CHAPTER – 6

Formulation and Characterization of Ezetimibe Ternary Cyclodextrin Complexes

ABSTRACT

The purpose of this part of study was to investigate the potential synergistic effect of two novel hydrophilic auxiliary substances, D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and L-ascorbic acid-2-glucoside (AA2G) on hydroxypropyl-β-cyclodextrin (HPBCD) solubilization of a poorly water soluble hypolipidemic drug, ezetimibe (Eze). In solution state, the compositions of the binary and ternary systems were optimized by phase solubility and Job's plot. The solid complexes prepared by freeze-drying were characterized by FTIR, DSC, PXRD, H-NMR and SEM to confirm the formation of inclusion complexes in the binary and ternary systems and to note the physical changes in new solid phases due to interaction between ternary components and drug-CD complex. The log P value, aqueous solubility, dissolution, pharmacokinetics and antihypercholesterolemic activity were studied. HPBCD complexation significantly improved ($p < 0.05$) the solubility and dissolution properties of Eze and the addition of ternary components produced further significant improvement in the dissolution of the drug even compared to binary system ($p < 0.05$). Both the ternary complexes significantly enhanced ($p < 0.05$) pharmacokinetic and antihypercholesterolemic performance of Eze.

6.1 INTRODUCTION

Low aqueous solubility has been a major challenge for pharmaceutical industry since the past two decades and up to 40% of the new chemical entities in development have been reported to be 'poorly water soluble' [Porter et al., 2008]. Oral route has been the major route of drug delivery and the oral absorption of 50% drugs is hampered because of low solubility and high lipohilicity and the consequent low and variable bioavailability [Gursoy and Benita, 2004]. It has been estimated that the development of about 25-30% active pharmaceutical ingredients has been dropped due to low aqueous solubility and poor bioavailability causing significant economic loss and therapeutic opportunity [Friesen et al., 2008]. This situation generates urgent need to advance multiple solubilizing approaches and achieve better oral bioavailability of BCS class II and class IV drugs [Yuvaraja and Khanam, 2014].

Eze is a model BCS class II drug with low water solubility. Eze is chemically 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone [Rosenblum et al., 1998] and the structural formula is shown in Figure 6.1. It is the first of its kind hypocholesterolemic that serves as a cholesterol absorption inhibitor unlike other marketed lipid lowering agents that act by inhibiting the synthesis of cholesterol. Eze inhibits cholesterol absorption by small intestine, but, being a P-gp substrate, the *in-vivo* absorption of Eze is lowered by P-gp efflux at the small intestinal brush border. The oral bioavailability of Eze is lowered to as low as 35% due to its low aqueous solubility and P-gp efflux [Bandyopadhyay et al., 2012].

Cyclodextrin (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. CDs are highly water soluble polymers that can

improve the solvation of dissolved drug molecules with ability to stabilize supersaturated solutions and inhibit precipitation [Augustijns et al., 2009].

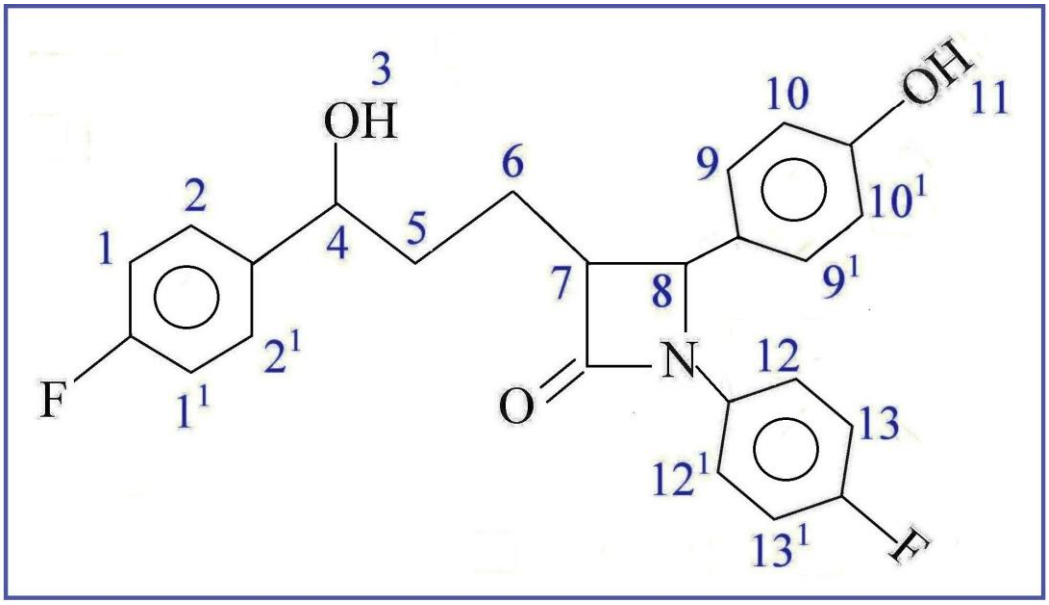

Figure 6.1. Structure of ezetimibe showing proton assignments.

Hydroxypropyl-β-cyclodextrin (HPBCD) presents very high aqueous solubility (> 60% at 25 ˚C) among the natural CDs and their derivatives and has been employed in several marketed pharmaceutical products. HPBCD monograph is official in both the Ph. Eur. and the USP/NF [Zoeller et al., 2012]. Literature also reports the ability of HPBCD to maintain cholesterol homeostasis [Peake and Vance, 2012]. Therefore, it has been conceived that formulating Eze as HPBCD complexes might serve to not only improve the aqueous solubility and dissolution but also to provide additive hypolipidemic effect and improved oral bioavailability of Eze.

Though the low single dose of Eze (10 mg) is very suitable for this study and a formulation with HPBCD of high molecular weight can still be administered easily by

oral route, from the pharmaceutical and economy point of view, it is recommended to keep the CD amounts in formulations to minimum. Attempts were made in the past to study the benefits of Eze-CD complexation. Patel et al. [2008] was the first to prepare incompletely amorphous complexes of Eze with β-CD and HPBCD by coevaporation and kneading methods and pointed out influence of method of complex preparation on solubility and dissolution behaviors [Patel et al., 2008]. Taupitz et al. [2013] also prepared incompletely amorphous Eze-HPBCD complex by freeze-drying method and clearly stated that incomplete amorphous state relates to incomplete complexation of the sample [Taupitz et al., 2013]. Such incomplete complexation may be in turn related to the choice of solvents used to solubilize Eze as explained by Selic and Ham [2008]. Methanol or ethanol was used in the afore-mentioned studies and these solvents were said to be associated with precipitation of either Eze or HPBCD during mixing or lyophilization process [Selic and Ham 2008]. Tertiary butyl alcohol (TBA) was shown as a suitable solvent by Selic and Ham [2008] where complete amorphous state of Eze-HPBCD complex was reported and the same was used in the present study and an elaborated analysis of the complexes was performed. Proton nuclear magnetic resonance (H-NMR) spectroscopy, scanning electron microscopy (SEM), saturation solubility and dissolution studies were not studied by Selic and Ham [2008]. Also, the antihypercholesterolemic activity of neither binary nor ternary CD complexes of Eze was reported till date.

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. Use of water soluble

polymers to improve the solubility and apparent stability constant of drug-CD complexes had been known for long. The ternary systems may further improve the physicochemical and transport properties of drugs in comparison to binary complexes [Loftsson et al., 1994]. Literature reports several studies on the effect of water soluble substances like polymers [Chowdary and Srinivas, 2006; Hirlekar et al., 2009; Loh et al., 2014], surfactants [Nogueiras-Nieto et al., 2012], metal salts [Sami et al., 2010] and amino acids [Jadhav et al., 2013] on CD solubilization of drugs. In the present study, the effect of two hydrophilic auxiliary substances namely, D-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS or simply TPGS) and L-ascorbic acid-2-glucoside (AA2G), as third components to the CD complexes, was evaluated for the first time. Their effect on HPBCD solubilization of Eze was investigated.

TPGS is a novel lipid based highly water soluble non-ionic surfactant that has been approved as a safe excipient by USFDA. It is a product of esterification of Vitamin E succinate with polyethylene glycol 1000 and has CMC as low as 0.02% w/w. It also exhibits P-gp inhibitory action and has been widely known to increase the solubility of water-insoluble drugs by many folds [Srivalli and Lakshmi, 2012; Guo et al., 2013]. The potential of TPGS as auxiliary substance has been studied in the present work and the use of this excipient has been assumed to aid in preventing the P-gp efflux of Eze *in-vivo*.

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 [Inoue et al., 2007]. AA2G is a highly stable derivative of antioxidant, ascorbic acid (AA) and it undergoes rapid conversion to AA in blood and liver cells by the action of α-glucosidase enzyme. Inoue et al. [2007]

reported improvement in the aqueous solubility and dissolution properties of clarithromycin on co-grinding with AA2G. Current study aims to investigate the role of AA2G as a ternary component in Eze-HPBCD complex.

Eze is a hypocholesterolemic P-gp substrate, HPBCD is known to maintain cholesterol homeostasis [Peake and Vance, 2012], TPGS is a P-gp inhibitor and AA2G is an efficient solubilizer. So, we hypothesized that the complexes may serve to not only improve the solubility and release properties of Eze *in-vitro* but also to enhance the *in-vivo* performance of Eze by improving its *in-vivo* absorption or offering synergistic hypocholesterolemic effect (superior biological activity and therapeutic efficacy) at the small intestinal brush border. The objective of the current part of study was to prepare binary, Eze-HPBCD (E-CD) and ternary, Eze-HPBCD-TPGS (E-CD-TPGS) and Eze-HPBCD-AA2G (E-CD-AA2G) complexes and study their solubility and dissolution properties. Freeze drying was the method of preparation and the complexes were evaluated for solid state characteristics, pharmacokinetic and pharmacodynamic performance.

6.2 MATERIALS

Eze (purity = 99.3%) was a kind gift from Lupin Ltd. (Pune, India). HPBCD (DS = 5.04), TPGS, and AA2G were received as generous gift samples from Gangwal Chemicals Pvt. Ltd. (Mumbai, India), Antares Health Products, Inc. (Illinois, USA) and Nagase Pvt. Ltd. (Mumbai, India), respectively. All other materials of analytical reagent grade were purchased locally and used as received.

6.3 METHODS

6.3.1 Optimization of the composition of binary Ezetimibe - Hydroxypropyl-β-cyclodextrin (E-CD) complex and ternary, Ezetimibe - Hydroxypropyl-β-cyclodextrin - D-α-tocopheryl polyethylene glycol 1000 succinate (E-CD-TPGS) and Ezetimibe - hydroxypropyl-β-cyclodextrin - L-ascorbic acid-2-glucoside (E-CD-AA2G) inclusion complexes

6.3.1.1 Evaluating the effect of increasing concentration of TPGS and AA2G with fixed concentration of HPBCD

A previously reported method was adopted with modifications [Soares-Sobrinho et al., 2012]. Excess Eze (20 mg) was added to 10 mL USP acetate buffer solutions of pH 4.5, containing a fixed concentration of HPBCD (2% w/v) and increasing amounts of TPGS (0.01 – 0.25% w/v) or AA2G (0.01 – 0.5% w/v). TPGS and AA2G were studied at different concentrations owing to their differing molecular weights. The suspensions were shaken on rotary shaker continuously for one week to obtain equilibrium at room temperature (25±1 ˚C). The unsolubilized drug in the suspensions was then filtered with syringe through a nylon membrane filter (0.45µm). The filtrates, after appropriate dilutions, were analyzed by UV at 232 nm.

6.3.1.2 Phase solubility studies

Phase solubility studies were conducted for two reasons: to study the effect of increasing concentration of HPBCD on the solubility of the drug and; to study the effect

of addition of fixed concentration of auxiliary substances with the increasing concentration of HPBCD, on the solubility of the drug.

The method reported by Higuchi and Connors [Higuchi and Connors, 1965] was followed. Excess amount of Eze (20 mg) was added to 10 mL USP acetate buffer solutions of pH 4.5, containing 2 – 14 mM HPBCD (liquid state E-CD system) with or without the addition of 0.05% w/v TPGS (liquid state E-CD-TPGS system) or 0.1% w/v AA2G (liquid state E-CD-AA2G system). The suspensions were continuously shaken on rotary shaker for one week at room temperature (25±1 ˚C) to obtain equilibrium. The suspensions were then filtered, appropriately diluted, and analyzed by UV at 232 nm. The experiments were performed in triplicate and the straight line portions of the phase solubility curves were used to calculate the apparent stability constants (K) of the binary and ternary complexes as per the following equation [same as Equation (2.1)].

$K = slope/S_0(1\text{-}slope)$

(S_0 is the intrinsic solubility of Eze).

Job's and Benesi–Hildebrand plots were also constructed to confirm the stoichiometric ratio of E:CD in the binary and ternary systems [Duran-Meras et al., 1994; Misiuk and Zalewska, 2011; Jug et al., 2014].

6.3.1.3 Job's Plot and Benesi–Hildebrand plots

The stoichiometric ratio of the E:CD in the binary, E-CD and the ternary, E-CD-TPGS and E-CD-AA2G complexes was determined by continuous variation Job's plot method using UV spectrophotometry. The method was experimentally implemented by mixing equimolar solutions of Eze and CD (0.005 mM) in varying molar ratios (1 ml of Eze:9 ml of HPBCD; 2 ml of Eze:8 ml of HPBCD; 3 ml of Eze:7 ml of HPBCD and so on) to

a standard volume containing a fixed total concentration of the components. The effect of addition of minute amounts of auxiliary substances on the molar ratio of E-CD was studied by adding 0.05% w/v TPGS and 0.1% w/v AA2G to the HPBCD solution. After stirring the mixed solutions of Eze and HPBCD, the absorbance of Eze in each of the solutions was measured at 232 nm by UV. The difference in absorbance (ΔA) in the presence and absence of HPBCD solutions was plotted against R (R=[Eze]/{[Eze]+[HPBCD]}). The maximum difference in the absorbance occurs at the stoichiometric ratio.

Benesi–Hildebrand plots further confirm the stoichiometric ratio of E:CD in the binary and ternary systems. The graphs were constructed using the CD concentration and the corresponding absorbance values noted from the above continuous variation method. The linearity of graphs was examined by plotting reciprocal and double reciprocal plots as 1/ΔA v/s 1/[CD] and 1/ΔA v/s $1/[CD]^2$, respectively [Duran-Meras et al., 1994; Misiuk and Zalewska, 2011; Jug et al., 2014].

6.3.2 Preparation and characterization of solid inclusion complexes

6.3.2.1 Preparation of inclusion complexes in solid state

The optimal ratio of Eze and HPBCD in the binary and ternary systems was determined based on the phase solubility studies as well as the UV-visible spectroscopy based continuous variation method (Job's plot). The complexes were prepared by the widely employed freeze drying method [Zeng et al., 2011; Wang et al., 2013]. 1 mmol Eze dissolved in TBA and 2 mmol HPBCD (1 mmol HPBCD for E-CD-AA2G system) in distilled water, were mixed at 30 ˚C and stirred for 30 min to ensure a homogenous

solution. The concentration of the drug solution was 25 mg/ml and that of the HPBCD solution was 300 mg/ml. The solution was cooled to room temperature, filtered, pre-frozen and then lyophilized. The preparation of ternary complexes was similar to that of binary complex except that 0.05% w/v TPGS, in case of E-CD-TPGS ternary system and 0.1% w/v AA2G, in case of E-CD-AA2G system, were added, respectively, to the 300 mg/ml HPBCD solution, before mixing it with the drug solution. The resultant product was a fine, white powder in all the cases.

