CHAPTER – 1

Introduction

Despite of enormous progress in drug delivery, oral route of administration of drugs is the most usual and convenient way due to low cost of therapy, self-medication, non-invasivity and ease of taking medication leading to high level of patient compliance. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism and susceptibility to P-glycoprotein (P-gp) efflux mechanisms [Alam et al., 2013]. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability, as they determine to a large extent, the bioavailability of a drug substance. According to the literature, between 60 and 70 % of the active pharmaceutical ingredients (APIs) in the pipeline show sufficient membrane permeability but a low water solubility [Rehder, 2013]. APIs with poor solubility lead to the failure of many drug candidates because poor bioavailability, limited by the solubility of the final drug is a common problem that occurs during drug development. Substantial amount of time and effort is being spent by formulation development researchers on improving the solubility of the new chemical entity without tampering with its molecular structure or altering the biological activity.

The drug chosen for the present study, ezetimibe (Eze) is one model low soluble and highly permeable drug. Pharmacologically, Eze is a hypocholesterolemic agent. Eze acts by inhibiting the small intestinal absorption of cholesterol. The oral absorption of Eze

shows inter-subject variability and its bioavailability could be as low as 35% due to its poor solubility and P-gp efflux [Bandyopadhyay et al., 2012]. The P-gp molecules at the intestinal brush border cause P-gp efflux [Srivalli and Lakshmi, 2012] of Eze and thus interfere with the absorption of Eze. Since Eze is a hypocholesterolemic agent, development of suitable formulations to improve the oral bioavailability of Eze may find application in treating the hypercholesterolemic conditions and the associated coronary heart disease (CHD) conditions like atherosclerosis, more effectively.

CHD is the leading cause of death in adults in many countries across the world. It has been associated with elevated levels of plasma cholesterol levels [Gaziano et al., 2010]. Premature coronary atherosclerosis is a major manifestation of hypercholesterolemia. Because atherosclerosis is one of the major causes of CHD, controlling plasma lipid levels is essential in treating this condition. Clinical trials have demonstrated that decreasing cholesterol concentrations in the plasma has a major impact on cardiovascular morbidity and mortality [Patel et al., 2003].

Cholesterol levels in the body come from two sources, dietary intake and biosynthesis. The majority of cholesterol utilized by healthy adults is synthesized in the liver, which produces up to 70% of the total daily cholesterol requirement (≈1 gram). The other 30% comes from dietary intake. The amount of cholesterol that is synthesized in the liver is tightly regulated by dietary cholesterol levels. When dietary intake of cholesterol is high, synthesis is decreased and when dietary intake is low, synthesis is increased. Cholesterol is transported throughout the body in the form of cholesterol esters. Excess cholesterol is also stored intracellulary as cholesterol esters. The enzyme cholesterol esterase controls the hydrolysis of these stored cholesterol esters yielding bioavailable cholesterol and fatty acids. Cholesterol esterase hydrolyzes long chain and unsaturated

fatty acid esters at a greater rate than short chain saturated fatty acids. Cholesterol esterase also contributes to the incorporation of cholesterol into mixed micelles and aids in the transport of free cholesterol into the enterocyte. Since cholesterol is a water-insoluble molecule it must be packaged for transport within the plasma. The particles that package cholesterol, cholesteryl esters, and triglycerides for transport, are called lipoproteins. There are five main classifications of lipoproteins based on their size and density. The higher the ratio of protein to lipid content, the higher the density. The largest and the least dense lipoproteins are chylomicrons and the order is chylomicrons > very low density lipoproteins > low density lipoproteins > intermediate density lipoproteins > high density lipoproteins. Chylomicrons predominately transport triacylglycerols to adipose tissue and muscle as fatty acids, but also deliver dietary cholesterol taken up by enterocytes in the lumen to the liver. The liver excretes excess cholesterol in the form of bile acids. Bile acids serve two purposes: to remove unwanted cholesterol from the body and to aid in lipid digestion in the intestine. Controlling blood cholesterol levels has an important therapeutic role as hypercholesterolemia often leads to the development of atherosclerosis and consequently to cardiovascular pathologies, which might result in myocardial infarction and stroke. Recent evidence suggests that a disturbance of cholesterol homeostasis contributes to the development of a chronic inflammatory state.

