CHAPTER – 3

Objective and Plan of work

The main purpose of any formulation research is to deliver any particular drug in its most bioavailable form by designing a most suitable formulation that involves use of minimum number of excipients and easiest possible technology so as to keep the cost of development as low as possible.

The core objective of the present research work was to improve the oral bioavailability and therapeutic efficacy of drug, ezetimibe (Eze) by employing three formulation approaches namely, cocrystallization, ternary complexation and nanonization. As aimed, Eze cocrystals (CoCs) were prepared using nicotinic acid (NA) and nicotinamide (ND) as coformers; ternary cyclodextrin (CD) complexes of Eze were prepared with hydroxypropyl-β-cyclodextrin (HPBCD) using D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) or L-ascorbic acid-2-glucoside (AA2G) as a ternary substance; and Eze drug nanocrystals (NCs) were prepared using TPGS and AA2G as stabilizers.

With the above conceived objective on mind, the agenda of the present research work was to:

- Formulate Eze as CoCs (Eze-NA and Eze-ND); ternary CD complexes (Eze-HPBCD-AA2G and Eze-HPBCD-TPGS); and NCs (using AA2G and TPGS as stabilizers)
- Characterization of all the formulations prepared using each formulation approach and to compare the optimized formulation from each formulation approach with each other and also with a marketed tablet formulation, at *in-vitro*

and *in-vivo* levels to propose the most suitable formulation approach to deliver Eze with highest bioavailability

- ❖ Target highest improvement in oral bioavailability and therapeutic efficacy of Eze
- ❖ Study the possible dose reduction efficiency of the optimized formulations

Eze is a hypocholesterolemic, low water soluble, P-glycoprotein (P-gp) substrate. The excipients employed to formulate Eze were hydrophilic with or without additional potentials like P-gp inhibitory action or hypocholesterolemic action. We hypothesized that the selected formulations may serve to not only improve the solubility and dissolution properties of Eze *in-vitro* but also to enhance the *in-vivo* performance of Eze by either offering a synergistic hypocholesterolemic effect or improving its *in-vivo* absorption at the site of action, the small intestinal brush border.

As per the above stated objective, agenda and hypothesis, to improve the oral bioavailability and therapeutic efficacy of Eze, the study was planned in the following steps:

Step I: Preparation and characterization of Eze CoCs

- ➢ Development of UV-VIS analytical method for Eze analysis during the optimization preformulation studies and to report the *in-vitro* aqueous solubility and dissolution test results of the formulations – applicable to all the following studies and steps to follow
- ➢ Development of (HPLC-UV analytical method and) HPLC-UV bioanalytical method for bioanalysis of Eze in plasma samples following *in-vivo* pharmacokinetic studies in Male Albino Wister rats – applicable to all the following studies and steps to follow

- Formulation of Eze CoCs, Eze-NA and Eze-ND
- Optimization of the composition of Eze CoCs by solution state phase solubility and Job's plot studies
- Preparation of the optimized solid CoCs, Eze-NA and Eze-ND
- Solid state characterization of the optimized Eze-NA and Eze-ND using fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) and thermogravimetric analysis
- Microscopic examination of the optimized Eze-NA and Eze-ND using scanning electron microscopy (SEM)
- Assessment of *in-vitro* aqueous solubility and dissolution of the optimized Eze-NA and Eze-ND in USP acetate buffer of pH 4.5, containing 0.45% w/v sodium lauryl sulphate (SLS) as the medium as suggested by the FDA Dissolution Methods Database guide for Eze
- Assessment of stability of the optimized Eze-NA and Eze-ND at 30±2 ˚C/70±5% RH in terms of appearance, drug content, aqueous solubility and dissolution
- *In-vivo* pharmacokinetic studies of the optimized Eze-NA and Eze-ND following oral administration of formulations to Male Albino Wister rats
- *In-vivo* antihypercholesterolemic activity study of the optimized Eze-NA and Eze-ND following oral administration of formulations to Male Albino Wister rats
- Arriving at the best performing solid CoC considering the cumulative *in-vitro* and *in-vivo* performances

