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Research Article

DISSOLUTION RATE ENHANCEMENT OF ATORVASTATIN, FENOFIBRATE AND EZETIMIBE BY INCLUSION COMPLEX WITH β-CYCLODEXTRIN

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ABSTRACT

Dissolution rate of inclusion complex of Atorvastatin calcium (AT) (10.23mg equivalent to 10mg of Atorvastatin), Fenofibrate (FE) (160mg) and Ezetimibe (EZ) (10mg) with β-cyclodextrin (β-CD) were investigated. The phase solubility profiles of AT, FE and EZ with β-CD were classified as A_L-type, which indicated the formation of 1:1 stoichiometry inclusion complexes. Stability constants with 1:1 molar ratio obtained from the phase solubility diagrams were 550.60 M^{-1}, 2020.61 M^{-1} and 1604.05 M^{-1} for AT, FE and EZ respectively. Quaternary systems of AT, FE and EZ with β-CD prepared by kneading and co-evaporation method were characterized by Differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRD). The dissolution profiles of inclusion complexes were determined and compared with those of AT, FE and EZ and physical mixtures with β-CD. The dissolution rate of AT, FE and EZ were increased by β-CD inclusion complexation. Highest dissolution rate was obtained by co-evaporation method followed by kneading method. However, the dissolution rate were increased significantly ($p < 0.05$) by co-evaporation method compared to kneading method.

Keywords: Atorvastatin, Fenofibrate, Ezetimibe, β-cyclodextrin, Inclusion complex.

INTRODUCTION

Cyclodextrins (CDs) are popular for their ability to form inclusion complex that increase the aqueous solubility and driving force for diffusion across the biological membrane for lipophilic

Drugs (J.A. Bruce *et al*., 2000; M. Mar *et al*., 1999) however, while forming inclusion complex with hydrophobic drugs, they do not alter their molecular structure and permeability characteristics (H. Brun *et al*., 2006). Complexation with cyclodextrins has been extensively used to enhance the aqueous solubility and dissolution rate of poorly water-soluble drugs (C.M. Fernandes *et al*., 2002). CDs have become popular due to their hydrophilic nature and ability to improve the solubility of poorly water-soluble drugs, enhancement in physicochemical properties and chemical stability of drugs (W. Xianhong *et al*., 2004). Due to hydrophobic central cavities, CDs are capable of forming stable complexes with properly sized guest molecules (L. Longxiao *et al*., 2006).

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis (Lennernas, 2003). Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. Atorvastatin calcium [R-(R*,R*)]-2-(4-fluorophenyl)- β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt, is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH (Lennernas, 1997; Wu et al., 2000). Fenofibrate, chemically 2-[4-(4-chlorobenzoyl) phenoxy] - 2-methyl-propanoic acid 1-methylethyl ester, is a lipid regulating agent. It is a highly lipophilic drug clinically used to lower lipid levels. However, its therapeutic efficacy has been compromised for years due to the virtual insolubility in water and physiological fluids (Guay, 2002). Ezetimibe, chemically (1-(4- flurophenyl)-3(R) - [3(S)-(4-flurophenyl) –3-hydroxy propyl] –4(S) (4 – hydroxyphenyl) azetidin- 2- one), belongs to a group of selective and very effective 2-azetidione cholesterol absorption inhibitors, acts at the level of cholesterol entry into enterocytes. By virtue of its extremely hydrophobic nature, the drug shows highly inconsistent dissolution profile in the gastrointestinal fluids (Bali *et al*., 2010). It is insoluble in water. Its bioavailability is highly unpredictable (Patel *et al*., 2008). Combination of AT, FE and EZ produces significant increase in HDL-C and reduction in TG with reduction in LDL-C compared with AT, FE and EZ alone (Peter *et al*., 2010). AT, FE and EZ are BCS class-II drugs. The present aim of the work was to enhance the dissolution rate of AT, FE and EZ in combination using inclusion complexation with β-cyclodextrin.

MATERIALS AND METHODS

Materials

AT was obtained from Torrent Research Center (India), FE and β-cyclodextrin were obtained from Zydus Research Center (India). EZ was obtained from Astron Research Center (India). All the reagents used were of analytical grade. Double distilled water was used throughout the experiments.

