Abstract

The aim of the present study was to enhance the dissolution rate of fenofibrate using its complexation with Hydroxy prypyl β – Cyclodextrin (HPβCD). The phase solubility behavior of fenofibrate was studied in various concentrations of (HPβCD) in aq. Solution at 37^0c. The solubility of fenofibrate increased with increase in amount of HPβCD in aq. Solution. Gibbs free energy $(\Delta G^0)_{tr}$ values were all negative. The complexes of fenofibrate with HPβ – Cyclodextrin were prepared at 1:1 ratio by kneading and co-precipitation method. Fenofibrate – HPβCD complexes were evaluated by dissolution studies, Fourier transform infrared (FTIR) spectroscopy and Differential scanning calorimetry (DSC) studies. The complexation of fenofibrate with HPβ – Cyclodextrin exhibited enhanced dissolution rate of fenofibrate. The mean dissolution time of fenofibrate decreases significantly in complexation. The Fourier transform infrared (FTIR) spectroscopy studies showed formation of intermolecular hydrogen bonding between fenofibrate and HPβ – Cyclodextrin. The Differential scanning calorimetric studies indicated the loss in crystalline state of fenofibrate in complexes of fenofibrate with HPβ – Cyclodextrin.

Keywords: - Fenofibrate, HPβ – Cyclodextrin, solubility, Gibbs free energy, dissolution rate.

INTRODUCTION:-

Fenofibrate, propan-2-yl 2-[4-(4-chlorobenzoyl) phenoxy]- 2- methylpropanoate is a fibric acid derivative useful as antilipidemic agent. Fenofibrate is a hypolipemiant drug that reduces the amount of lipids (fats) in the blood. It is a white crystalline powder, practically insoluble in water (log P= 5.24)[1]. In any case, its low water solubility and poor dissolution rate causes problems in formulation development and restricts its therapeutic application by influencing the rate of absorption and the onset of action.

Consequently, its bioavailability is incomplete, irregular and often varies from one person to another. As a result, commercially available doses are of higher strength and require repeated dosing. From an economic point of view, this low bioavailability of drug leads to wastage of more amount of drug after oral administration; increasing the cost of medication. Therefore, it is very important to find appropriate formulation approaches to enhance the aqueous solubility, dissolution rate and thus the bioavailability of poorly soluble drugs.Nowadays, many approaches are used to enhance the solubility and dissolution rate of poorly soluble drugs by the use of pharmaceutical technology[2]. Physical modification often aims to increase the surface area, solubility and /or wettability of the powder. Other approaches include cosolvency by using various solvent blends, cyclodextrin complexation[3], use of surfactants[4], salt forms[5], prodrugs[6], alteration of crystal properties[7,8,9].

A number of different microorganisms and plants produce certain enzymes called cyclodextrin glucosyltransferases (CGTs), which degrade starch to cyclic products called cyclodextrins. These cyclodextrins are cyclic oligosaccharides involve (α-1,4)-associated α-D-glucopyranose units and contain a genuinely lipophilic cavity and a hydrophilic external surface. They are shaped like a truncated cone rather than perfect cylinders. In light of such qualities, cyclodextrins are able to form inclusion complexes both in solid state and in solution state, in which every guest entity is surrounded by the hydrophobic environment of the cyclodextrin cavity. Upon inclusion, the water solubility of the guest can increase as well as its bioavailability.[10,11] This inclusion complex formation leads to alteration of the physic-chemical and biological properties of the guest molecules and may eventually have considerable pharmaceutical potential.[12,13,14]

The naturally occuring α-, β- and γ-cyclodextrin consist of six, seven, and eight glucopyranose units, respectively. The natural cyclodextrins like β-cyclodextrin is of limited aqueous solubility and the formed complexes from interaction of lipophiles/ hydrophobic drugs with these cyclodextrins may be of limited solubility. It may reuslt in precipitation of solid cyclodextrin complexes from water and other aqueous systems. Cyclodextrin derivatives of pharmaceutical interest include the derivatives of these naturally occurring β- and γ-cyclodextrin. Out of these cyclodextrin derivatives, Hydroxy propyl β-cyclodextrin (HPβCD) appears most useful as a pharmaceutical complexing agent because of its complexing ability, low cost and other properties. The approach of cyclodextrin complexation can be used to increase water solubility and dissolution rate of poorly soluble drug and to solve bioavailability problems.