6.3.2.2 Characterization by FTIR, DSC, PXRD, SEM and H-NMR

The FTIR, DSC, PXRD and SEM were carried out as described under the section 5.3.2.4. The CD complexes were analysed by nuclear magnetic resonance (NMR) spectroscopy instead of TGA. The proton nuclear magnetic resonance (H-NMR) spectral data was obtained on a 300 MHz NMR spectrometer (Joel-FT NMR-AL300, Japan) at 25 ˚C. Samples were dissolved in dimethylsulphoxide (DMSO) and degassed using liquid nitrogen (N_2). Tetramethylsilane was used as an internal reference and the chemical shifts were reported in ppm.

6.3.2.3 Drug content

Known amounts of binary and ternary systems equivalent to 10 mg of drug were dissolved in 5 mL methanol, sonicated for 1 min and filtered. After appropriate dilutions, the solutions were assayed for Eze content by UV at 232 nm. The readings were taken in triplicate and the average was noted.

6.3.2.4 Measurement of octanol–water partition coefficient (P) and log P

Five milliliters aqueous solutions of each system (pure Eze, binary and ternary complexes), at 10^{-4}mol/L concentration, were respectively, mixed with five milliliters octanol at room temperature and shaken vigorously in a separating funnel to reach equilibrium. The systems were allowed to stand under gravity to separate the two phases and the amount of drug in each phase was quantified by UV at 232 nm. Log P, the logarithm of partition coefficient (P) was calculated using the following equation, Equation (6.1):

Log P = Log (Drug concentration in Octanol phase/ Drug concentration in Aqueous phase) ……. **Equation 6.1.**

6.3.2.5 Saturation aqueous solubility studies

Excess amounts of drug, binary and ternary systems were added to 10 mL distilled water. The suspensions were shaken continuously on rotary shaker for one week at room temperature, filtered and the drug amount was measured by UV at 232 nm.

6.3.2.6 Dissolution

The procedure followed was the same as described under the section 5.3.2.8. Dissolution was studied for 120 min and the dissolution efficiency (DE) representing the area under the dissolution curve up to 120 min was calculated for each of the systems. Additionally, $t_{80\%}$ and $t_{90\%}$ values were noted for the binary and ternary systems.

6.3.2.7 Stability

The optimized ternary complexes, E-CD-AA2G and E-CD-TPGS were subjected to stability studies at 30±2 ˚C/70±5% RH for 6 months. Six batches of dry powder formulations, each of E-CD-AA2G and E-CD-TPGS, were stored in air-tight glass vials, sealed and placed in stability chamber (Narang Industries, New Delhi, India) at 30±2 ˚C/70±5% RH. The freshly prepared batches were tested for drug content, aqueous solubility and dissolution tests and the results were noted. After 6 months, the samples were drawn and examined again for drug content, solubility and dissolution performance and the results obtained before and after 6 months were compared.

6.3.2.8 *In-vivo* preclinical pharmacokinetic study

6.3.2.8.1 Animals

The study protocol was approved and guided by the Central Animal Ethical Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Male Albino Wister rats (200 – 250 g) were used and the animals were divided into three groups of six animals each. The standard - group I, test - group II, and test – group III, received pure drug suspension, E-CD-TPGS, and E-CD-AA2G, respectively. The animals were housed in polypropylene cages and kept at standard laboratory conditions (25±2 ˚C and 55±5% RH). Six animals per cage were accommodated with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*.

6.3.2.8.2 Dosing and sampling

All the animals used were fasted overnight for the study and dosed orally using 18-gauge oral feeding needle. A single dose pharmacokinetic study was conducted and all the treatment group animals received 2 mL of 50 mg/kg body weight equivalent dose of

Eze, via oral administration. The animal groups, I, II, and III, received pure drug, E-CD-TPGS, and E-CD-AA2G, respectively, dispersed in 0.25% w/v NaCMC. After anaesthetizing the rats with diethyl ether, 500 µL blood samples were collected by retro-orbital puncture at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 4, 12, and 24 h, into heparinized microcentrifuge tubes. After blood sampling at each time point, the blood loss was compensated by immediately injecting same volume of normal saline. Plasma was immediately separated by centrifugation at 5000 rpm for 20 min, spiked with IS and stored at -20 °C until bioanalysis.

6.3.2.8.3 **Drug extraction** – same as described under the section 5.3.2.10.3.

6.3.2.8.4 **Plasma drug analysis** – same as described under the section 5.3.2.10.4.

6.3.2.8.5 **Pharmacokinetic parameters** – same as described under the section 5.3.2.10.5.

6.3.2.9 Antihypercholesterolemic activity

The study protocol was approved and guided by the Central Animal Ethical Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Male Albino Wister rats (200 – 250 g) were used and the animals were divided into seven groups of six animals each. The control - group I, standard - group II, test – group III, test – group IV, test – group V, test – group VI and test – group VII received cholesterol, pure drug suspension plus cholesterol, HPBCD plus cholesterol, E-CD PM plus cholesterol, E-CD plus cholesterol, E-CD-TPGS plus cholesterol and E-CD-AA2G plus cholesterol, respectively. The animals were housed in polypropylene cages and kept at standard laboratory conditions (25±2 ˚C and 55±5% RH). Six animals per cage were accommodated with free access to standard laboratory diet (Lipton feed, Mumbai, India)

and water *ad libitum*. The study was carried out for total period of 8 weeks wherein the first four weeks, all the animals were fed 200 mg cholesterol in 2 mL coconut oil as high fat diet for inducing hypercholesterolemia. At the end of fourth week, the plasma cholesterol levels were measured for all the groups and the values were considered as baseline values for the following four week study, the actual antihypercholesterolemic activity study. The first four weeks is a stage 1, hypercholesterolemic induction study and the next four weeks is the stage 2, antihypercholesterolemic activity study.

The antihypercholesterolemic study was carried out for 28 days and the animals were fed and dosed orally using 18-gauge oral feeding needle. To carry out the study, all the groups were induced with hypercholesterolemia by administering them with high fat diet (200 mg cholesterol in 2 mL coconut oil) [Srivalli and Mishra, 2016]. Two hours following the administration of high fat diet, the treatment groups, II, III IV, V. VI and VII were respectively fed with pure drug, HPBCD, E-CD PM, E-CD, E-CD-TPGS and E-CD-AA2G, dispersed in 0.25% w/v NaCMC. The daily dose for rats was calculated by considering the rat to human being surface area ratio [Dixit and Nagarsenker, 2008; Bandyopadhyay et al., 2012]. Volume of vehicle (200 µL) and dose levels of 1 mg Eze or equivalent formulation/kg body weight/day were kept constant in each case. For pure HPBCD, the dose was equivalent to the amount of HPBCD in 1 mg Eze equivalent weight of E-CD complex. Blood samples were collected on day 7, 14, 21 and 28, after anaesthetizing the rats with diethyl ether, by retro-orbital puncture, into anticoagulated microcentrifuge tubes (heparin treated). The plasma was separated by centrifugation at 5000 rpm for 20 mins and stored at 2 ˚C until further use. Percent reduction in the levels of total plasma cholesterol was analysed using *in-vitro* Cogent diagnostic kit (Span Diagnostics Ltd., Surat, India).

6.3.2.10 Statistical analysis

All the results were shown as Mean±SD. The data pertaining to solubility, log P, dissolution and pharmacokinetic investigations were analyzed by one-way ANOVA followed by post hoc Tukey multiple comparison test (*p* value set 0.05). The antihypercholosterolemic study results were analyzed by two-way ANOVA followed by post hoc Bonferrroni multiple comparison test.

6.4 RESULTS AND DISCUSSION

6.4.1 Optimization of the composition of binary E-CD complex and ternary, E-CD-TPGS and E-CD-AA2G inclusion complexes

6.4.1.1 Evaluating the effect of increasing concentration of TPGS and AA2G with fixed concentration of HPBCD

This study confirmed that the solubility of Eze could be increased not only by complexation with HPBCD but also by possible interactions with the water soluble auxiliary substances. The study also suggested the concentration of auxiliary component to be used to obtain a stable as well as more soluble ternary system.

The solubility of Eze, in a fixed 2% w/v HPBCD solution (pH 4.5) in the presence of increasing concentrations of TPGS, increased until an optimum level and declined beyond that level (Figure 6.2). The best and minimum concentration of TPGS that could be used was selected as 0.05% w/v. The solubility of Eze, in a fixed 2% w/v HPBCD solution (pH 4.5) in the presence of increasing concentrations of AA2G, increased with

the increase in AA2G concentration (Figure 6.3). The best and minimum concentration of AA2G was selected as 0.1% w/v.

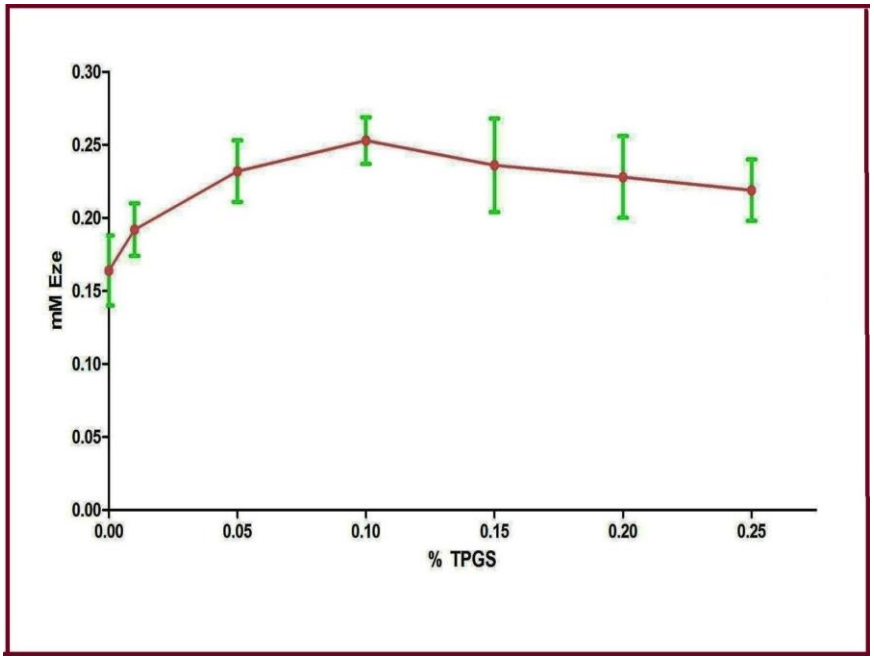

Figure 6.2. Eze solubility diagram in a fixed concentration of HPBCD (2% w/v) with increasing concentrations of TPGS.

The liquid state basic solubility analysis confirmed the increase in solubility pattern of Eze with the increase in the amounts of TPGS/AA2G in presence of fixed concentration of HPBCD suggesting the existence of possible intermolecular interactions among Eze, HPBCD and TPGS/AA2G. The study also suggested the concentration of auxiliary component to be used to obtain a stable as well as more soluble ternary system. Both the ternary systems studied showed encouraging solubility results in liquid state and the solubility studies were extended to evaluate the effect of the fixed (selected) optimum concentration of ternary components obtained in this study on the drug-CD phase solubility.

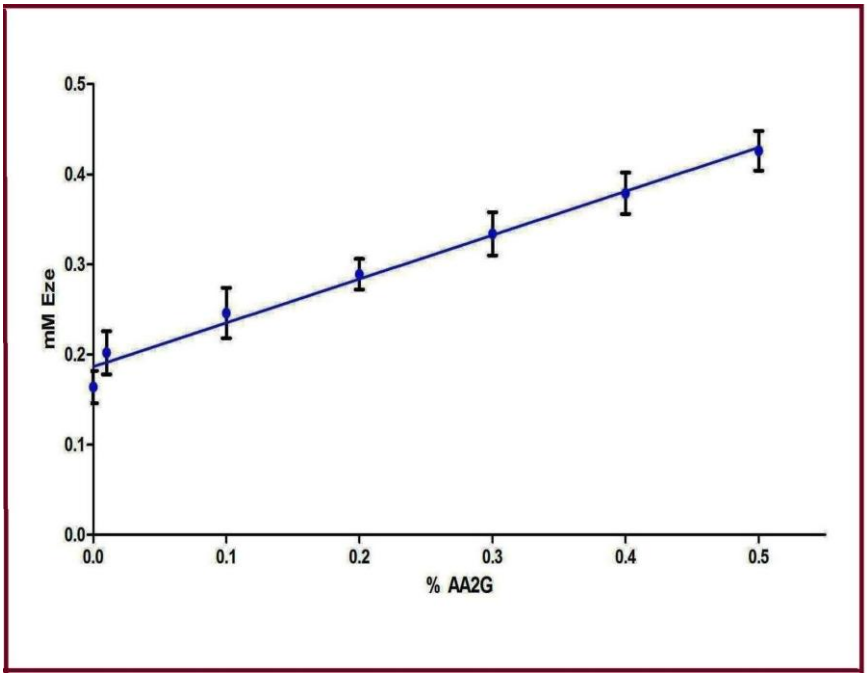

Figure 6.3. Eze solubility diagram in a fixed concentration of HPBCD (2% w/v) with increasing concentrations of AA2G.

It has been confirmed during preformulation studies that there was no interference of the employed excipients with UV drug absorption i.e. Eze analysis at 232 nm. Literature also offered supporting publications to the inferences on the non-interference of excipients with the UV-VIS Eze analysis at 232 nm wavelength. HPBCD has no absorption in the wavelength range 225-400 nm and hence would not interfere with the UV analysis of drug [Misiuk and Zalewska, 2009]. TPGS presents a UV absorption λ_{max} at 284 nm in distilled water and USP acetate buffer [Wang et al., 2009]. AA2G shows a UV absorption λ_{max} at 260 nm in distilled water and USP acetate buffer [http://www.nicnas.gov.au/__data/assets/pdf_file/0016/10393/STD1056FR.pdf; Muto et al., 1990].

6.4.1.2 Phase solubility studies

The phase solubility diagram of Eze in 4.5 pH USP acetate buffer in the presence of HPBCD, alone and in combination with TPGS/AA2G was presented in Figure 6.4. According to Higuchi and Connors [Higuchi and Connors, 1965], the phase solubility curves of the liquid state E-CD, E-CD-TPGS and E-CD-AA2G systems were classified as A_P, A_P and A_L types, respectively. The results indicated the occurrence of Eze:HPBCD complex in the ratios, 1:2, 1:2 and 1:1, in the systems, E-CD, E-CD-TPGS, and E-CD-AA2G, respectively. The ratios were further confirmed by constructing Job's plot (Figure 6.5). Benesi–Hildebrand plots were provided in Figures, 6.6 and 6.7.