Phase solubility studies

Phase solubility studies were carried out in water in triplicate according to the method described by Higuchi and Connors (Higuchi et al, 1965). Excess amount of AT (10.23mg), FE (160mg) and EZ (10mg) were added to 20ml of aqueous solutions containing various concentrations of β-cyclodextrin (0, 2, 4… 16mM) and were shaken on rotary shaker at 25±2 °C for 4 days. After equilibrium was achieved, the suspensions were filtered through 0.45 µm membrane filter and appropriately diluted with methanol. The concentration of AT, FE and EZ were simultaneously determined by HPLC with PDA detector at 248nm, 288nm, 234nm respectively (A. Ajmera, et al., 2012). The apparent stability constants, K_s were calculated from phase solubility diagrams with the assumption of 1:1 stoichiometry according to the following equation:

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad \text{..... (eq. 1)}$$

Where K_S is the apparent binding/stability constant, and S_0 (intercept) is the intrinsic solubility of the compound (AT, FE and EZ) in absence of complexing agent.

Preparation of solid quaternary systems

The following quaternary systems of AT (10.23mg), FE (160mg) and EZ (10mg) with β-cyclodextrin (541.05mg) were prepared in 1:1 molar ratio.

Preparation of physical mixtures of AT, FE and EZ with β-cyclodextrin

Physical mixture (PMBC) was prepared by geometric mixing of AT, FE and EZ (AFE) with β-CD without applying pressure that had previously been sieved through sieve no.60.

Preparation of inclusion complex by kneading method

AFE and β-cyclodextrin (KNBC) were accurately weighed and transferred to mortar. The mixtures were triturated in a mortar with a small volume of water: ethanol (1:1 v/v) solution till a homogenous paste was formed. The paste formed was kneaded for 45 min and dried at 45 ^{0}C in an oven. The dried masses were pulverized and sieved through sieve no.60.

Preparation of inclusion complex by co-evaporation method

AFE and β-cyclodextrin (CEBC) were accurately weighed and dissolved in ethanol and water respectively. Both the solutions were mixed and solvents were evaporated by controlled heating at 45^{0}C - 50°C. The dried mass were pulverized and sieved through sieve no.60.

X-ray powder diffractometry (XRD)

The XRD patterns of AFE, AFE–β-cyclodextrin inclusion complexes and physical mixtures were recorded by using Philips Analytic X-Ray—PW3710s diffractometer with tube anodeCu over the interval 2–50^0/2Θ and scanning speed 1^0/min.

Differential scanning calorimetry (DSC)

A DSC model 60 from Shimadzu Corporation, Japan was used for recording DSC thermograms of the AT, FE and EZ, inclusion complexes, and their physical mixtures. Samples (2-8 mg) were accurately weighed and heated in open aluminum cells, at a rate of 10°C/min between a temperature range of 50°C and 300°C. Reproducibility was checked by running the sample in triplicate.

Dissolution studies

The dissolution rate studies of AT pure, FE pure, EZ pure, PMBC, KNBC and CEBC were performed in a dissolution apparatus (model: Electrolab 08L tablet dissolution test apparatus, Mumbai, India) using the paddle method (USP Type II). Dissolution studies were carried out using 900ml of phosphate buffer (pH 6.8) with 0.05M SLS at 75 rpm at 37°C ± 0.5°C. Samples of 5ml were withdrawn at time intervals of 5, 10, 20, 30, 50, 60 and 120 min. The volume of dissolution medium was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5ml of phosphate buffer (pH 6.8) with 0.05M SLS and simultaneously determined AT, FE and EZ by HPLC with PDA detector at 248nm, 288nm, 234nm respectively(A. Ajmera, *et al*; 2012). Dissolution studies were performed in three times, and calculated mean values of cumulative percent release.

RESULTS AND DISCUSSION

Phase solubility curves of AT, FE and EZ with β-CD

The phase solubility curves of AT, FE and EZ with β-CD is shown in Figure 1. The drug solubility increased linearly with increasing β-CD concentration indicative of the A_L type of solubility phase diagram ($r^2 > 0.95$), which indicated that 1:1 (AFE-β-CD) inclusion complex was formed in solution. Solution of β-CD was not prepared beyond 16 mM because of limited solubility of cyclodextrin in water (*i.e.*1.85gm/100ml). Beyond this limit addition of β-CD will lead to decrease in solubility of drug. The values of stability constants (K_s) for the complexes of AT, FE and EZ at 37^0 ± 0.5^{0}C, assuming a 1:1 (molar ratio) stoichiometry, calculated from the slope of the phase solubility diagram were 550.60 M^{-1}, 2020.61 M^{-1}, 1604.05 M^{-1} respectively, which indicated a suitable and stable complex formation. It is reported that cyclodextrin-drug complexes with the values of K_s in the range of 200 to 5000 M^{-1} show improved dissolution properties (Higuchi *et al.*, 1965).