Biosynthesis of cholesterol generally takes place in the endoplasmic reticulum of hepatic cells. Dietary cholesterol is obtained from foods derived from animal sources that are rich in fat content. A healthy adult only needs to ingest about 30% of the daily cholesterol requirement. Obtaining more than this amount from dietary cholesterol can lead to increased cholesterol levels and serious health risks. Preventing the absorption of

this dietary cholesterol has become a key area in cholesterol related research. Dietary cholesterol is absorbed within the lumen of the small intestine. Bile salts produced from cholesterol in the liver interact with phospholipids to produce a biliary micelle that is transported via bile into the lumen. Dietary cholesterol in the lumen is easily incorporated into these micelles and together with the already present biliary cholesterol can now be absorbed into the enterocytes that make up the walls of the lumen. The micelles enter the cell by a channel know as Niemann-Pick C1 Like 1 protein (NPC1L1). Once in the cells the cholesterol can either be pumped back out into the lumen or it can be esterified for transport within chylomicrons. The inhibitors that block the absorption of the biliary micelles into the enterocytes could be of interest to block the uptake of dietary cholesterol. Eze is one such inhibitor that inhibits cholesterol absorption by binding to NPC1L1 [https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/2/biofiles_issue12.pdf].

Eze is the first of a new class of lipid-lowering drugs known as cholesterol absorption inhibitors. Eze localizes at the brush border of the small intestine and selectively inhibits the absorption of cholesterol from the intestinal lumen into enterocytes. Eze does not affect absorption of fatty acids, bile acids, or fat-soluble vitamins, including vitamins A, D, E, and K and α and β carotenes. Eze is commercially available in the market as a tablet formulation and the oral bioavailability of this useful drug, Eze, could be as low as 35% due to its poor solubility and P-gp efflux.

So far, Eze has been formulated as cocrystal (CoC) formulations [Snehal et al., 2012], cyclodextrin (CD) complexes [Patel et al., 2008], and colloidal drug delivery systems (CDDS) [Bandyopadhyay et al., 2012; Dixit and Nagarsenker, 2008; Bali et al., 2010 and 2011]. While the studies on CD complexes and CoC formulations were limited only

to *in-vitro* characterization, the CDDS formulations were studied extensively at *in-vivo* level too. Therefore, the *in-vivo* behavior of Eze CD complexes and CoC systems lack an in-depth study report.

The CDDS formulations reported improvement in *in-vitro* dissolution as well as *in-vivo* bioavailability of Eze which signified the effect of nanosize on the improved performance of Eze. Among the different CDDS, Eze was formulated as self nanoemulsifying systems reported in liquid [Bandyopadhyay et al., 2012] and solid forms [Dixit and Nagarsenker, 2008] and as nanoemulsion formulations [Bali et al., 2010; Bali et al., 2011]. All these formulations contained several components which made the optimization of their preparation laborious and time taking. Furthermore, their preparation involved use of large amounts of surfactants and cosurfactants, which, from the toxicological stand point, is a legitimate concern. So, though nanosize proved advantageous in improving the performance of Eze, there is still a need to develop a simpler, less toxic, less laborious and economic nanoformulation.

The current research work aimed to improve the oral bioavailability of Eze by formulating the drug as CoCs, CD ternary complexes and drug nanocrystals (NCs). The main purpose of any formulation research is to deliver a 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 basic requirement for such a formulation research development is to consider the following three aspects in parallel: the physicochemical properties of the drug; selection of a suitable formulation; and the nature of excipients.

Eze is a poorly soluble P-gp substrate with low oral bioavailability. The formulation approaches chosen in the present investigation to improve the oral bioavailability and

therapeutic efficacy of Eze were cocrystallization, CD ternary complexation and nanonization.

Cocrystallization is a supramolecular pure crystal engineering technique and is one of the approaches to enhance solubility and other physicochemical properties of poorly soluble drugs. Cocrystallization involves building a periodic crystal lattice out of two or more neutral molecular species that are solids at room temperature, without making or breaking covalent bonds. Cocrystallization modifies the solid-state structural organization and results in powders with finely tuned end-use properties like solubility, dissolution, crystallinity, etc [Gagniere et al., 2009a].

CDs are highly water soluble polymers and CD complexation of non-polar drug molecules has been well-known to render the drugs more soluble by several orders of magnitude when compared to the parent or uncomplexed drug molecules. Minimizing the amounts of high molecular weight CDs in formulations without compromising on the solubility advantage of CD complexes is of pharmaceutical importance and it may be possible by introducing auxiliary substances into binary inclusion complexes to form supramolecular ternary systems. The ternary systems may further improve the physicochemical properties and absorption of drugs in comparison to binary complexes [Srivalli and Mishra, 2016].

NCs are one of the CDDS that reached the market fastest as their formulation involves simple dispersion of drug in either aqueous or nonaqueous media containing one or more GRAS stabilizers (drug:stabilizer ratio is generally considered between 2:1 and 20:1 on a weight basis). NCs are encapsulating-carrier free nanoparticles and are known for their manufacturing simplicity. NCs are produced either by top down fragmentation or bottom up amalgamation. NCs exist at the epicenter of the CDDS because of their

formulation simplicity, lack of drug loading problems, upscalability and ability to reduce bioavailability variations. The potential benefit of NCs in improving the solubility, dissolution and oral bioavailability of poorly soluble drugs has been well established over the last two decades [Srivalli and Mishra, 2014, 2015a and 2015b].