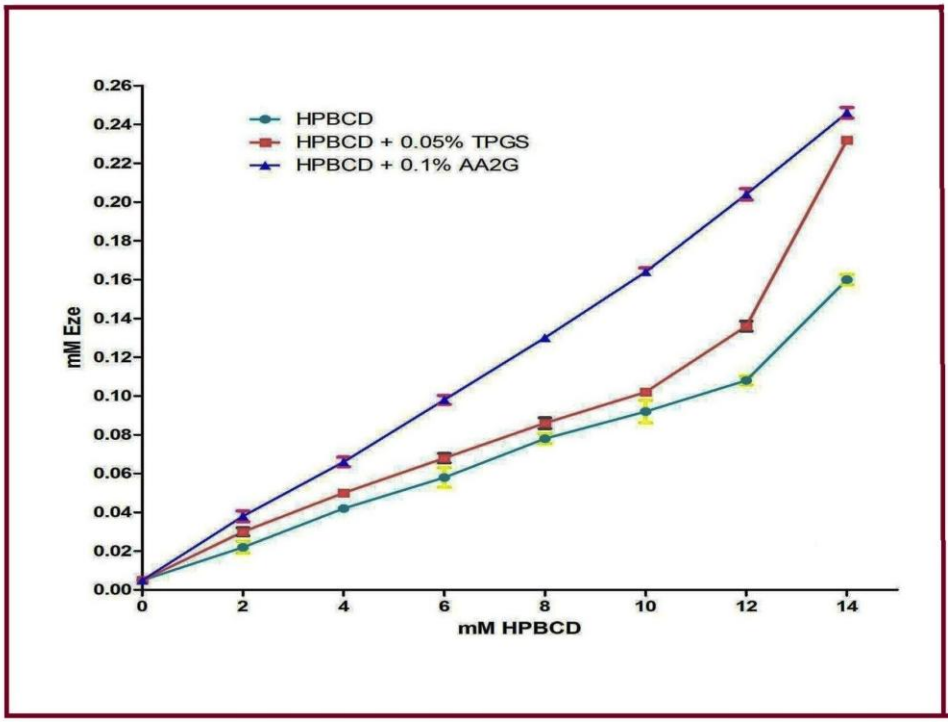

Figure 6.4. Phase solubility curves of liquid state binary and ternary systems.

The results of the study indicated that the solubility of Eze increased with the increase in HPBCD concentration when HPBCD was used alone or in combination with TPGS. In

the presence of TPGS, the solubility of Eze was further increased. However, the solubility of Eze increased non-linearly with a positive deviation in case of E-CD binary and E-CD-TPGS ternary liquid state systems. When HPBCD was used in combination with AA2G, not only the solubility of drug improved, but the phase solubility curve also shifted from A_P to A_L. The shift in the phase solubility curve of liquid state E-CD-AA2G to A_L as opposed to A_P nature of E-CD binary system indicated the plausibility of formation of a water soluble 1:1 Eze:HPBCD complex in presence of AA2G. The possibility that the addition of a water soluble auxiliary substance can cause a shift in the phase solubility curve has already been suggested in literature [Jug et al., 2011 and 2014]. Further confirmation on the Eze:HPBCD ratio was drawn after constructing Job's plot (Figure 6.5) and Benesi–Hildebrand plots (Figures, 6.6 and 6.7).

The slopes of the straight line portions of the phase solubility curves of the liquid state E-CD, E-CD-TPGS and E-CD-AA2G systems were 0.0091, 0.01, and 0.0169, respectively. The stability constants were calculated from the straight line portions of the phase solubility curves. The stability constant values of the liquid state E-CD, E-CD-TPGS and E-CD-AA2G systems were 1836.7±9.2 M^{-1}, 2020.2±10.1 M^{-1}, and 3438.1±9.9 M^{-1}, respectively. Both the employed hydrophilic auxiliary substances, TPGS and AA2G caused statistically significant improvement in the stability constant ($p < 0.05$) compared to the binary E-CD system indicating the formation of more stable ternary complexes in comparison to the binary complex.

With respect to the binary E-CD system, the altered pH could be the possible reason for the higher stability constant obtained in this part of study (statistically significant, $p < 0.05$) compared to the value reported earlier (1316 M^{-1}) by Patel et al. [2008]. All the liquid state analyses in the present part of study were performed using USP acetate

buffer of pH 4.5 in contrast to the distilled water used by Patel et al. [2008] because the pH of the FDA recommended dissolution media for Eze is also the same. Both TPGS and AA2G further enhanced the solubility and stability of E-CD. The Eze:HPBCD complex occurred in the ratios, 1:2, 1:2 and 1:1, in the systems, E-CD, E-CD-TPGS, and E-CD-AA2G, respectively. The phase solubility results, Job's and Benesi–Hildebrand plots confirmed that the addition of AA2G successfully decreased the amount of HPBCD required to complex and completely solubilize Eze.

6.4.1.3 Job's Plot and Benesi–Hildebrand plots

The mole-ratio plots were constructed according to the continuous variation Job's method.

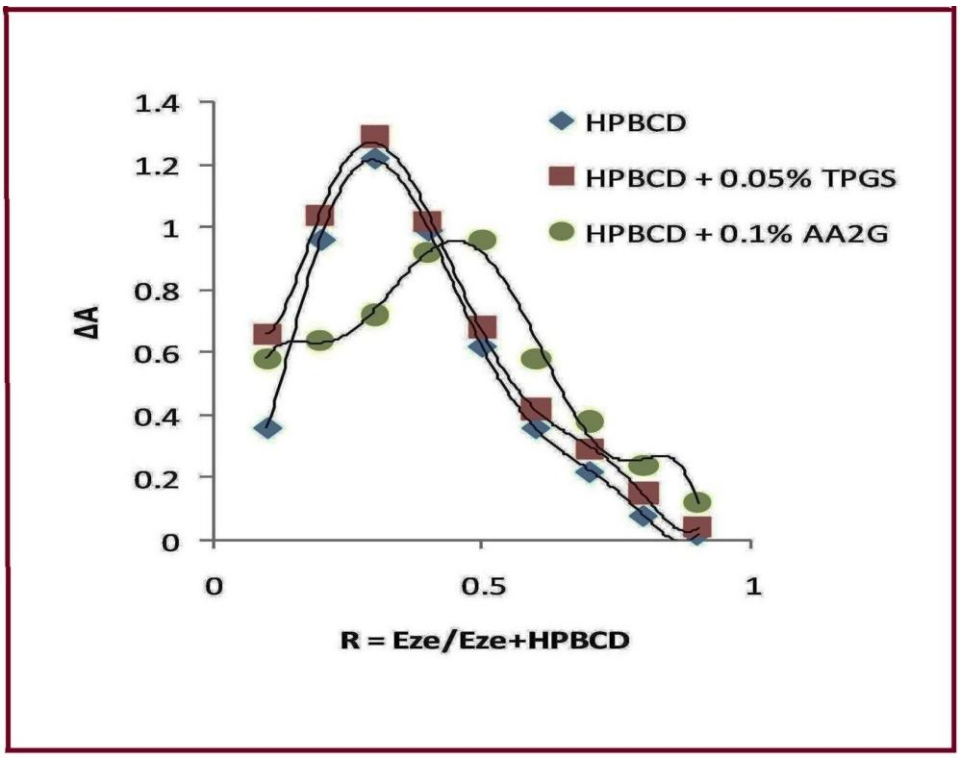

Figure 6.5. Job's plots of liquid state binary and ternary systems.

Solutions with varying mole fractions of Eze and HPBCD were prepared such that the total concentration of the two species in each of the solutions remained constant, 0.005 mM. The absorption values were measured at 232 nm. The observed absorbance values of these solutions were different from the sum of the corresponding values of their components and the difference was calculated. This difference in absorbance was considered an evidence for the complex formation between Eze and HPBCD. As in the Figure 6.5, the calculated absorbance difference was plotted against mole fraction of Eze and HPBCD, R (R=[Eze]/{[Eze]+[CD]}).

For a fixed total concentration of two species of a complex, the complex exists in its highest concentration at that point where the two species are combined in the ratio in which they occur in the complex. As depicted in the Figure 6.5, significantly abrupt changes in the absorbance difference occurred at 0.33 mole fraction of Eze indicating that 1:2 is the optimal stoichiometric ration of E-CD complex. Similar studies carried out with addition of minute amounts of ternary substances to the CD solutions indicated 0.33 and 0.5 mole ratios of E-CD in presence of TPGS and AA2G, respectively. The ratios were further confirmed by constructing Benesi–Hildebrand plots (Figures, 6.6 and 6.7), according to which, the 1:1 complexes result in a straight line for the plot of $1/\Delta A$ v/s $1/[CD]$ and the 1:2 complexes result in a straight line for the plot of $1/\Delta A$ v/s $1/[CD]^2$. E-CD and E-CD-TPGS systems were confirmed as 1:2 complexes of Eze and HPBCD and the E-CD-AA2G system was confirmed as 1:1 complex.

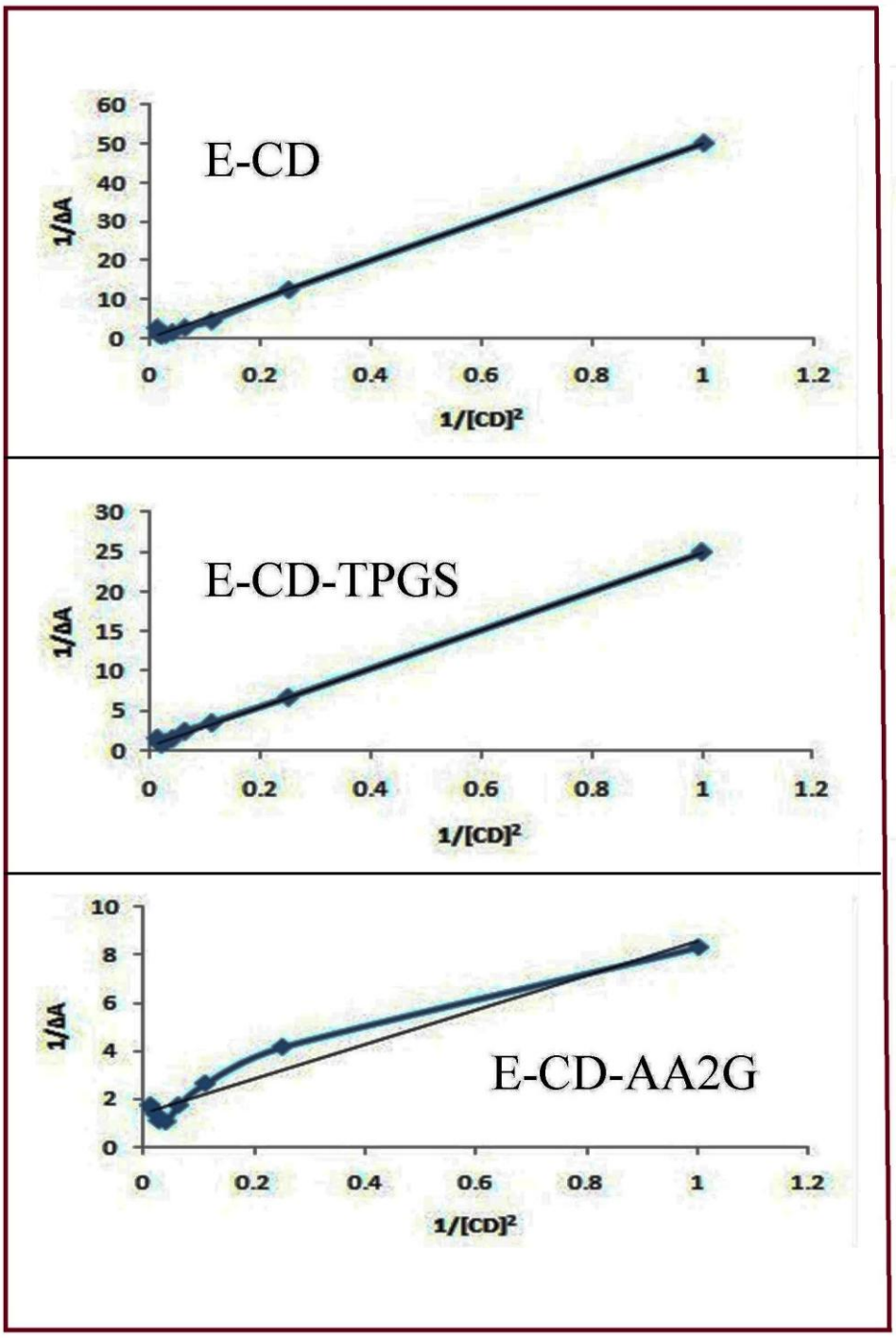

Figure 6.6. Benesi–Hildebrand plots - Double reciprocal plots of binary and ternary systems.

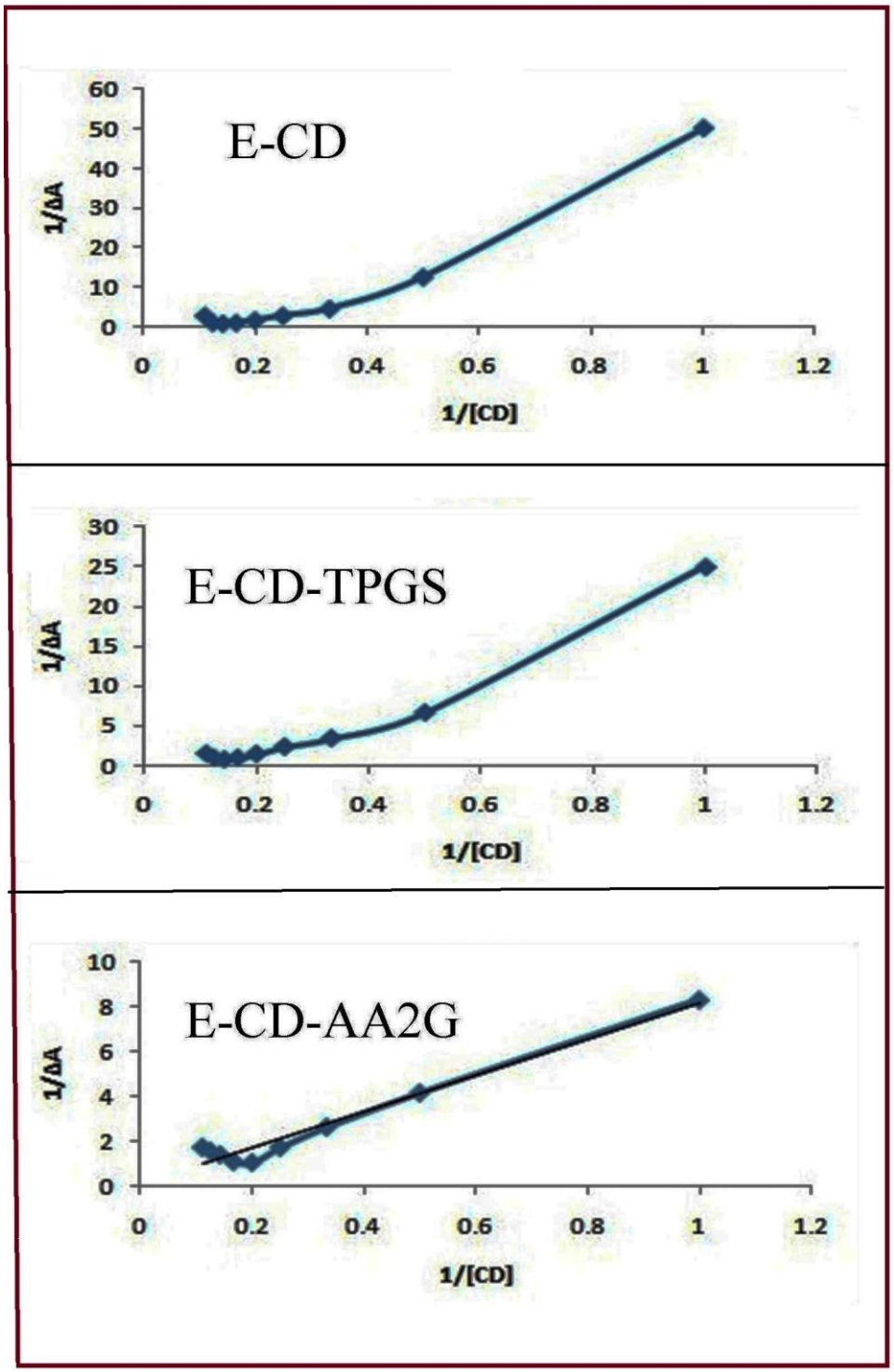

Figure 6.7. Benesi–Hildebrand plots - Reciprocal plots of binary and ternary systems.