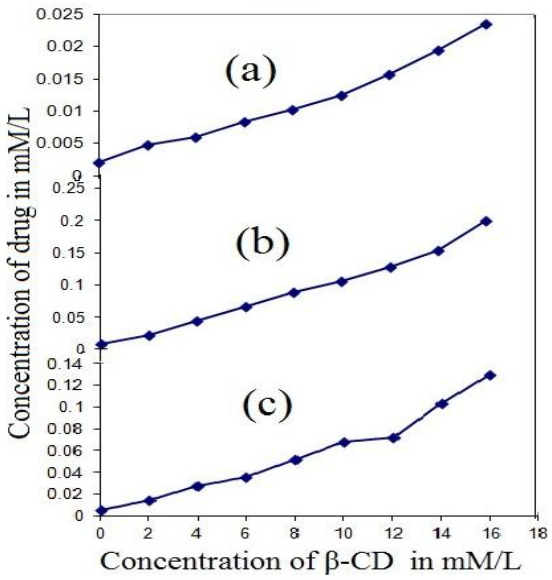

Fig. 1: Phase solubility curve of (a) AT- β-CD, (b) FE- β-CD and (c) EZ- β-CD

X-ray powder diffractometry (XRD)

The powder X-ray diffraction patterns of the pure AFE, PMBC, KNBC and CEBC are illustrated in Fig. 2. The sharp peaks of the AFE appeared in the 2θ range of 2–50^0 (Fig. 2a), indicating that AFE were crystalline material. The highest peak intensity of AFE was 12553 at 12.6^0. In case of PMBC (Fig. 2b), the highest peak intensity was 3001, but the sharp peaks of the pattern indicated the retention of the crystalline structure of the AFE in the physical mixture. However, the highest peak intensity of KNBC (Fig. 2c) and CEBC (Fig. 2d) were 2650 and 1521, respectively at 12^0 and not at 12.6^0. More over the peaks were broader. The reduction of intensity and sharpness of KNBC and CEBC peaks could be attributed to the entrapment of AFE into the β-CD cavity through the formation of inclusion complex and reduction of crystallinity of AFE.

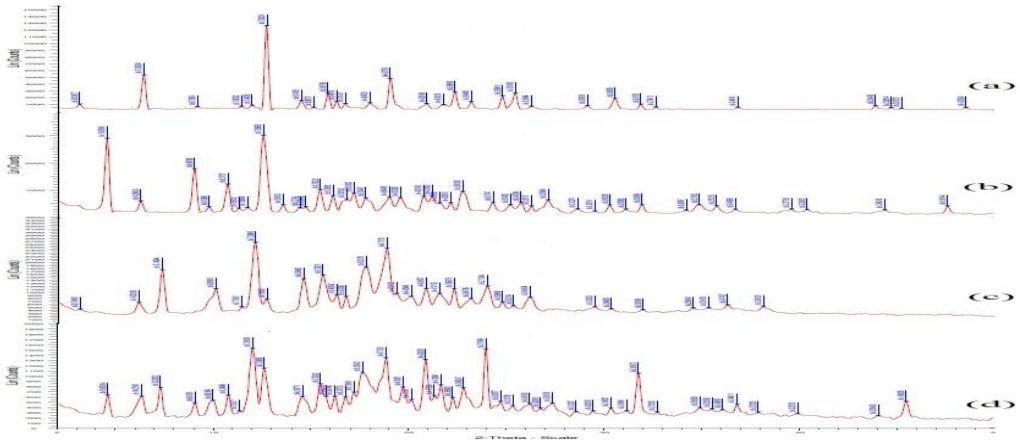

Fig. 2: XRD patterns of (a) AFE (b) PMBC (c) KNBC and (d) CEBC

Differential scanning calorimetry (DSC)