Step II: Preparation and characterization of ternary CD complexes of Eze

- Formulation of Eze ternary CD complexes, Eze-CD-AA2G and Eze-CD-TPGS
- Optimization of the composition of Eze-CD-AA2G and Eze-CD-TPGS by solution state phase solubility, Job's plot studies and Benesi–Hildebrand plots
- Preparation of the optimized solid ternary CD complexes, Eze-CD-AA2G and Eze-CD-TPGS
- Solid state characterization of the optimized Eze-CD-AA2G and Eze-CD-TPGS using FTIR, DSC and PXRD
- Microscopic examination of the optimized Eze-CD-AA2G and Eze-CD-TPGS using SEM
- Assessment of *in-vitro* aqueous solubility and dissolution of the optimized Eze-CD-AA2G and Eze-CD-TPGS in USP acetate buffer of pH 4.5, containing 0.45% w/v SLS as the medium as suggested by the FDA Dissolution Methods Database guide for Eze
- Assessment of stability of the optimized Eze-CD-AA2G and Eze-CD-TPGS at 30±2 ˚C/70±5% RH in terms of appearance, drug content, aqueous solubility and dissolution
- *In-vivo* pharmacokinetic studies of the optimized Eze-CD-AA2G and Eze-CD-TPGS following oral administration of formulations to Male Albino Wister rats
- *In-vivo* antihypercholesterolemic activity study of the optimized Eze-CD-AA2G and Eze-CD-TPGS following oral administration of formulations to Male Albino Wister rats
- Arriving at the best performing optimized solid ternary CD complex considering the cumulative *in-vitro* and *in-vivo* performances

Step III: Preparation and characterization of Eze drug NCs

- Formulation of Eze NCs, AA2G NCs and TPGS NCs
- Optimization of AA2G NCs and TPGS NCs by application of suitable experimental designs (Plackett-Burman and Box-Behnken design were applied to optimize AA2G NCs as AA2G was being studied as an NC stabilizer for the first time and a complete knowledge about NC formulation development employing AA2G as an NC stabilizer was unavailable and also, there was a need to screen with a higher number of factors. Similarly, optimization of TPGS NCs was accomplished by applying Central composite design)
- Preparation of the optimized solid AA2G NCs and TPGS NCs with low mean particle size, low polydispersity index and high optimum zeta potential
- Solid state characterization of the optimized AA2G NCs and TPGS NCs using FTIR, DSC and PXRD
- Microscopic examination of the optimized AA2G NCs and TPGS NCs using SEM and atomic force microscopy
- Assessment of *in-vitro* aqueous solubility and dissolution of the optimized AA2G NCs and TPGS NCs in USP acetate buffer of pH 4.5, containing 0.45% w/v SLS as the medium as suggested by the FDA Dissolution Methods Database guide for Eze
- Assessment of stability of the optimized AA2G NCs and TPGS NCs at 30±2 ˚C/70±5% RH in terms of appearance, mean particle size, polydispersity index, zeta potential, drug content, aqueous solubility and dissolution
- *In-vivo* pharmacokinetic studies of the optimized AA2G NCs and TPGS NCs following oral administration of formulations to Male Albino Wister rats

- *In-vivo* antihypercholesterolemic activity study of the optimized AA2G NCs and TPGS NCs following oral administration of formulations to Male Albino Wister rats
- Arriving at the best performing optimized solid Eze NC considering the cumulative *in-vitro* and *in-vivo* performances

Step IV: Evaluating the effect of each formulation approach on the *in-vitro* and *in-vivo* performance of Eze

- The *in-vitro* aqueous solubility test results of the best performing solid CoC, best performing solid ternary CD complex and best performing solid Eze NC were compared
- The *in-vitro* dissolution of the above three optimized formulations and a marketed tablet formulation were studied in three different media - 0.01 HCl (pH 2) with 0.45% w/v SLS; USP acetate buffer of pH 4.5, containing 0.45% w/v SLS; and distilled water (measured pH 6.8) with 0.45% w/v SLS
- Assessment of stability of the optimized formulations at 30±2 ˚C/70±5% RH in terms of FTIR, DSC and PXRD
- *In-vivo* pharmacokinetic performances of the optimized formulations and a marketed tablet formulation following oral administration of formulations to Male Albino Wister rats were evaluated
- *In-vivo* pharmacodynamic performances (in terms of lipid profile values and atherogenic indices) of the optimized formulations and a marketed tablet formulation following oral administration of formulations to Male Albino Wister rats were evaluated

- The possible dose reduction efficiency of each of the optimized formulation was studied
- The best performing formulation that presented superior *in-vitro* and *in-vivo* test results with greater dose reduction efficiency was proposed

To summarize, as a first step, the UV-VIS and HPLC-UV methods were developed for Eze analysis. Both the methods were discussed under chapter 5 - preparation and characterization of Eze CoCs and the same are applicable to all the following sections discussed in this research work. Eze CoCs (chapter 5), ternary CD complexes (chapter 6) and NCs (chapter 7) were formulated, optimized and characterized to arrive at the best performing formulation at *in-vitro* and *in-vivo* levels. Finally, all the three optimized formulations were compared with each other and also with a marketed tablet product to study the possible dose reduction efficiencies of the optimized formulations (chapter 8). The best performing formulation that presented superior *in-vitro* and *in-vivo* test results with greater dose reduction efficiency was proposed as the most suitable formulation for the oral delivery of Eze.