6.4.2 Characterization of solid inclusion complexes

6.4.2.1 Characterization by FTIR, DSC, PXRD, SEM and H-NMR

6.4.2.1.1 FTIR

The occurrence of drug-CD inclusion complexation could be provided by the variations in the shape, frequency shift, and intensity of the FTIR absorption peaks of the guest or host [Ge et al., 2012]. The FTIR spectra of all the samples were shown in Figure 6.8 and the spectral band assignments of the parent compounds were listed in Table 6.1. The FTIR spectrum of E-CD PM was a replica of the spectrum of pure Eze which suggests that Eze was compatible with HPBCD and that there was no evidence of intermolecular interactions between Eze and HPBCD when a physical mixture was compounded.

Most of the principle absorption bands of pure Eze disappeared in the FTIR spectra of binary and ternary inclusion complexes but none of the inclusion complexes exhibited new peaks dismissing the possibility of formation of chemical bonds during the CD complexation. The spectra of the binary and ternary inclusion complexes were similar to that of HPBCD. HPBCD spectrum indicated that none of the bands in the wave number range 500 – 1500 cm^{-1} rose from one single type of molecular vibration. This is because HPBCD is a macrocyclic molecule and there occurs strong coupling of vibrations caused by the neighboring bonds that vibrate with similar frequencies. The FTIR spectrum of HPBCD is characterized by intense bands between 3300 and 3500 cm^{-1} due to O–H stretching vibrations. The vibrations of the –CH and CH_2 groups appear between 2800 and 3000 cm^{-1} region.

The spectra of the binary and ternary inclusion complexes were similar to that of HPBCD due to the following cumulative factors: (a) each HPBCD molecule produces

intense absorption bands since it has a relatively large number of polar groups like O–H, C–O, etc.; (b) in most of the spectral regions, the absorption bands of both the host and guest molecules coincide; (c) an excess of free HPBCD may be present in each of the inclusion complex samples [Misiuk and Zalewska, 2009].

Table 6.1. FTIR data table presenting characteristic peak assignments of parent compounds of Eze CD complexes.

Parent compound	FTIR spectral bands and assignments
Eze	As elaborated in chapter 5, Table 5.4.
HPBCD	Intense bands between 3300 and 3500 cm^{-1} (O–H stretching vibrations) and between 2800 and 3000 cm^{-1} region (–CH and CH_2 group vibrations)
AA2G	3292.60 cm^{-1} (O–H stretch), 1770.71, 1705.13 cm^{-1} (C=O stretch), and 1400.27 cm^{-1} (O–H bending)
TPGS	3493.20 cm^{-1} (terminal O–H), 2868.24 cm^{-1} (CH_2 groups), and 1732.13 cm^{-1} (C=O stretch)

However, the presence of Eze in the complexes was undoubtedly and consistently confirmed by the bands corresponding to C=O stretch of lactone ring, ring C–C stretch, C–N stretch, C–F stretch and the para-disubstituted benzene ring vibration though the intensities of the same above bands were drastically attenuated in the binary and ternary complexes. The band signals of C=O, C–F, para-disubstituted benzene ring, C–N and the lactone ring C–C stretch were observed in the inclusion complexes at the following wave numbers: (a) 1726.35, 1255.70, 879.57, 1458.23 and 1510.31, respectively, for the E-CD binary complex; (b) 1716.70, 1220.98, 879.57, 1458.23 and 1516.10, respectively, for the E-CD-AA2G ternary complex; (c) 1728.56, 1224.84, 835.21, 1456.30, 1514.17, respectively, for the E-CD-TPGS ternary complex.

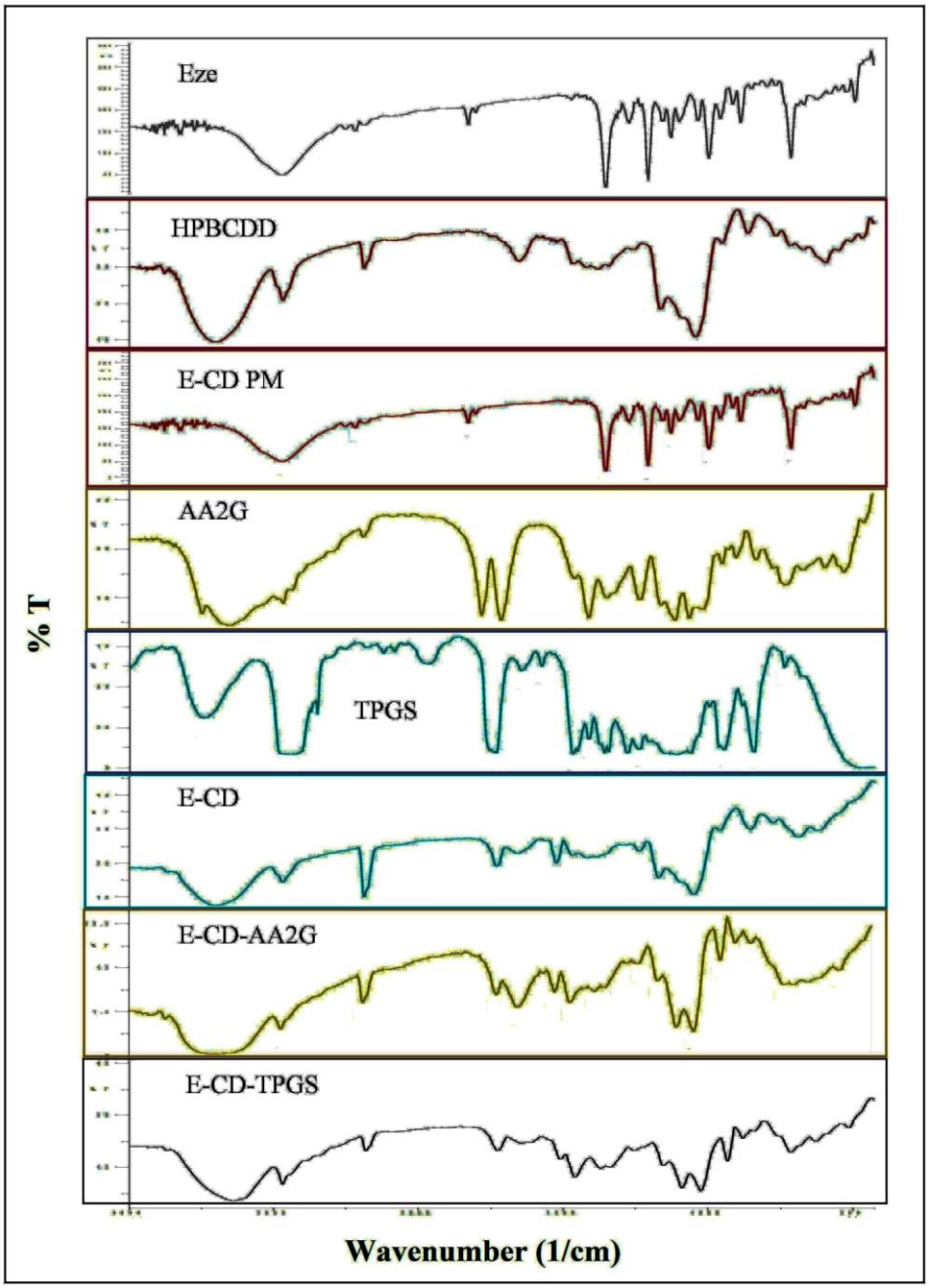

Figure 6.8. FTIR spectra of parent compounds, physical mixture and complexes.

The binary and ternary systems showed lack of the overtone band of the lactone ring of Eze molecule which suggests the possible entrapment of the lactone ring into the interior of the CD cavity. The participation of the O–H moieties of the drug molecule

could not be confirmed by FTIR due to overlapping of the O–H signals of the drug with those of the CD molecule.

In all binary and ternary systems, no new FTIR peaks different from that of parent compounds were noted but, modifications like broadening, attenuation and frequency shifts in the characteristic bands of the drug were observed. These results indicated that the vibrations and stretching of Eze molecule were restricted due to the formation of inclusion complex. The hydrophobic regions of Eze are likely to get placed into the lipophilic core of HPBCD.

6.4.2.1.2 DSC

DSC detects phase transformations involving endothermic or exothermic changes. DSC serves as an alternative tool to investigate the host-guest interactions. The inclusion of guest molecules in CD cavity generally causes extinction or shift in the melting points of the guest molecules [Shah et al., 2012]. As shown in Figure 6.9, the DSC curve of Eze was characterized by a very sharp peak at 163.56^0C with an onset at 163.07^0C and end set at 165.74^0C. The broad endotherm of HPBCD between 50^0C and 110^0C suggested loss of water molecules from the CD cavity [Sinha et al., 2005].

The DSC thermogram of E-CD PM retained the characteristic peak of Eze which means that the amorphous nature of HPBCD had no influence on the crystal state of drug in physical mixture form. AA2G and TPGS displayed peaks at 170.72^0C and 40.43^0C, respectively. The thermograms of all the formulations were similar to HPBCD alone.

All the formulations presented the characteristic broad endotherm of HPBCD and peaks corresponding to the pure drug and the ternary substances totally disappeared. These changes in the thermal behavior confirmed the formation of amorphous inclusion

complexes through molecular interactions between drug and HPBCD. The auxiliary components could have synergistically favored the drug entrapment into HPBCD cavity.

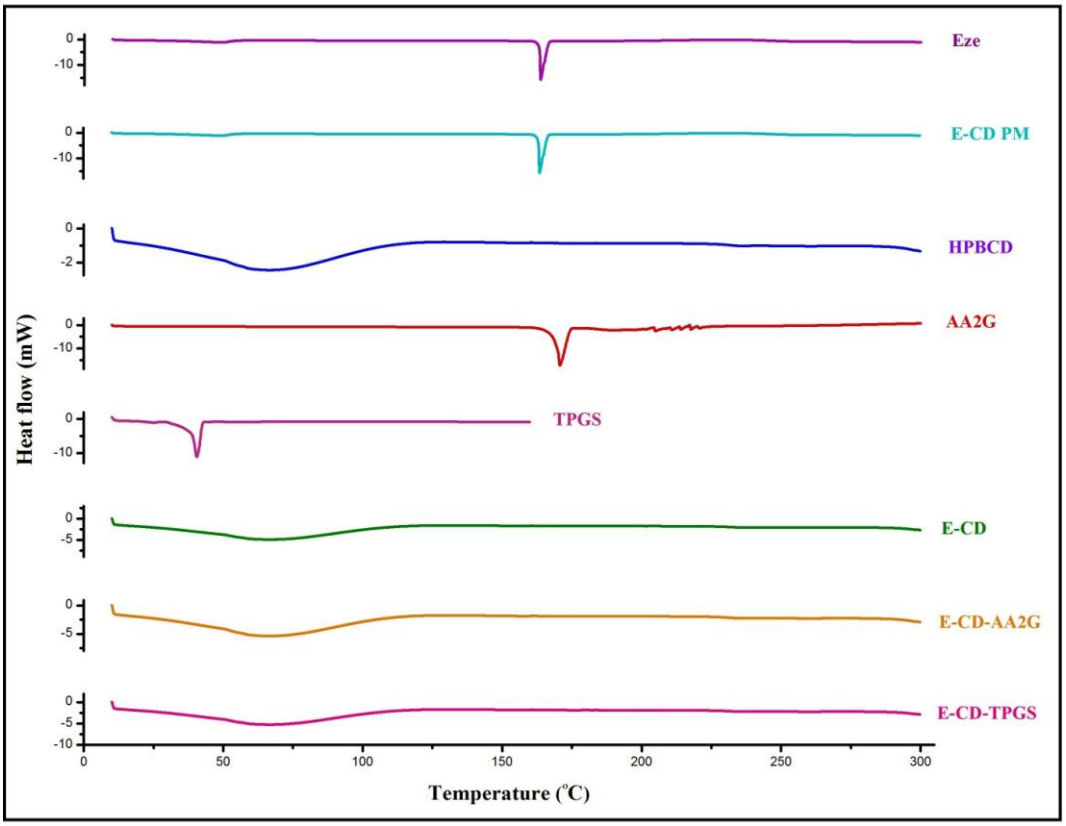

Figure 6.9. DSC thermograms of parent compounds, physical mixture and complexes.

6.4.2.1.3 PXRD

Further evidence for the formation of E-CD, E-CD-AA2G and E-CD-TPGS systems was obtained from XRD. Powder XRD is a useful technique for detecting drug-CD complexation in powder state and is powerful in determining the crystal habit modifications. When an intrinsic inclusion complex is formed, the diffraction pattern of

the newly formed complex would be clearly distinct from that of pure drug and that of the superposition of each of the components of the system [Nikolic et al., 2004]. Figure 6.10 shows X-ray diffractograms of all the samples. The graph of drug showed characteristic sharp diffraction peaks confirming the crystalline nature. Major intense peaks of drug were recorded at 2θ values of 7.825, 13.859, 15.733, 17.136, 18.589, 19.345, 19.845, 21.719, 22.866, 23.363, 25.21, 26.96, 28.16, 30.045 and 32.96. The XRD pattern of AA2G and TPGS showed peaks characteristic of their respective crystal habit whereas that of HPBCD was diffused and scattered depicting its amorphous nature. The PXRD pattern of E-CD PM retained the peaks and corresponding intensities of pure Eze which confirmed that the amorphous nature of HPBCD did not affect the crystal state of drug in physical mixture form.

The absence of characteristic drug peaks in the profiles of the binary and ternary complexes suggested loss of drug crystallinity due to complete entrapment into the CD cavity. The XRD profiles of the binary and ternary systems assumed the amorphous halo-pattern typical of HPBCD indicating formation of new complexes and complete amorphization of the drug which highlighted the efficient complexing power of HPBCD toward the drug. The results from the DSC analysis also strongly supported the XRD measurements and established evidence for the formation of binary and ternary complexes in the solid state. The amorphization demonstrated by the complexes may contribute to solubility and dissolution properties of the complexes. The solid state characterization suggested that all the graphs of binary and ternary systems were similar to the graphs of HPBCD because with the increase in complexation efficiency and addition of TPGS/AA2G, an excess of free HPBCD may have been present in each of the inclusion complex samples [Misiuk and Zalewska, 2009].

