FBX in Albino rabits resulted from Freeze dried inclusion complex was found 3.08 fold and 2.29 fold greater than plain FBX and marketed formulation, respectively.

The obtained reults justified the selection of cyclodextrin, molar ratio and method of preparation for the formulation of efficient and stable inclusion complex of FBX with cyclodextrin. The outcome was supported by FTIR, DSC and XRD studies which further

lead to enhanced dissolution properties, low cytotoxicity and improved bioavailability of FBX in inclusion complex with HP-β-CD.

6.3 Conclusions

The results of present investigations conclusively indicate the remarkable enhancement in oral bioavailability of selected drugs in prepared nanosuspension and cyclodextrin inclusion complex formulations. Hence the developed formulations of Diacerein and Febuxostat can be potentially useful in clinical treatment of Osteoarthritis and chronic Gout, respectively. Thus these formulations hold promise as better alternative to the conventional dosage forms. However, further investigations in human beings under clinical conditions are necessary before they can be commercially exploited.