The thermal properties of various samples were investigated by differential scanning calorimetry (DSC) and the DSC curves are depicted in Fig. 3. DSC is widely used to characterize the inclusion complexation of active substances with CD and thermoanalytical results for various inclusion compounds are summarized in a review article (Giordano et al., 2001). The AT (Fig. 3a), FE (Fig. 3b) and EZ (Fig. 3c) showed a characteristic endothermic melting peak with their onset at 162.10^{0}C, 82.93^{0}C and 163.43^{0}C respectively and the heat required to melt the drug were 89.51 mJ, 126.61 mJ and 399.49 mJ respectively. The AT with FE (Fig. 3d), AT with EZ (Fig. 3e) and FE with EZ (Fig. 3f) showed reduction of melting point of drugs. This was due to the drug melted at lower temperature in mixture. The mixture of AFE showed three different endothermic melting peaks with their onset at 128.46^{0}C, 80.60^{0}C and 151.55^{0}C. This was due to FE melted first and AT and EZ tended to decrease their melting point (Fig. 3g). β-CD exhibited an endotherm at 289^{0}C (Fig. 3h). The DSC curve for PMBC showed two endotherms at 83.63^{0}C and 120.48^{0}C with 226.68 mJ and 11.63 mJ heat requirement respectively (Fig. 3i). The peak of AT and EZ tended to merge in FE as FE melted first and its concentration was high in mixture.

However, in the case of KNBC (Fig. 3j) and CEBC (Fig. 3k) the endothermic peak of the drug was significantly reduced (62.02 mJ and 30.55 mJ respectively) or no longer observed. This result confirms that the AFE were entrapped in the β-CD cavity, through forming inclusion complex.

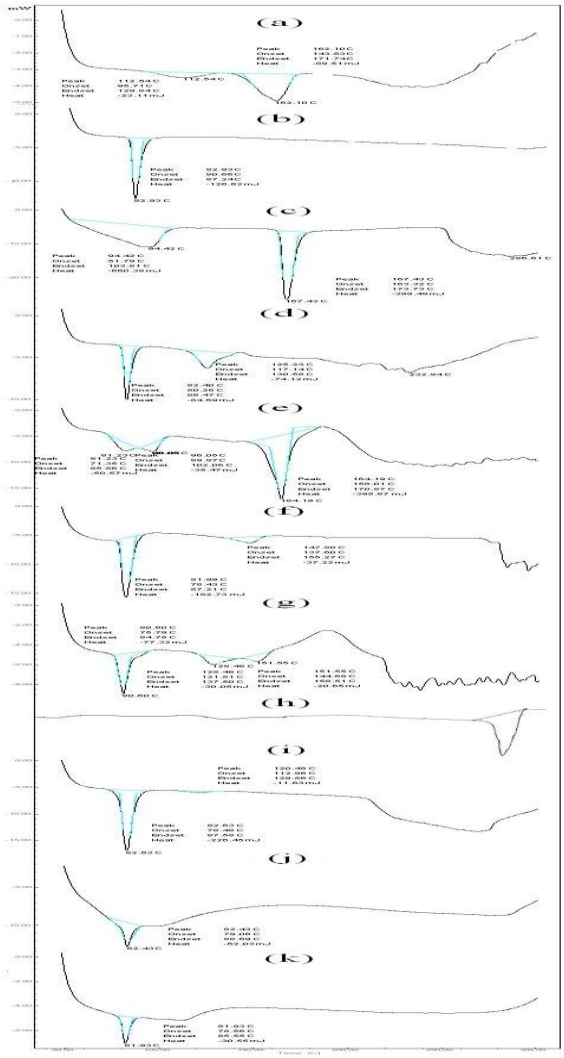

Fig. 3: DSC patterns of (a) AT, (b) FE, (c) EZ, (d) AT with FE, (e) AT with EZ, (f) FE and EZ, (g) AFE, (h) β-CD, (i) PMBC, (j) KNBC and (k) CEBC