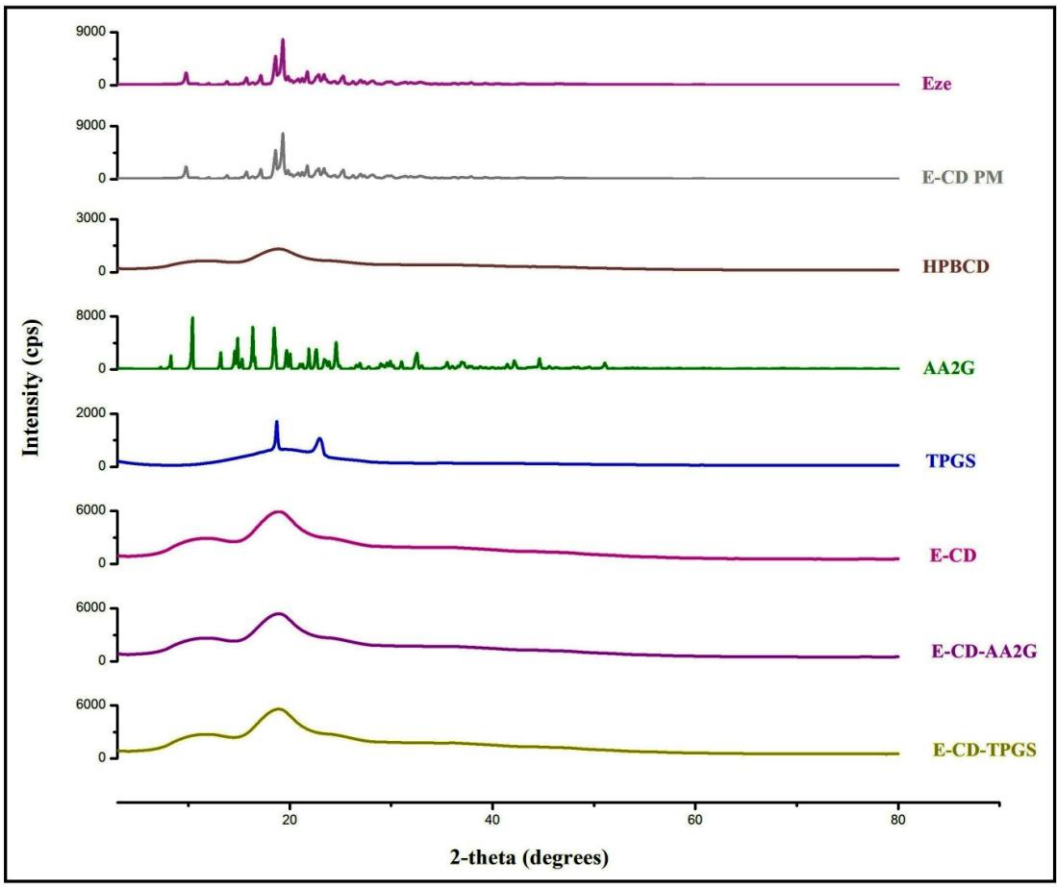

Figure 6.10. X-ray diffractograms of parent compounds, physical mixture and complexes.

The DSC and XRD graphs confirmed complete amorphization of binary and ternary systems similar to Selic and Ham [2008] and unlike the incompletely amorphous Eze:HPBCD complexes reported in the earlier works [Patel et al., 2008; Taupitz et al., 2013]. The auxiliary components could have synergistically favored the drug entrapment into CD cavity. The complete amorphization confirmed complete drug complexation with the use of TBA to solubilize Eze solution and highlighted the efficient complexing power of HPBCD toward the drug through molecular interactions among drug, HPBCD and TPGS/AA2G.

6.4.2.1.4 SEM

SEM is a qualitative method used to visualize the microscopic surface morphology and study the surface structural properties of formulation ingredients and the finished products. The SEM images of all the samples were shown in Figure 6.11. Pure Eze existed as small stone shaped crystals whereas HPBCD appeared as irregularly large and small compact block solids with a thick and non-smooth surface. The microphotographs of TPGS could not be drawn on account of its waxy consistency. AA2G appeared as crystalline cylindrical particles. The stone shaped drug appearance was completely disguised in the binary and ternary systems. E-CD complex appeared as glossy amorphous aggregates with smooth surface indicating the disappearance of the original morphology of drug and HPBCD and confirmed the formation of an intrinsic inclusion complex. E-CD-TPGS system looked like processed rectangular blocks with slightly rough surfaced appearance which might be due to the adsorption offered by TPGS as waxy layering in the ternary complex. The thick surface adherence of AA2G was quite prominent in the microphotographs of E-CD-AA2G ternary system. AA2G, upon lyophilization might have assumed a more polished and radiant look and the same was reflected in the SEM pictures of E-CD-AA2G.

The SEM photomicrographs clearly confirmed the formation of new binary and ternary complexes of Eze with hydrophilic HPBCD and ternary components in the solid state as all the processed samples exhibited unique morphological properties. All the three systems, E-CD, E-CD-TPGS, and E-CD-AA2G, appeared not only different from the parent compounds but also different from each other. The altered particle shape, surface characteristics and intimate bonding with the hydrophilic excipients in complexes may

be expected to contribute to enhanced solubility and dissolution rate of Eze [Yadav et al., 2012].

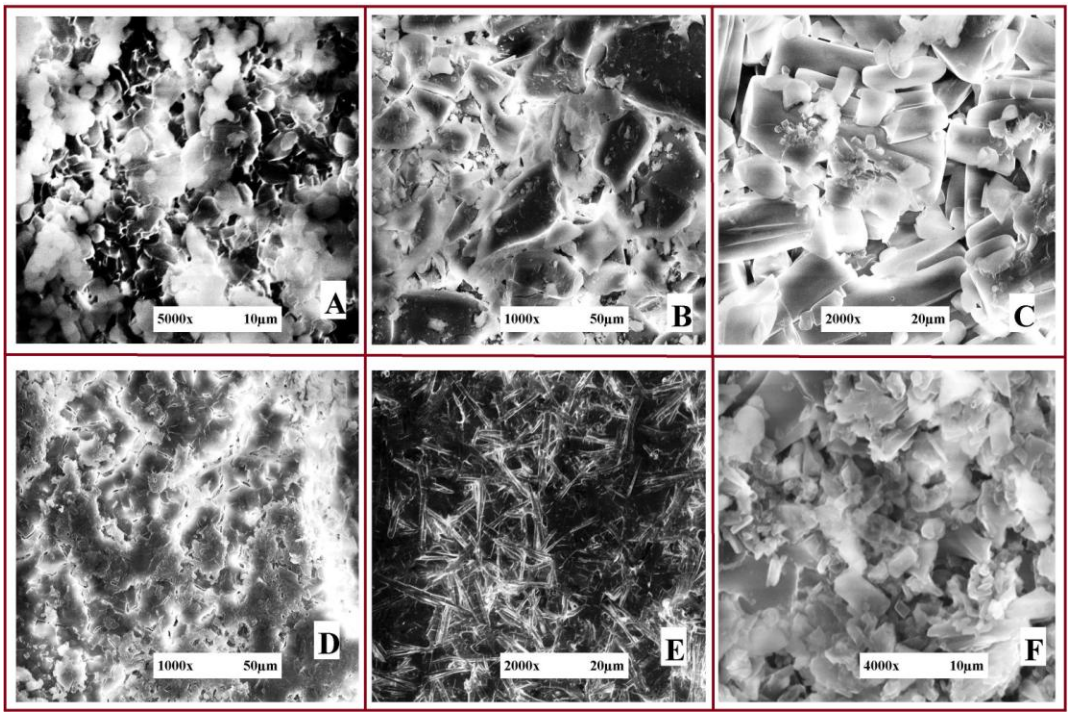

Figure 6.11. SEM photomicrographs of A:Eze; B:HPBCD; C:AA2G; D:E-CD; E:E-CD-AA2G; F:E-CD-TPGS.

6.4.2.1.5 H-NMR

It was quite evident from the FTIR, DSC and PXRD that the inclusion complexes contained HPBCD. The possible reason for the appearance of the graphs of inclusion complexes similar to HPBCD was also detailed. HPBCD is a high molecular weight compound and due to an increase in complexation efficiency, a little excess of free HPBCD may have been present in each of the inclusion complex samples [Misiuk and Zalewska, 2009].

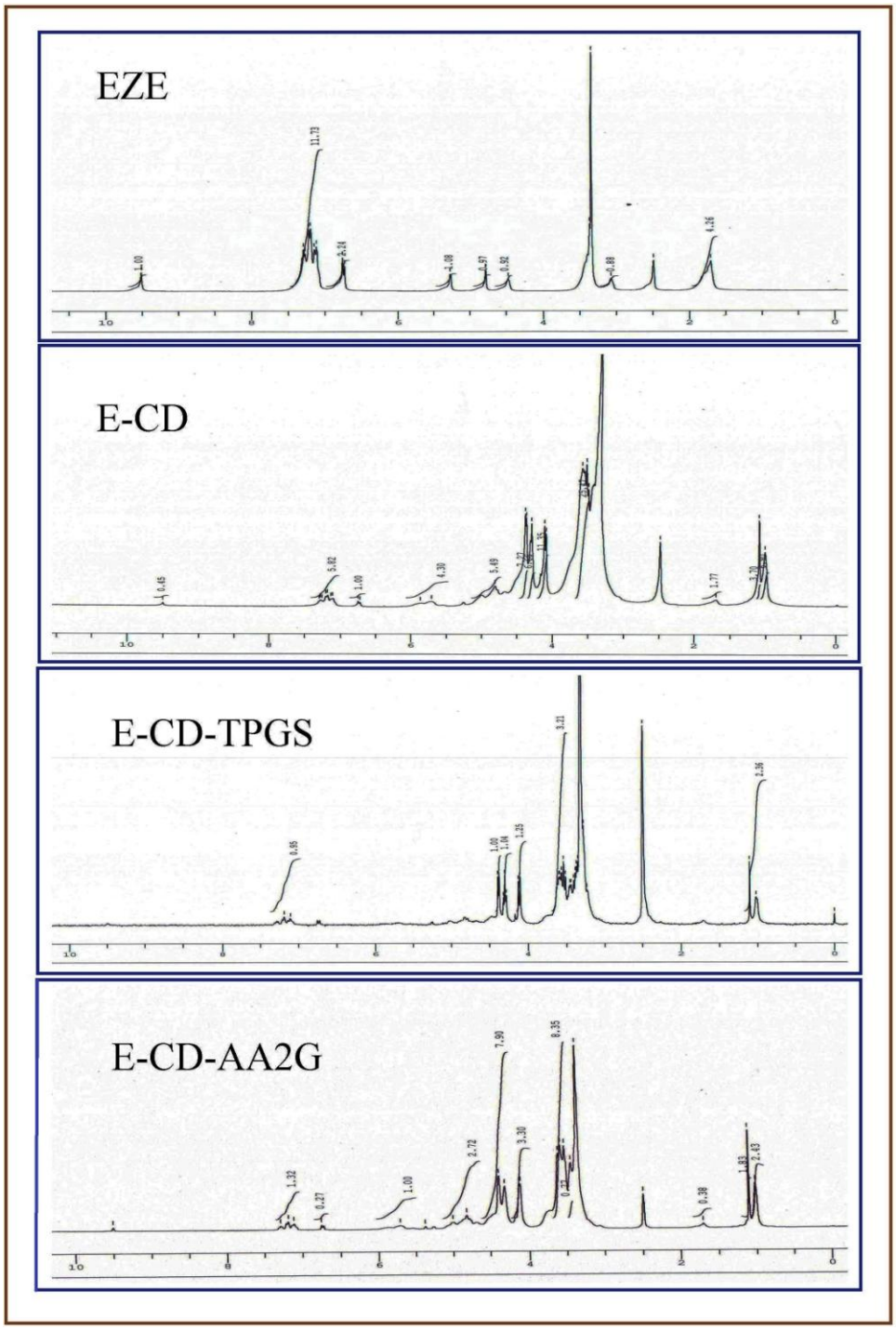

Figure 6.12. NMR spectra of pure Eze and complexes.

Parallely, the presence of Eze in the complexes was also undoubtedly and consistently confirmed by the FTIR spectral bands corresponding to the characteristic peaks of Eze. The formation of multicomponent systems was still clearly confirmed by SEM pictures. The SEM photomicrographs clearly confirmed the formation of new binary and ternary complexes of Eze with hydrophilic HPBCD and ternary components in the solid state as all the processed samples exhibited unique morphological properties. All the three systems, E-CD, E-CD-TPGS, and E-CD-AA2G, appeared not only different from the parent compounds but also different from each other. Additionally, the H-NMR study was an attempt to obtain a further confirmation on the presence of Eze in the inclusion complexes as well as the formation of inclusion complexes.

The H-NMR spectrum of pure Eze was compared with the spectra of the binary and ternary systems. As far as the application of NMR is concerned, there are two ways to interpret the CD complexation – one is to compare the NMR spectra of pure drug and complexed drug (binary/ternary system) and identify changes and; the other is to compare the NMR spectra of plain CD and complex (binary/ternary system) and identify changes [Wu et al., 2010; Zeng et al., 2011]. The first procedure was observed in this study.

H-NMR spectroscopy is the most effective method for studying the CD inclusion complexes. The protons of the guest and host molecules are likely to feel the physical and chemical environment affected due to the inclusion phenomenon and the formation of inclusion complexes can be proved by the complexation induced changes in the chemical shifts recorded in the one-dimensional H-NMR spectra. The proton chemical shift values of pure/free Eze and the binary and ternary systems prepared were recorded at 300 MHz and 25 ˚C. Samples were dissolved in DMSO and degassed by bubbling N_2

directly in the NMR tubes. The structure of Eze molecule with the proton numbering used for H-NMR was presented in Figure 6.1. The one-dimensional H-NMR chemical shifts were reported as ppm. The chemical shift values of the various protons of Eze (Figure 6.1) were given in Table 6.2. The H-NMR spectra of pure Eze and complexes were shown in Figure 6.12.

Table 6.2. NMR data table presenting protonic shifts of Eze protons after binary and ternary complexation (chemical shifts values in ppm).

Proton	**Free Eze**	**E-CD**	**ΔE-CD**	**E-CD-AA2G**	**ΔE-CD-AA2G**	**E-CD-TPGS**	**ΔE-CD-TPGS**
1, 1^1	7.110	7.135	0.025	7.125	0.015	7.134	0.024
2, 2^1	7.187	7.206	0.019	7.206	0.019	7.218	0.031
3	5.275	5.866	0.591	5.706	0.431	5.764	0.489
4	4.48	4.4	0.084	4.391	0.093	4.394	0.09
5	1.708	1.703	0.005	1.701	0.007	1.715	0.007
6	1.708	1.703	0.005	1.701	0.007	1.715	0.007
7	3.337	3.391	0.054	3.372	0.035	3.389	0.052
8	4.792	4.782	0.01	4.786	0.006	4.793	0.001
9, 9^1	7.187	7.206	0.019	7.206	0.019	7.218	0.031
10, 10^1	6.725	6.747	0.022	6.747	0.022	6.758	0.033
11	9.511	9.505	0.006	9.505	0.006	9.498	0.013
12, 12^1	7.263	7.283	0.02	7.281	0.018	7.287	0.024
13, 13^1	7.110	7.135	0.025	7.125	0.015	7.134	0.024

FTIR and NMR spectra are drawn to confirm the formation of inclusion complexes. It has been described even in the past publications that the formation of ternary complexes is hard to be determined by either of the spectroscopic techniques due to the overlapping spectral readings [Valero et al., 2003; Ammar et al., 2006; Taupitz et al., 2013]. Only the existence of the Eze:HPBCD complex in all the systems may be inferred. The possibility that the inclusion complexation could have involved formation of hydrogen bonds between the drug and HPBCD as suggested by FTIR studies was confirmed by the proton shifts recorded in H-NMR studies.