The excipients chosen for this study were GRAS and FDA approved ingredients like nicotinic acid (NA) and nicotinamide (ND); hydroxypropyl-β-cyclodextrin (HPBCD); D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS); and L-ascorbic acid-2-glucoside (AA2G). NA, also known as niacin (vitamin B3), is a lipid lowering agent that acts by inhibiting lipolysis in adipose tissue [Athimoolam and Rajaram, 2005]. ND is an amide of niacin. It is safe and extensively used in human and is highly hydrophilic in nature [Berry et al., 2008]. HPBCD is the most commonly used CD derivative and has been employed in several marketed pharmaceutical products [Srivalli and Mishra, 2016]. TPGS is a novel lipid based highly water soluble non-ionic surfactant that has been approved as safe excipient by US FDA. It also exhibits P-gp inhibitory action and has been widely known to increase the solubility and bioavailability of water-insoluble drugs by many folds. AA2G is a facile hydrophilic excipient that has been approved as a food additive and is expected to be used as a principle ingredient for solubilization in fat-soluble vitamin formulations and in other cosmetic products [Srivalli and Mishra, 2015b and 2016].

In the present study, Eze CoCs were prepared using NA and ND as coformers, for the first time; CD ternary complexes of Eze were prepared with HPBCD using TPGS/AA2G as ternary substance, for the first time [Srivalli and Mishra, 2016]; and Eze NCs were prepared using TPGS and AA2G as stabilizers, for the first time [Srivalli and Mishra, 2015b].

The excipients employed to formulate Eze were hydrophilic with or without additional potentials like P-gp inhibitory action or hypocholesterolemic action. Since Eze is a hypocholesterolemic drug with very low oral bioavailability due to problems like poor water solubility and its P-gp substrate nature, the current research work aimed to improve the oral bioavailability of Eze by formulating CoCs, CD ternary complexes and NCs, employing the above mentioned problem fitting relevant excipients. It has been 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 small intestinal brush border.

The whole research work has been detailed in four chapters. 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, for each formulation approach. 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 each of the optimized formulations (chapter 8).

The aim of chapter 5 was to prepare and characterize two new CoCs of Eze, prepared using NA and ND. The preliminary solution state studies included phase solubility and Job's plot for optimizing the drug:coformer ratio. Thus optimized ratios of Eze-NA CoC and Eze-ND CoC were prepared and characterized by or for the following: flow

properties, fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), aqueous solubility, dissolution, bioavailability and antihypercholesterolemic activity.

The purpose of chapter 6 was to study the ternary CD complexes of Eze. The potential synergistic effect of two novel hydrophilic auxiliary substances, TPGS and AA2G on HPBCD solubilization of Eze was investigated. In solution state, the compositions of binary and ternary systems were optimized by phase solubility studies and Job's plot. Thus optimized solid binary and ternary complexes were prepared and characterized by FTIR, DSC, PXRD, SEM, and for aqueous solubility, dissolution, bioavailability and antihypercholesterolemic activity.

In chapter 7, NCs of Eze were developed using bottom up precipitation methods. TPGS and AA2G were the two stabilizers whose potential in developing Eze NCs was investigated. The systematically optimized NC formulations (using suitable and applicable experimental design approach) were evaluated for *in-vitro* solubility and dissolution, solid state characters and *in-vivo* bioavailability and antihypercholesterolemic activity.

Though Eze contains ionisable groups, literature suggests that the drug essentially shows a pH independent solubility characteristic across the gastrointestinal pH range. Thus, pH-based strategies to improve the solubility/dissolution characteristics (e.g. salts, addition of pH modifiers) were not a first-line option for a drug like Eze [Taupitz et al., 2013]. For the same reason, the formulations in this study were optimized under each formulation approach (chapter 5, 6 and 7) by studying their dissolution in just one pH media, the USP acetate buffer medium of pH 4.5, containing 0.45% w/v sodium lauryl

sulphate, as suggested by the USFDA Dissolution Methods Database guide for Eze (marketed Eze tablet). However, the pH independent solubility/dissolution characteristic of Eze was confirmed by conducting the dissolution of the optimized formulations in three different pH media in chapter 8.

In chapter 8, the optimized formulations prepared by the above three approaches (chapter 5, 6 and 7) were compared at *in-vitro* and *in-vivo* levels. Additionally, the dose reduction efficiencies offered by the optimized formulations were studied and reported in terms of the ability of each of the optimized formulation to manage the plasma lipids and atherogenic indices. Also, in 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.