As fenofibrate dissolves very slightly in water; the present study was undertaken to overcome the limitations existing in available fenofibrate products so as to improve the dissolution profile, absorption characteristics and bioavailability and to reduce the dose required for administration to attain a desired effect.

The study also aimed to develop a method for preparation of an inclusion complex of fenofibrate with HPβ – Cyclodextrin which is efficient and economical, simple and less time consuming than other methods

So, the present study was performed to enhance the solubility and dissolution rate of fenofibrate using complexation with HPβ – Cyclodextrin in order to attain a therapeutic effect. The possible interactions between fenofibrate and HPβ – Cyclodextrin in both solid state and liquid states were investigated. Solid state Interaction was investigated by Fourier transform infrared (FTIR) spectroscopy, Differential scanning calorimetry (DSC) studies. Interaction in solution was studied by phase solubility analysis and dissolution experiments.

MATERIALS AND METHODS

Materials

A gift sample of fenofibrate was received from Shreya Life Sciences, (Aurangabad, India). HPβ – Cyclodextrin was received from Wockhardt pharmaceuticals (Aurangabad, India). All other solvents and ingredients used were of analytical grade.

Methods

Phase solubility studies

Phase solubility studies were performed in triplicate according to the method reported by Higuchi and Connors.[15] An excess of drug was added to 5ml portions of distilled water in vials each containing variable amount of HPβ-CD (2mM to10mM). All the above solutions were subjected to sonication for 30mins and then allowed to stand at room temperature (~25 °C) for 48 hrs without disturbance to attain saturation equilibrium. These saturated systems were carefully filtered through Whatmann filter paper (No.41) and were analyzed spectrophotometrically at 287 nm after appropriate dilutions on UV-Visible Spectrophotometer. Solubility of fenofibrate in every HPβ-CD solution was calculated and phase solubility diagram was drawn between solubility of fenofibrate and different concentrations of HPβ-CD. The apparent stability constant (Kc) was calculated by using formula[15].

$$Stability\ constant\ (Kc) = \frac{Slope}{So(1-Slope)} \quad \text{..... 1}$$

Where,

So= Aqueous solubility of fenofibrate

The Gibbs free energy of transfer $(\Delta G^0)_{tr}$ of fenofibrate from pure water to the aqueous solution of carrier was calculated as[16]

$$\Delta Gtr = 2.303\ RT\ LogSo/\ Ss \quad \text{.....2}$$

Where

$So/\ Ss$ is the ratio of molar solubility of fenofibrate in aqueous solution of HPβ-CD to that of the same medium without HPβ-CD.

Preparation of solid binary systems

Preparation of physical mixture of fenofibrate with HPβ-CD

The physical mixture of fenofibrtae with HPβ-CD containing molar weight ratio 1:1 (Fenofibrate: HPβ-CD) were prepared and followed by passing through sieve (No.72) with minimum abrasion.

Preparation of inclusion complex by kneading method[17]

Stochiometric quantities (1:1) of fenofibrate: HPβ-CD was accurately weighed. HPβ-CD was added to the mortar, and a small amount of ethanol: water (1:1 v/v) was added while triturating to get slurry like consistency. Then slowly drug was incorporated into the slurry, and trituration was continued further for 45 mins. The slurry was then dried at 50^0C for 24 hours, pulverized and passed through sieve No. 72 and stored in desiccators until further use.

Preparation of inclusion complex by co – precipitation method

Fenofibrate and HPβ-CD with1:1 molar ratio was accurately weighed. Saturated cyclodextrin solution was prepared with HPβ-CD and water. Then, fenofibrate solution in methanol was added slowly and suspension was formed. The suspension was stirred at 40 ^{0}C for 30 min and the stirring was continued at room temperature (25^0C) for 30 min. The obtained masses were filtered through Whatmann filter paper no.41 and dried at 50 ^{0}C in an oven for 24 h. The dried complexes were pulverized and passed through sieve No. 72 and stored in desiccators until further use[18].

The yield for HPβCD complex was not significant. Therefore, we opted following method for co-precipitation.