The NMR spectrum of free drug was the same as reported by Guntupalli et al. [2014]. Eze has 13 different types of protons and some protons experienced upfield shifts and others, downfield. Such variations might have occurred possibly due to the steric effects from HPBCD, slight variations in local polarity and differential shielding undergone due to vander Waals interactions with HPBCD and TPGS/AA2G. The inclusion could have become stabilized by the formation of hydrogen bonding (a) between the electronegative atoms of the Eze molecule and the protons of the HPBCD molecule (the interior H3 proton located at the wide side of the CD cavity and the H5 proton located at the narrow side of the cavity) [Bernini et al., 2004], and; (b) between the oxygen atom of HPBCD and the hydrophobic alkyl or aryl protons of Eze or may be even the alkyl hydroxyl protons of Eze. The ppm shifts quantitatively demonstrated the stability of inclusion complexes and the depth of ligand penetration into CD cavity. Eze has 13 different types of protons and the chemical shifts underwent by each of those protons upon binary and ternary complexation were listed in Table 6.2.

Some protons experienced upfield shifts and others, downfield. Such variations might have occurred possibly due to the steric effects from HPBCD. Significant shifts were

noted for protons, H1, H2, H3, H4, H7, H9, H10, H12 and H13 and the justifications for such observations were as follows. The H3 of alkyl hydroxyl group experienced drastic downfield shift which could be due to the participation of this proton in the hydrogen bonding with the oxygen atom of glucopyranose unit of HPBCD. Similar explanation could be extended to the downfield shift felt by the H7 proton (the hydrogen atom of the –CH– group might have hydrogen bonded with the oxygen of CD). The H4 proton recorded upfield shifts which may be on account of slight variations in local polarity. The low upfield shifts normally indicate weak interactions of the hydrogen atoms and shielding undergone due to vander Waals interactions with the CD molecules. The rest of the protons (H1, H2, H9, H10, H12 and H13) were aromatic and any shift in their ppm could be considered reasonable since they represent the hydrophobic regions of the drug molecule (benzene rings) and they could have got placed into the lipophilic core of HPBCD.

Based on the chemical shift values, it may be inferred that Eze molecule could have been captivated by HPBCD cavity in all the binary and ternary sytems. An intrinisic inclusion complex formation may be inferred though the exact orientation of Eze in the CD cavity needs to be established.

The NMR studies have been basically conducted to confirm the presence of Eze in the complexes. It has been observed from all the afore mentioned characterization studies, FTIR, DSC and PXRD that the graphs of all the binary and ternary complexes were replicates of HPBCD graphs. This observation may, though, be understood as complete amorphization of samples due complete entrapment of Eze inside the HPBCD cavity, a valid confirmation as NMR was desirable. The purpose of the H-NMR study was to reaffirm the presence of drug in the prepared binary and ternary complexes.

Accordingly, the NMR studies established the presence of Eze in all the prepared complexes and indicated the complexation of drug with HPBCD in all the binary and ternary complexes.

6.4.2.2 Drug content

The drug content analysis was performed in triplicate and the average was reported. The percentages of drug content in E-CD, E-CD-TPGS and E-CD-AA2G were found to be 98.9±1.89% w/w, 100.21±1.02% w/w and 99.99±1.32% w/w, respectively. The results indicated that the drug was uniformly distributed in all the complexes.

6.4.2.3 Octanol–water partition coefficient (P) and log P

The octanol–water partition coefficient, P is the measure of the differential solubility of drug in octanol and water. It is the ratio of drug concentration in octanol to that in aqueous phase. The logarithm of this octanol–water partition coefficient, log P is a major physicochemical descriptor and is otherwise known as lipophilicity or Hansch factor. This parameter is widely used in the quantitative structural activity relationship studies of organic compounds for modeling biochemical and pharmacological processes, transports across biological membranes, pharmacokinetics and toxicity studies [Misiuk and Zalewska, 2009]. Since literature suggests that CD complexes are likely to alter the transport properties of parent drug molecules [Loftsson et al., 1994; Misiuk and Zalewska, 2009; Loftsson and Brewster, 2010], a test of log P values that affect the transport of drug molecules across biological membranes, was made part of this segment of the research work.

The experimental results of log P measurements were tabulated in Table 6.3. Δ log P (the relative hydrophilicity enhancement) was also determined to express the improved hydrophilicity brought about by HPBCD complexation and addition of auxiliary substances to the binary complex. The Δ log P can be defined as Δ log P = log P guest (pure drug) – log P complex (binary/ternary) [Misiuk and Zalewska, 2009].

Table 6.3. Saturation solubility and log P values of pure Eze and Eze CD complexes. Data shown as Mean±SD (n = 3).

System	Saturation aqueous solubility (10^{-3} mg/mL)	Log P	Δ log P
Eze	1.99±0.62	1.602±0.029	-
E-CD	12.01±0.23 [a***]	0.765±0.061 [a***]	0.837±0.032
E-CD-TPGS	14.55±0.18 [a***, b**]	0.686±0.032 [a***]	0.916±0.003
E-CD-AA2G	15.26±0.74 [@***]	0.646±0.054 [a***]	0.956±0.025
Symbols and statistical representations = ***$p < 0.001$ and **$p < 0.01$; a = compared to pure Eze; b = compared to Eze-CD; @ = compared to pure Eze and Eze-CD (One way ANOVA followed by Tukey's post hoc test).			

The octanol–water partition coefficient, P is the measure of the differential solubility of drug in octanol and water and log P is Hansch factor and indicates the lipophilicity [Misiuk and Zalewska, 2009 and 2011]. The log P results were in the order Eze > E-CD > E-CD-TPGS > E-CD-AA2G ($p < 0.05$ for each comparison against pure Eze). Eze depicted low hydrophilicity and this property was significantly improved by complexation with HPBCD and with the use of ternary components. The results suggested improvement in the hydrophilicity of drug by 2 – 2.5 times by binary and ternary complexation and E-CD-AA2G system showed the highest hydrophilicity. The

enhanced hydrophilicity may contribute to improved bioavailability of drug from the prepared formulations.

6.4.2.4 Saturation aqueous solubility studies

The aqueous saturation solubility results of pure drug, binary and ternary systems were shown in Table 6.3. Beyond the results from solid state analysis, it is the remarkable enhancement in solubility and release properties that could be seen as proof for the ternary complex formation and for possible interactions between HPBCD/Eze and TPGS/AA2G. The solubility of Eze in distilled water improved significantly up to 6 – 7.7 folds by binary and ternary complexation in comparison to pure drug ($p < 0.05$ for each complex in comparison to pure Eze). CD complexation caused amorphization of drug and significantly improved the solubility of the drug by decreasing the surface tension between the medium and drug. Introduction of TPGS or AA2G successfully increased HPBCD solubilization of Eze. E-CD-TPGS and E-CD-AA2G systems might be novel, but, there are no reported results for the log P and aqueous solubility of binary E-CD system too, in the literature. The solubility of each of the ternary complex was significantly higher than that of E-CD ($p < 0.05$) but, the difference in the solubilities of E-CD-AA2G and E-CD-TPGS was not statistically significant ($p > 0.05$). However, it needs to be noted that the amount of HPBCD employed in E-CD-AA2G was half that of E-CD-TPGS system.

TPGS is an amphiphilic molecule which means it contains both hydrophilic and lipophilic groups. The lipophilic portion of the molecule may tend to get attracted toward the CD cavity and may pose competition to the drug. However, CD-guest complexes are formed at definitive ratios and the ratio is characteristic of the guest

molecule. In liquid state solubility study, section 6.4.1.1., a decrease in the solubility of drug was observed with increasing concentration of TPGS beyond 0.1%. When used above 0.1%, TPGS might have competed with the drug for CD cavity and the free TPGS and CD molecules available for drug molecules could have been insufficient to solubilize the drug efficiently and eventually caused decrease in drug solubility. Such phenomenon of competition in the presence of surfactant excipients has already been reported [Nogueiras-Nieto et al., 2012]. Even if TPGS had not competed for CD cavity, there could have been some interaction between TPGS and the external surface of HPBCD which would have interfered with the drug affinity to the inner cavity of CD. In case there was no prevalence of competition or interference posed by TPGS, the presence of two solubilizers could have contributed to a significant additive effect on the drug solubilization which did not happen. However, with the concentration of TPGS kept optimum in the solid ternary system (0.05%), it was assumed that the competition phenomenon subsided. The concentration of TPGS chosen was 0.05% w/v because this concentration it fells on the ascending side of the solubility graph. Both CD and TPGS could have been available to the drug as solubilizers by inclusion complexation and by surfactant (micellar solubilization) effect, respectively [Nogueiras-Nieto et al., 2012]. While HPBCD is also known to cause non-inclusion type of micellization/self-association mode of solubilization of drugs, the surfactant activity of TPGS is likely to prevent the former possibility. Surfactant excipients, when used as ternary components, are known to prevent the formation of HPBCD aggregates [Nogueiras-Nieto et al., 2012]. TPGS could have completely coated the E-CD inclusion complex (indicated by SEM studies) by interacting with both the drug and CD by weak intermolecular bonds and formed a stable E-CD-TPGS ternary system. The optimization of the proportions of

ingredients of the solid ternary complex could have brought about the aqueous solubility enhancement in case of E-CD-TPGS ternary system.

AA2G is a completely hydrophilic molecule that lacks any affinity to the lipophilic CD cavity. This glucosidic water soluble substance could have had a definitive synergistic effect on the CD drug solubilization as indicated by the liquid state solubility study, section 6.4.1.1. AA2G may not have diffused into the CD cavity but there is a possibility of hydrogen bond formation or dipole-dipole interaction between CD and AA2G which explains the shifting of phase solubility curve of this liquid state ternary system to A_L type as compared to the A_P type exhibited by the liquid state binary system. Another possibility is that AA2G could have formed weak hydrogen or vander Waals bonding with drug or might have also favored the HPBCD's non-inclusion type of micellization/self-association mode of solubilization of the drug. With the increasing AA2G concentration, the solubility of drug also increased and an optimal minimum quantity (0.1%) of AA2G was chosen for the preparation of solid ternary complex in order to keep the amount of ternary component to an optimal minimum. The chemical structure of AA2G is enriched with several hydroxyl groups which offer higher possibility to hydrogen bond with drug as well as HPBCD. As such, AA2G could have totally coated the E-CD inclusion complex (indicated by SEM studies) by interacting with both the drug and CD by weak intermolecular bonds and formed a stable E-CD-AA2G ternary system. The proportions of the components of solid ternary system chosen to formulate the E-CD-AA2G system were sufficient to achieve greater aqueous solubility of drug even at half the CD concentration (compared to E-CD and E-CD-TPGS).

6.4.2.5 Dissolution

Though Eze contains ionisable groups [Figure 2.17 or Figure 6.1], 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 [Taupitz et al., 2013]. For the same reason, the formulations in this study were optimized 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 FDA Dissolution Methods Database guide for Eze.

The dissolution efficiency (DE), $t_{80\%}$ and $t_{90\%}$ values were calculated for each of the systems and tabulated in Table 6.4. The dissolution profiles of all the systems were shown in Figure 6.13.

Table 6.4. Dissolution data of pure Eze and Eze CD complexes shown as Mean±SD (n = 6).

System	DE (%)	$t_{80\%}$ (mins)	$t_{90\%}$ (mins)
Eze	24.91±1.65	Not achieved	Not achieved
E-CD PM	25.93±1.14	Not achieved	Not achieved
E-CD	73.54±1.32^{a***}	57.14±1.2	84.38±1.32
E-CD-TPGS	82.08±1.44 $^{@***}$	40.91±1.33 ^{b***}	56.25±1.28 ^{b***}
E-CD-AA2G	85.92±1.19 $^{@***, \#*}$	29.27±1.36 $^{\$***}$	42.19±1.23 $^{\$***}$

Symbols and statistical representations = ***$p < 0.001$ and *$p < 0.05$; a = compared to pure Eze and Eze-CD PM; b = compared to Eze-CD; @ = compared to pure Eze, Eze-CD PM and Eze-CD; # = compared to E-CD-TPGS; $ = compared to Eze-CD and E-CD-TPGS (One way ANOVA followed by Tukey's post hoc test).

The drug dissolution rate was greater for both binary and ternary systems and the pure drug obviously presented the lowest dissolution rate because of its low solubility. DE was improved by 3 – 3.5 folds on binary and ternary complexation as compared to pure Eze. The DE values were significantly higher in the order: E-CD-AA2G > E-CD-TPGS > E-CD > E-CD PM ≈ Eze ($p < 0.05$ for each comparison except E-CD PM and Eze). Pure drug did not dissolve more than 36.2±4.2% during 120 mins dissolution study and the difference in dissolution of E-CD PM and Eze was insignificant ($p > 0.05$). Both $t_{80\%}$ and $t_{90\%}$ values were significantly lower in the order: E-CD-AA2G < E-CD-TPGS < E-CD ($p < 0.05$ for each comparison).

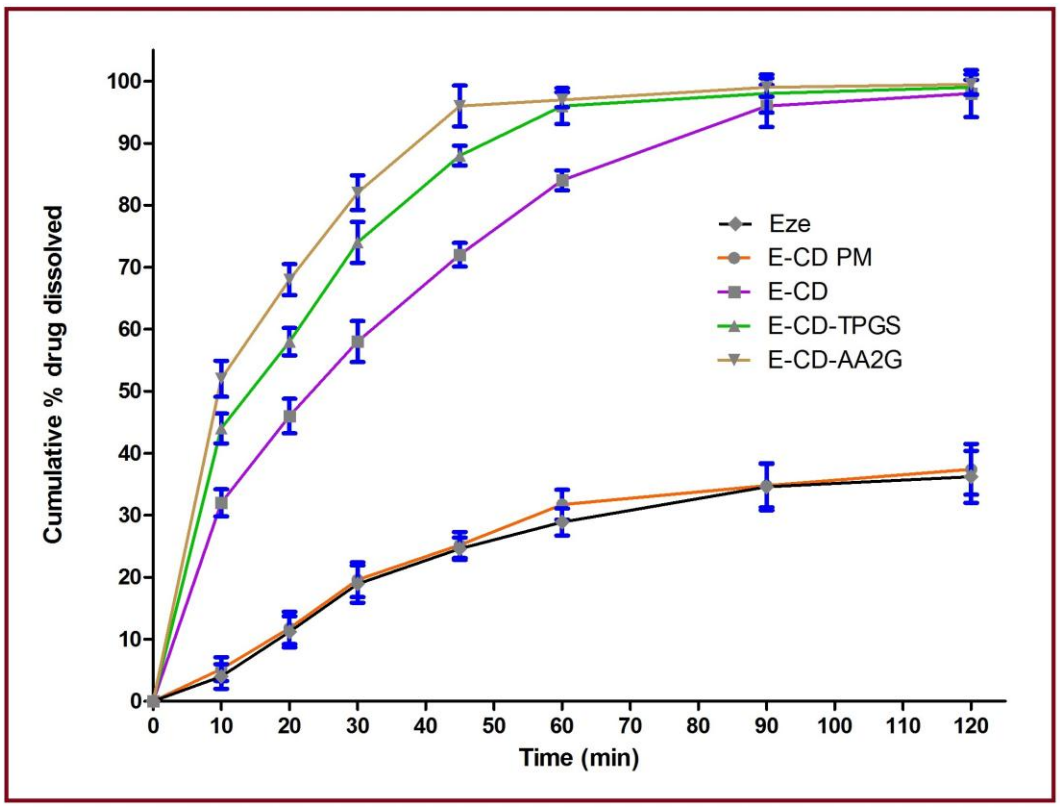

Figure 6.13. Dissolution profiles of pure drug, binary and ternary complexes (vertical bars represent SD, n = 6).