Dissolution Studies

The dissolution rate profiles of AT- β-CD (Fig. 4A), FE- β-CD (Fig. 4B) and EZ- β-CD (Fig. 4C) were expressed as the cumulative percent release (vs.) time. Percentage drug dissolved at 5min (DP_5), at 10min (DP_{10}), at 30min (DP_{30}) and at 60min (DP_{60}) of AT, FE and EZ from pure drug, PMBC, KNBC and CEBC were determined in Table. CEBC and KNBC showed higher dissolution rate as compared to the physical mixtures and pure drug at DP_5, DP_{10}, DP_{30} and DP_{60} ($p < 0.01$). However, CEBC showed significant effect at DP_5 ($p < 0.05$) and DP_{10} ($p < 0.05$) with respect to KNBC. The moderate enhancement in dissolution rate was attributed to the formation of inclusion complexes in the solid state with reduction in the crystallinity of AT, FE and EZ, as conformed by XRD studies. The increased dissolution rate for inclusion complexes was due to greater hydrophilicity, higher wetting effect, mechanical treatment, which increase the contact between the drugs and the carrier. (C.M.Fernandes *et al*., 2002)

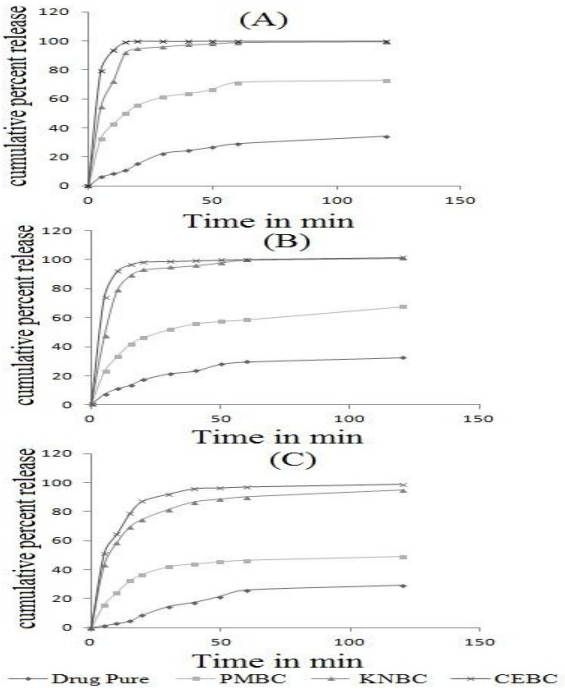

Fig.4: Dissolution diagram of (A) AT– β-CD systems at 37±0.5 ^{0}C, (B) FE– β-CD systems at 37±0.5 ^{0}C and (C) EZ– β-CD systems at 37±0.5 ^{0}C

Table:1 The dissolution parameters of pure drug, PMBC, KNBC and CEBC

SYSTEM	DP_5*±SD	DP_{10}*±SD	DP_{30}*±SD	DP_{60}*±SD
		AT		
AT pure	6.15±2.05	8.44±3.65	22.46±1.23	29.18±3.21
PMBC	32.44±2.25	42.65±2.31	61.25±2.45	71.24±2.65
KNBC	54.26±3.15	72.33±1.12	96.02±2.14	98.99±1.25
CEBC	79.64±1.54	93.43±1.23	99.79±1.49	99.93±1.68
		FE		
FE pure	7.24±2.31	10.97±2.10	21.34±2.96	29.64±3.12
PMBC	22.64±3.78	32.99±2.17	51.63±1.15	58.44±1.23
KNBC	46.96±2.01	78.69±3.02	94.37±2.01	99.59±2.04
CEBC	73.86±2.65	91.92±2.25	98.63±1.24	99.97±1.05
		EZ		
EZ pure	1.56±2.25	2.94±1.36	14.57±2.64	25.78±1.65
PMBC	15.86±2.12	24.36±3.23	41.96±2.89	46.36±2.58
KNBC	43.40±2.65	58.76±2.56	81.39±2.15	89.95±1.14
CEBC	51.33±2.10	84.69±2.15	92.22±3.21	97.15±1.54

***Indicates mean of three experiments; S.D.: standard deviation**

CONCLUSION

The present investigation revealed that AFE can form inclusion complex with β-CD in solid state. The stoichiometry of complex formation is in 1:1 molar ratio with better stability constant. From these results, it can be assumed that the formation of the inclusion complex of AFE with β-CD can increase the aqueous solubility of AFE. The improved dissolution rate may be due to increase in solubility, brought about by complexation, amorphizing power of β-CD and mechanical treatment. From these evidences it can be concluded that the aqueous solubility and dissolution rate of AFE can be significantly increased by forming an inclusion complex with β-CD. Further, inclusion complexes prepared by co-evaporation method is found to be superior with respect to enhancement in aqueous solubility than those obtained by kneading method.

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