Fenofibrate and HPβCD with1:1 molar ratio was accurately weighed. Cyclodextrin solution was prepared with HPβCD and water. Then, fenofibrate solution in methanol was added slowly to above solution and suspension was formed. The suspension was stirred at 40 ^{0}C for 30 min and kept stirring at room temperature for 12 hrs. The obtained masses were refrigerated for 24 hrs. Then, these masses were filtered through Whatmann filter paper No.41 and dried at 50 ^{0}C in an oven for 24 hrs. The dried complexes were pulverized and passed through sieve No. 72 and stored in desiccators until further use[19].

Dissolution Studies

As Fenofibrate is a lipophilic compound and practically insoluble in water; dissolution study of fenofibrate dosage forms necessitates modifications in the dissolution medium. SLS (Sodium Lauryl Sulphate) at concentration level of 20 mM and above provides sink conditions[20,21].

Dissolution studies of fenofibrate, physical mixture, kneaded product and co-precipitated product were performed by using the U.S. pharmacopoeia (USP) model digital tablet dissolution test apparatus – II (Electro lab, Mumbai) at paddle rotation speed of 75 rpm in 900ml of 20mM SLS at 37.0±0.5ºC.

The physical mixture equivalent to 145 mg of fenofibrate was weighed using a digital balance (Make Eagle, India) and added into dissolution medium. The 5 ml samples were withdrawn at predetermined intervals and replaced with fresh dissolution medium and suitably diluted. Diluted samples were then assayed for fenofibrate content by measuring the absorbance at 287 nm using the UV- visible spectrophotometer (Jasco model V630, Japan). The dissolution studies were either performed until all the solids were completely dissolved or stopped at 2 hrs if the duration of dissolution was longer. Studies were performed in triplicate (n=3). Mean values of cumulative drug release were calculated for plotting the release curve.

Fourier transformation-infrared spectroscopy

Fourier transformation-infrared (FT – IR) spectra were obtained by using an FT – IR spectrophotometer- 4100 (Jasco, Japan). The samples were (Fenofibrate, physical mixture and drug:cyclodextrin complexes) previously ground and mixed thoroughly with potassium bromide, an infra-red transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. Forty scans were obtained from 4000 – 400 cm^{-1}.

Differential Scanning Calorimetry (DSC) studies

The DSC thermograms were obtained on a differential scanning calorimeter (Shimadzu DSC-60 thermal analyzer, Japan). The instrument was calibrated using indium as a standard. Samples (5 mg) were heated in sealed aluminium pans under nitrogen using the following program: hold for 10 min at 40^0C; heat from 40.0 to 250.0^0C at a scanning rate of 10^0C/min. Then the samples were subjected to DSC studies. Samples were

sealed in 40 µl aluminum pans. An identical empty pan was used as a reference. The samples were scanned at 10^0C/min with a 50 ml/min nitrogen purge.

RESULTS AND DISCUSSION

Phase solubility studies

The phase solubility profiles of Fenofibrate – HPβ-CD is presented in Fig. 1. This plot showed that aqueous solubility of the drug increases linearly as a function of HPβ-CD. The phase solubility profile of fenofibrate with HPβ-CD can be classified as A_L-type. The linear host–guest correlation coefficient r = 0.9969 (r^2 = 0.994) with a slope (m) of 0.004 suggested the formation of a 1:1 complex with respect to HPβ-CD concentrations. The apparent stability constants, $K_{1:1}$ obtained from the slope of the linear phase solubility diagram was 630.0006 M^{-1} for HPβ-CD (Eq.(1)). The $K_{1:1}$ value suggested that fenofibrate formed more stable complex with HPβ-CD.

An indication of the process of transfer of fenofibrate from pure water to the aqueous solution of HPβ-CD may be obtained from the values of the Gibbs free energy change (Table No. 1). The values of Gibbs free energy associated with the aqueous solubility of fenofibrate in presence of HPβ-CD (ΔGtr) were all negative for HPβ-CD at various concentrations indicating the spontaneous nature of the drug solubilization. The values decreased by increasing HPβ-CD concentration, demonstrating that the solubilization more favorable as concentration of HPβ-CD increased.

Dissolution studies

The results of the dissolution studies for individual samples (Fenofibrate alone, PMs and complexes) over the period of 2 hour are shown in Fig.2. Onset of dissolution of pure fenofibrate is very low about 13.29% of drug being dissolve din 120 mins. Complexes of fenofibrate with HPβ-CD considerably enhanced dissolution rates as compared to pure drug fenofibrate and PMs.