The percent drug dissolved in 30 mins for the systems, E-CD, E-CD-TPGS and E-CD-AA2G was 58.1±3.3%, 74.2±2.4% and 82.5±2.8%, respectively. Patel et al. [2008] conducted dissolution studies in phosphate buffer (pH 7.8 and containing 1% w/v SLS) and reported 60.7% drug dissolution ($p > 0.05$ compared to the E-CD system in the present part of study) and Bandyopadhyay et al. [2012] reported 67.04% drug dissolution ($p < 0.05$ compared to the E-CD system in the present part of study) in 0.5% w/v SLS solution, within 30 mins, from their respective binary Eze:HPBCD systems. The differences may be attributed to the altered dissolution medium employed.

In case of binary system, the hydrophilic HPBCD with its ability to form a stable inclusion complex might have been responsible for improved dissolution of drug. In the ternary systems, the hydrophilic auxiliary substances further improved the stability of inclusion complex (phase solubility investigations) by adsorption phenomenon which was confirmed by the SEM reports. The surface coating offered by AA2G, in particular, was clearly evident in the microphotographs and the same might have resulted in a relatively superior *in-vitro* performance of this ternary complex. The layering of these hydrophilic ternary components onto binary drug-CD complexes resulted in the formation of ternary complexes and imparted additional hydrophilicity to the systems (log P analysis) which offered synergistic positive effect on the aqueous solubility and dissolution rate of the drug. The ternary complexes performed better not only as compared to the pure drug but also as compared to the binary complex.

6.4.2.6 Stability

The aim of the stability testing was to study the variation in the quality of the drug product with time under the influence of environmental factors such as temperature and

humidity. The purpose of the stability study was to recommend, based on testing a minimum of three batches of the drug product, the storage instructions applicable to all future batches of the drug product manufactured and packaged under similar circumstances. In the present study, six batches of ternary systems were subjected to stability testing. The results of drug content, solubility and dissolution tests, observed before and after 6 months, were tabulated in Table 6.5.

Table 6.5. Stability study performances of optimized ternary complexes. Data shown as Mean±SD and n = 3 (n = 6 for dissolution data).

Tests		**Properties before stability storage studies**		**30±2 ˚C/70±5% RH storage evaluation after 6 months**	
		E-CD-TPGS (initial)	**E-CD-AA2G (initial)**	**E-CD-TPGS (final)**	**E-CD-AA2G (final)**
Description		Fine white powder	Fine white powder	Complies	Complies
Drug content by UV (%)		100.21±1.02	99.99±1.32	100.04±1.28	99.86±1.44
Saturation aqueous solubility (10^{-3} mg/mL)		14.55±0.18	15.26±0.74	14.36±0.22	15.14±0.56
Dissolution by UV	**DE_{120} (%)**	82.08±1.44	85.92±1.19	81.42±1.16	84.84±1.28
	$t_{80\%}$ (min)	40.91±1.33	29.27±1.36	41.38±0.96	29.63±1.3
	$t_{90\%}$ (min)	56.25±1.28	42.19±1.23	56.49±1.02	43.09±1.18

The dissolution profiles of stability batches in comparison to fresh batches were shown in Figure 6.14. On comparing the stored samples with the initial samples, it was observed that neither the physical appearance nor the drug content of the stored samples was influenced by the storage conditions.

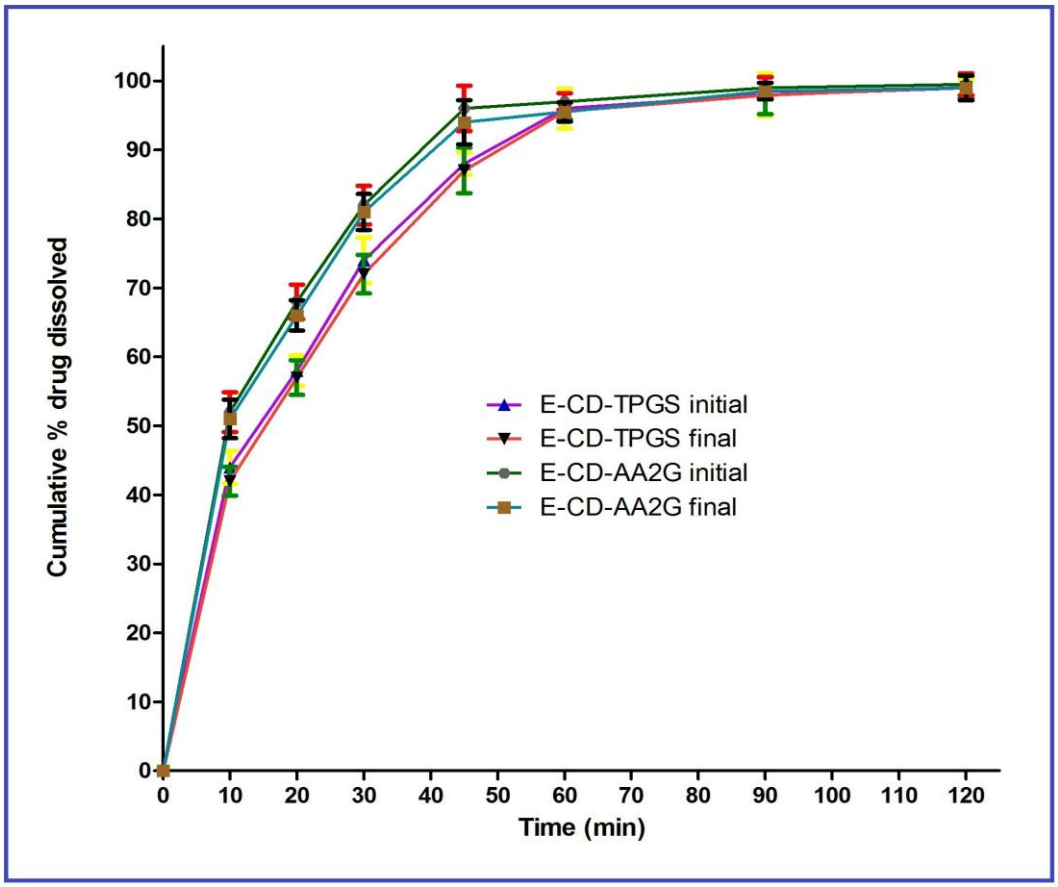

Figure 6.14. Dissolution profiles of ternary complexes before and after stability study (vertical bars represent SD, n = 6).

No significant change in solubility or drug release properties was noted after 6 months of storage. The observations indicated that the formulations were stable and capable of withstanding the environmental fluctuations during storage and handling. Both the ternary complexes, E-CD-TPGS and E-CD-AA2G, maintained their stability for the period of 6 months and showed neither noticeable change in physical appearance nor statistically significant variation in drug content, solubility or dissolution results suggesting that the room temperature storage as acceptable.

6.4.2.7 *In-vivo* preclinical pharmacokinetic study

6.4.2.7.1 HPLC-UV plasma drug analysis – method development and validation

validation – same as described under the section 5.4.2.8.1.

6.4.2.7.2 Pharmacokinetic parameters

The pharmacokinetic parameters were determined using Kinetica 5.0 pharmacokinetic software (Trial version, PK-PD analysis, Thermofischer) and Graphpad Prism software (version 5.03, GraphPad Software, USA). The plasma profiles of total Eze quantified in adult male Albino Wistar rats following single dose oral administration of pure drug suspension, E-CD-TPGS and E-CD-AA2G were reported in Table 6.6.

Table 6.6. Pharmacokinetic parameters derived for pure Eze and optimized ternary complexes (n = 6). Data shown as Mean±SD.

Parameter/Treatment	Pure drug	E-CD-AA2G	E-CD-TPGS
T_{max} (h)	2±0.00	1.5±0.00	1.5±0.00
C_{max} (ng/mL)	1912±195.92	2339±242.68	3380.5±359.97$^{@**}$
AUC_{0-24h} (ng. h/mL)	17848±1306.54	19591±1420.26	24145±1421.89
$AUC_{0-\infty}$ (ng. h/mL)	18664.43±1324.18	20481.69±1492.44	25215.6±1476.92$^{a**; b*}$
$AUMC_{0-24h}$ (ng. h^2/mL)	163568±3801.65	172128±4608.46	206846±4654.93
MRT_{0-24h} (h)	9.15±1.02	8.76±1.68	8.55±1.79
% $RB_{(0-24h)}$	100±0.00	109.76±1.47	135.28±2.04
% $RB_{(0-\infty)}$	100±0.00	109.73±1.73	135.1±2.21

Symbols and statistical representations = $^{**}p < 0.01$ and $^{*}p < 0.05$; a = compared to pure Eze; b = compared to E-CD-AA2G; @ = compared pure Eze and E-CD-AA2G (One way ANOVA followed by Tukey's post hoc test). % RB = % Relative bioavailability with respect to pure drug.

The peak plasma concentration (C_{max}) and the time to attain C_{max}, T_{max}, were recorded directly from the plasma concentration – time curve. The area under the plasma concentration – time curve was determined by trapezoidal method. It was observed that the plasma concentration time profile of Eze for ternary complexes represented improved drug absorption compared to the simple drug suspension.

Pharmacokinetic study was performed to quantify the total plasma Eze concentration (free plasma Eze + plasma EzeG) following single dose oral administration of pure drug suspension and ternary formulations as 0.25% w/v NaCMC dispersions to male Albino Wister rats. After oral administration, the absorbed Eze is extensively conjugated to EzeG, a pharmacologically active metabolite. Addition of β-glucuronidase (minimum 100,000 units/mL) to the plasma samples aided in the conversion of EzeG to Eze and the total Eze in plasma was estimated and reported.

The T_{max} was observed in the order, pure drug > E-CD-TPGS = E-CD-AA2G. The T_{max} of pure drug was seen at 2 h and that for both, E-CD-TPGS and E-CD-AA2G was recorded at 1.5 h. Both the ternary complexes reduced the T_{max} of pure Eze. The parameters, C_{max}, $AUC_{0\text{-}24h}$, $AUMC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$ were observed in the order, E-CD-TPGS > E-CD-AA2G > pure drug. The mean C_{max} of pure drug was improved by 2 and 1.2 times, respectively, by E-CD-TPGS and E-CD-AA2G. The C_{max} of E-CD-AA2G was higher than pure Eze, though, not statistically significantly ($p > 0.05$); the C_{max} of E-CD-TPGS was significantly higher ($p < 0.01$) than either pure Eze or E-CD-AA2G. The mean values of each of the following three parameters, $AUC_{0\text{-}24h}$, $AUMC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$ of pure drug were improved by 1.4 and 1.1 times, respectively, by E-CD-TPGS and E-CD-AA2G. The $AUC_{0\text{-}\infty}$ of E-CD-AA2G was higher than pure Eze, though, not statistically significantly ($p > 0.05$); the $AUC_{0\text{-}\infty}$ of E-CD-TPGS was significantly higher

compared to pure Eze ($p < 0.01$) as well as E-CD-AA2G ($p < 0.05$). The $AUMC_{0\text{-}24h}$ of E-CD-AA2G was higher than pure Eze and that of E-CD-TPGS was significantly higher than either pure Eze or E-CD-AA2G. The MRT values of pure drug, E-CD-TPGS and E-CD-AA2G were insignificantly different on comparison.

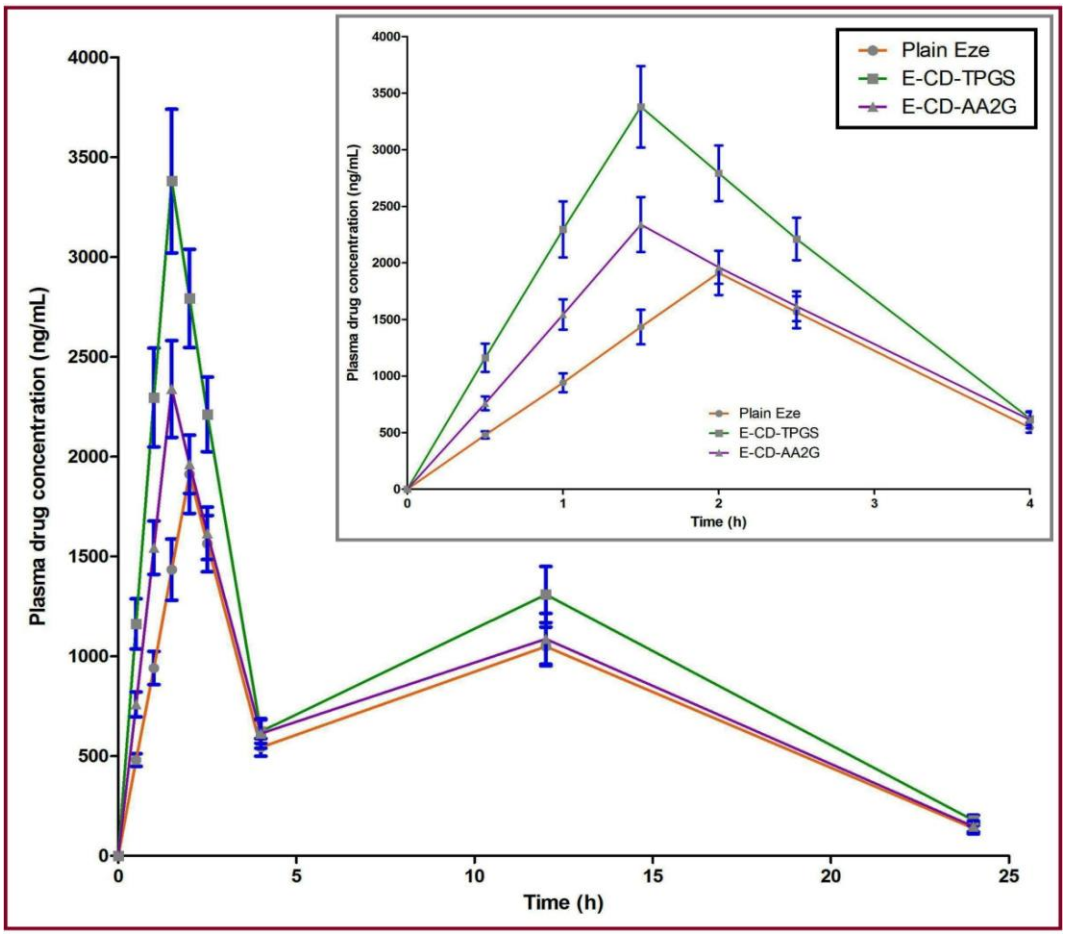

Figure 6.15. Pharmacokinetic profiles of pure Eze and optimized ternary complexes (vertical bars represent SD, n = 6). Inset shows the profile up to 4 h.

The plasma concentration time profile of Eze from the drug suspension and both the ternary complexes as depicted Figure 6.15 indicated that Eze reached its peak followed by a rapid decline and this pattern repeated leading to the occurrence of multiple peaks

in the plasma concentration time graph. These multiple peaks were due to the fact that Eze is subjected to extensive glucuronidation to a phenolic glucuronide at its site of action, the intestine and is then excreted into the bile. It may be possible that Eze experiences enterohepatic recirculation and is repeatedly delivered back to its site of action, the intestinal tract lumen, after undergoing reabsorption in the ileum [Bali et al., 2010 and 2011]. This reabsorption and recirculation processes have the potential to enhance the residence time of Eze in the lumen of the intestinal tract, thereby improving its cholesterol-lowering activity.

The T_{max} for both the ternary complexes was found to be 1.5 h while that of pure drug suspension was 2 h. A reduction in the value of T_{max} was observed in case of both the ternary complexes which could be due to the fact that ternary complexes presented the drug already in a solubilized form to the GIT and the major rate-limiting step in absorption process, the drug dissolution, might have been accomplished in a very short duration. In case of pure drug suspension, the drug is suspended in the form of fine particles and is yet to undergo dissolution in gastrointestinal fluids to get absorbed. MRT is an intrinsic property of a drug and therefore, no change in the intrinsic property of Eze was observed when the drug was formulated into CD complexes [Bali et al., 2010 and 2011]. The ternary CD complexes of Eze presented enhanced bioavailability which may be attributed to the increased solubility and immediate drug dispersion from these formulations in the GIT. In addition, as the CD complexes are also known to improve the membrane permeability of drugs, both the ternary complexes could have aided in improving the intestinal membrane permeation of Eze and resulted in enhanced oral absorption of the drug.

6.4.2.8 Antihypercholesterolemic activity

6.4.2.8.1 Principle behind performing the plasma cholesterol determination test for pure Eze and formulations – same as described under the section 5.4.2.9.1.

6.4.2.8.2 Hypocholesterolemic potential of pure drug and optimized formulations

The study was conducted for a total period of eight weeks wherein the first four weeks, all the animal groups were fed with 200 mg cholesterol in 2 mL coconut oil as high fat diet for inducing hypercholesterolemia. At the end of fourth week, the plasma cholesterol levels were measured for all the groups and the values were considered as baseline values for the following four week study, the actual antihypercholesterolemic activity study. It was noted that all the animals together showed a mean 80-85% elevation in total plasma cholesterol levels at the end of four week hypercholesterolemic induction study, when compared to day one mean value.

The percent reduction values in the levels of total plasma cholesterol achieved by various treatment groups were presented in Table 6.7 and Figure 6.16. Since, HPBCD is also known for its cholesterol homeostasis, two treatment groups, one treated with HPBCD alone and the other with E-CD PM were studied to evaluate the influence of this excipient on the antihypercholesterolemic activity of Eze:HPBCD complex formulations. The E-CD complex was studied to ensure the difference in its performance compared to E-CD PM and to analyze its activity against both the ternary complexes. The antihypercholesterolemic performance of pure Eze or pure HPBCD was statistically insignificant in comparison to the control group ($p > 0.05$). The activity of E-CD PM was statistically insignificant in comparison to the pure Eze or pure HPBCD

groups ($p > 0.05$) on all days and insignificant compared to control ($p > 0.05$) on days, 7 and 14. The decrease in plasma cholesterol levels presented by this treatment was significant in comparison to control group ($p < 0.05$ on day 21 and $p < 0.01$ on day 28). The performances of all the three formulations, E-CD, E-CD-TPGS and E-CD-AA2G were significantly prominent when compared to either control ($p < 0.001$) or pure Eze ($p < 0.001$), or pure HPBCD ($p < 0.001$), or E-CD PM ($p < 0.001$), on each of the test day. The improved performance of the three systems was also suggested by their enhanced *in-vitro* solubility and dissolution profiles.

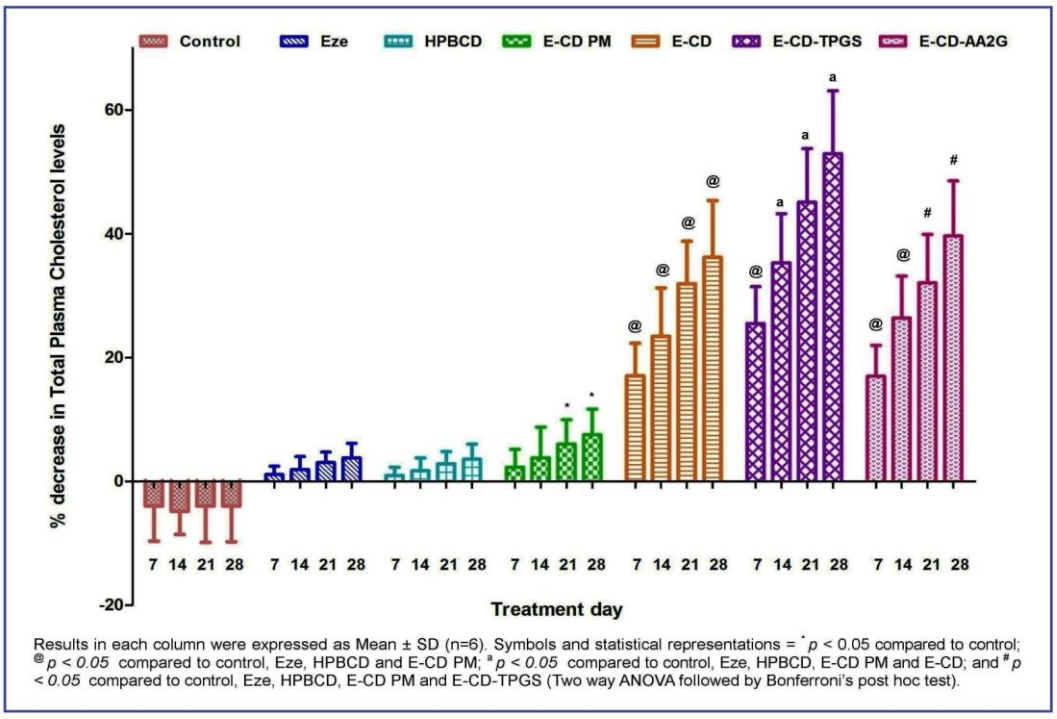

Figure 6.16. Percent reduction in the total cholesterol levels achieved by CD formulations (vertical bars represent SD, n = 6).

While the difference in the percent reduction in total plasma cholesterol levels achieved by E-CD and E-CD-AA2G was statistically insignificant ($p > 0.05$), the enhanced performance of E-CD-TPGS in comparison to either E-CD or E-CD-AA2G was

indicated in the Figure 6.16. The study showed that the cholesterol reduction efficiency of all the complexes, binary as well as ternary, were far superior compared to pure Eze or pure HPBCD or E-CD PM. The E-CD and E-CD-AA2G systems had the contribution of HPBCD to the pharmacological activity (HPBCD maintains cholesterol homeostasis) [Peake and Vance, 2012] of Eze.

Table 6.7. Percent reduction in the total cholesterol levels achieved by pure Eze and optimized ternary complex formulations. Results were expressed as Mean±SD (n = 6).

Treatment/Day	% Decrease in total plasma cholesterol levels			
	Day 7	**Day 14**	**Day 21**	**Day 28**
Control	-4±5.67	-4.8±3.78	-4±5.89	-4±5.75
Eze	1.13±3.21	1.89±5.18	3.02±4.16	3.77±5.84
HPBCD	0.94±3.28	1.7±5.12	2.83±4.84	3.58±5.92
E-CD PM	2.26±2.87	3.77±4.98	6.04±3.92^{a*}	7.55±4.14^{a*}
E-CD	17.02±5.26^{b***}	23.4±7.83^{b***}	31.91±6.87^{b***}	36.17±9.21^{b***}
E-CD-TPGS	25.49±5.98^{b***}	35.29±7.92$^{b***; c**}$	45.1±8.65$^{b***; c**}$	52.94±10.15$^{b***; c***}$
E-CD-AA2G	16.98±4.98^{b***}	26.42±6.74^{b***}	32.1±7.83$^{b***; d**}$	39.62±8.93$^{b***; d**}$

Symbols and statistical representations = ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$; a = compared to Control; b = compared to Control, Eze, HPBCD and E-CD PM; c = compared to E-CD; d = compared to E-CD-TPGS (Two way ANOVA followed by Bonferroni's post hoc test).

The non-surfactant solubilizer, AA2G played an insignificant role in improving the pharmacological action of Eze which suggests involvement of mechanisms other than solubility enhancement in explaining the superior hypocholesterolemic potential of E-CD-TPGS. The differentially superior performance of E-CD-TPGS in comparison to either E-CD or E-CD-AA2G may be explained by the agonistic contribution of HPBCD and P-gp inhibitory function of TPGS to the pharmacological action of Eze. Owing to

the surfactant action, TPGS could have not only improved the solubility *in-vitro* but have also altered the membrane permeability and inhibited the P-gp efflux of Eze at the intestine *in-vivo*. Though E-CD-AA2G presented superior *in-vitro* dissolution profile, the markedly superior pharmacological performance of E-CD-TPGS may be ascribed to reduced oral bioavailability variations of Eze. Also, considering the statistically insignificant difference in the aqueous solubilities of E-CD-AA2G and E-CD-TPGS, the latter may be noted as the most effective formulation for oral delivery of Eze.

6.5 SUMMARY

HPBCD solubilization of Eze was successfully improved by introducing TPGS/AA2G as ternary components in both solution and solid state. The surfactant properties of TPGS and the polyolic nature of AA2G served excellently in improving the drug aqueous solubility and dissolution properties. The use of solvent TBA enhanced the drug-CD complexation efficiency and caused complete amorphization of the drug in both binary and ternary systems. Use of auxiliary substances clearly increased the drug-CD complexing stability, solubility and dissolution even when compared to the binary system. HPBCD could have acted as a surfactant and increased the surface area and decreased the interfacial tension of drug particles on exposure to the dissolution medium and prevented the aggregation of drug particles. Both TPGS and AA2G favored the amorphous state and synergistically enhanced the surfactant action of HPBCD. The surfactant properties of TPGS and the sugar like properties of AA2G served excellently in improving the drug aqueous solubility and dissolution properties. Above all, with the ability of HPBCD to maintain cholesterol homeostasis [Peake & Vance, 2012], both the binary and ternary systems served as suitable formulations for Eze not only by

improving its aqueous solubility and dissolution but also by providing additive hypolipidemic effect and enhanced bioavailability. AA2G best served as ternary component at *in-vitro* level and decreased the amount of HPBCD required to solubilize Eze to half. However, at *in-vivo* level, E-CD-TPGS considerably improved the pharmacokinetics as well as antihypercholesterolemic action of Eze. Therefore, considering the cumulative *in-vitro* and *in-vivo* performances, E-CD-TPGS may be noted as the best ternary CD complex formulation in improving the solubility, oral absorption and reducing the bioavailability variations of Eze. TPGS is also known for its P-gp inhibitory action [Guo et al., 2013] and as such could have presented its advantage in improving the *in-vivo* absorption of P-gp substrate, Eze [Bandyopadhyay et al., 2012], at the small intestinal brush border.

6.6 GRAPHICAL SUMMARY

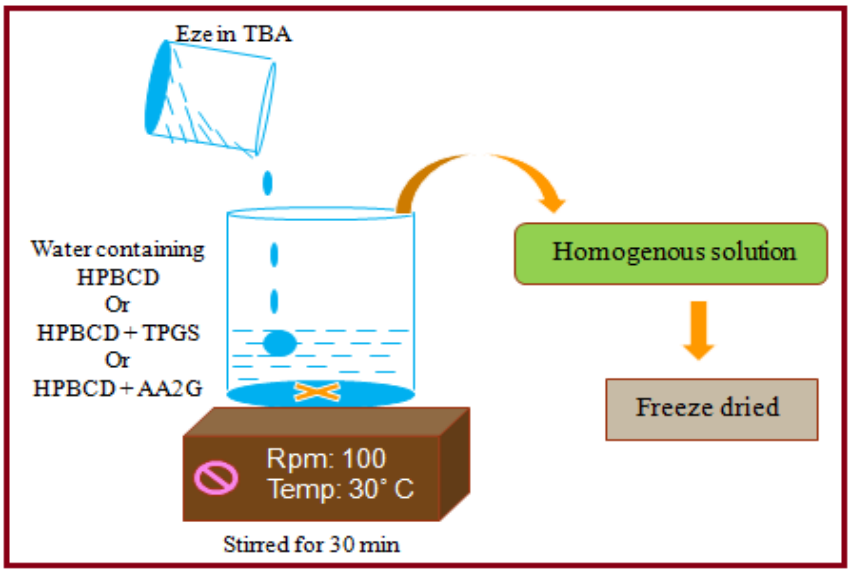

Figure 6.17. Schematic diagram showing formation of binary and ternary CD complexes of Eze.

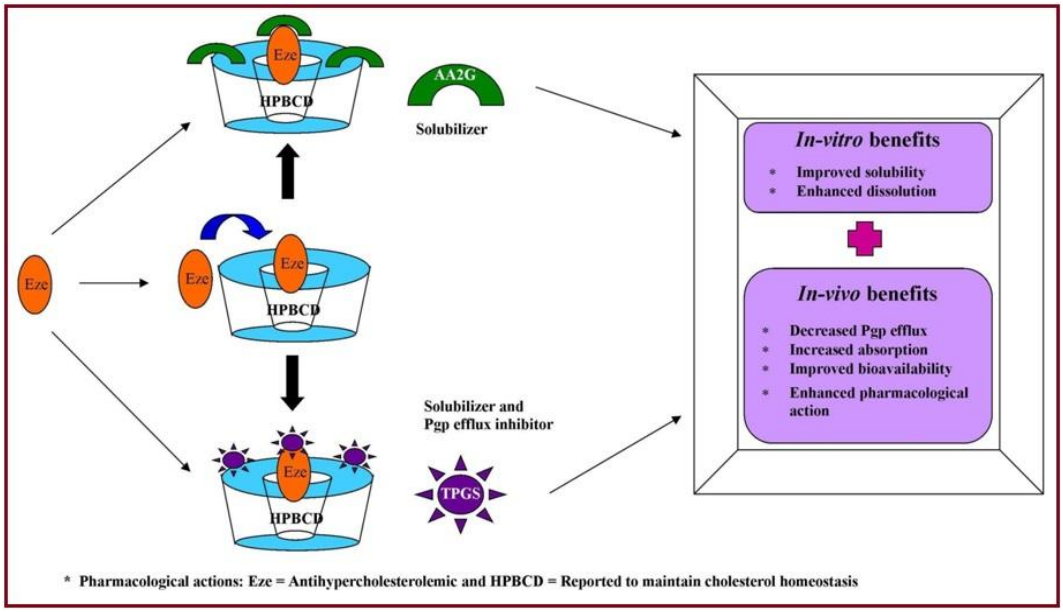

Figure 6.18. Gist of effect of ternary CD complexation formulation approach on Eze performance.