Percentage dissolution efficiencies (%DE) values were computed, for comparative analysis of all the formulations. The % DE values in the initial time period of dissolution study i.e. %DE 10_{min} provide comparative information for very fast releasing formulations, whereas $\%DE_{60min}$ provide relative information about both fast and slow releasing formulations. The values of $\%DE_{60min}$ for the pure drug was increased to

32.45% in PMs and up to 87.39 % in kneaded product and 56.46 % in co – precipitated products. The change of DE_{60min} of drug in its PMs and complexes is statistically significant. ($p< 0.05$).

The results of % dissolution, dissolution efficiency study indicate an improvement of dissolution rate of fenofibrate in cyclodextrin complexes by both the techniques. The improvement of dissolution rate is possibly caused by several factors. Such factors are

a) the strong hydrophilic character of HPβ-CD, which improves the water penetration and the wettability of the hydrophobic fenofibrate.

b) the optimal dispersion of fenofibrate to HPβ-CD

c) the absence of crystals corresponds to lower energy required for dissolution and

d) the intermolecular hydrogen bonds and the molecular dispersion of fenofibrate on HPβ-CD leads to partial miscibility, improving the hydrophilic characteristics of the drug substance via interactions with β-CD the improvement of dissolution rate of fenofibrate in physical mixture is due to increased wettability of the drug powder[22].

Kneading shown improved dissolution than coprecipitation. This could be attributed to the improved wetting provided by cyclodextrins in kneading than coprecipitation, as earlier reported by Mukne A P for triamterene[23] and Deshmukh S S for Ziprasodine[24] Thus it can be concluded kneading is better for complexation than coprecipitation.

Fourier transformation-infrared spectroscopy

The FTIR spectra of the systems Fenofibrate- HPβCD and those of pure components are shown in from fig.3. When the systems are compared, it can be observed that the ester group stretching band at 1727.91 cm^{-1} broadens and shifts towards higher wave numbers, indicating change in the intermolecular H- bonds of the drug upon complexation. Similar modifications have been in the combination signal of the ester group which point out change in the interaction of this group when the complex is formed. In addition, the bands at 1050- 1340 cm^{-1}, corresponding to antisymmetric vibrations of the aryl ether group and C-O stretching of esters broaden in some cases and in others peaks vanishes upon complexation. The decreased intensity and vanishing of the band is associated to the out of plane bending of the aromatic C-H bonds at 824-844 cm^{-1} evidences the inclusion of the benzene ring.

Finally, the C- H stretching seen at 3032-3052 cm^{-1} is vanished in the complexes indicating complexation has been occurred.

Differential Scanning Calorimetry (DSC) studies

DSC thermogram of HPβCD showed a straight line. The DSC curve of fenofibrate showed a broad endothermic peak in the range of 80–90 ^{0}C owing to the melting point of the drug. The peak of fenofibrate showed changes in terms of peak area and ΔH (heat of fusion) value (Table No. 2) in case of the complexes as compared to the physical mixture comprising of drug: HPβCD in the same ratio. This suggested that the presence of HPβCD resulted in complexation of fenofibrate. The change in peak height and broadening of peaks may be attributed to lost in crystallinity.

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CONCLUSIONS

The solubility and dissolution rate of fenofibrate can be enhanced by the use of complexes of fenofibrate with HPβ-CD. The solubilization effects of HPβ-CD, reduction of particle aggregation of the drug, loss in crystallinity, increased wettability and dispersibility and alteration of the surface properties of the drug particles might be responsible for the enhanced solubility and dissolution rate of fenofibrate from its complexes and physical mixtures.

Kneading shown improved dissolution than coprecipitation. This could be attributed to the improved wetting provided by cyclodextrins in kneading than coprecipitation.

It is concluded that Fenofibrate - HPβ-CD complexation results in an increase in solubility and dissolution rate of drug, suggesting a possible enhancement of its oral bioavailability.

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CONFLICT OF INTERES

There are no conflicts of interest.

ABBREVIATION USED

HPβCD - Hydroxy prypyl β – Cyclodextrin

PM – Physical Mixture

$(\Delta G^0)_{tr}$ - Gibbs free energy

FTIR - Fourier transform infrared spectroscopy

DSC - Differential scanning calorimetry

%DE - Percentage dissolution efficiencies

SLS - Sodium Lauryl Sulphate

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