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Improved Dissolution Rate of Leflunomide using Hydroxypropyl-β-Cyclodextrin Inclusion Complexation by Freeze-Drying Method

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Abstract

Inclusion complexes between leflunomide, a poorly water soluble antirheumatic drug, and HP-β-cyclodextrin (HP-β-CD) were used to improve the solubility and dissolution rate of the drug. Leflunomide forms an inclusion complex with HP-β-CD both in aqueous and in solid state. Phase solubility profile indicated that the solubility of leflunomide significantly increased in the presence of HP-β-CD and was classified as A_L-type, indicating the 1:1 stoichiometric inclusion complexes in aqueous medium. The solid complexes prepared by different methods such as physical mixture, co-grinding method and freeze dried method in 1:1 and 1:2 molar ratio, were characterized by differential scanning calorimetry (DSC) and X-RD. The dissolution profiles of solid complexes were determined and compared with leflunomide alone and their physical mixture, different mediums such as simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). The dissolution studies showed that, the freeze-dried complex exhibited higher dissolution rates than the drug, in the simulated gastric fluid (SGF pH 1.2) in 1:2 molar ratio.

Keywords: Leflunomide, dissolution rate, cyclodextrin, phase solubility, inclusion complex.

Introduction

From many years new chemical entities were discovered by the pharmaceutical industry. Out of these about 40% are poorly water soluble or lipophilic compounds. Not only these new drugs but also many existing drugs are also poorly water soluble, so they pose difficulties to the biopharmaceutical scientists in their formulation. [1,2] Oral delivery of poorly water-soluble drugs often results in low bioavailability since the rate-limiting step for absorption from the gastrointestinal tract is a significantly slower dissolution rate. Thus, many molecules that are biologically active *in-vitro* are ineffective *in-vivo* as a result of their limited solubility and slow rate of dissolution. Several techniques have been used to improve the solubility and dissolution rate of poorly water soluble drugs and among the possibilities, over recent years, the cyclodextrin and their derivatives have received considerable interest in the pharmaceutical field due to their potential to form complexes with a variety of drug molecules. Pharmaceutical modification of the drug molecules by inclusion complexation with cyclodextrin has been extensively developed to improve solubility, dissolution rate, chemical stability, absorption and bioavailability of the poorly water soluble drugs and reduce side effect and toxicity of the drug. [3,4] The cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight D-glucopyranose units (, β and γ CD) linked by (-1, 4) glycosidic bonds and arranged in a truncated cone shape structure. [5-7] These agents exhibit a hydrophilic exterior and a hydrophobic internal cavity in which drugs may form inclusion complexes, being trapped entirely or at least partially. [8, 9] Several driving forces have been proposed for the inclusion of CDs with substrates such as hydrogen binding, Van der Waals force, hydrophobic interaction and the release of "high energy water" molecules from the cavity, however, no covalent bonds exist between the CD and its guest. As a result of complex formation, the physicochemical properties of the guest molecules, such as solubility, thermal stability, melting point, chemical reactivity, spectroscopic and electrochemical properties will be changed. Out of three parent CDs, HP-β-cyclodextrin appears more useful as a pharmaceutical agent because of its complexing ability, cavity dimension, low cost, higher productive rate and other properties. The cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 g mol^{-1}. [10, 11]

Leflunomide is a pyrimidine synthesis inhibitor indicates in adults for the treatment of active rheumatoid arthritis. Rheumatoid

arthritis is an auto immune disease characterized by high T-cell activity. Chemically it is 5-methyl-N-[4-(trifluoromethyl) phenyl]-4-isoxazole carboxamide. Leflunomide is an isoxazole immunomodulatary agent which inhibits dihydroroate dehydorgenase (an enzyme in involved in de novo pyrimidine synthesis) and has antiproliferative activity. The leflunomide is a poorly water soluble drug. The poor aqueous solubility of leflunomide leads to variable oral bioavailability. [12,13] This limits several advantages of the drug with respect to its absorption, distribution and therapeutic efficacy. In this study, investigations were performed on the possibility of complexation of leflunomide with HP-β-CD for improving the solubility and dissolution rate of this drug, as well as to characterize the physicochemical properties of formed complexes. The complexes with HP-β-CD were prepared by different methods such as physical mixture, co-grinding method and freeze drying method in 1:1 and 1:2 molar ratios. The types and the stability constants of the complexation were established according to phase solubility studies. The differential scanning calorimetry and X-RD were used to characterize the solid state of all the products. The dissolution properties of the solid complexes were evaluated and compared with those of leflunomide alone and of a physical mixture between leflunomide and HP-β-CD.

Material and Methods

Leflunomide was obtained as gift sample from Aarti Drugs, Mumbai, HP-β-CD was obtained as gift sample from Signet Chemical Corporation, Pvt. Ltd., Mumbai, India., and the other chemicals used are of analytical grade.

Methods

Phase Solubility Studies

Solubility measurements were carried out and the stability constants (Ks) of the complex were determined according to the phase solubility method of Higuchi and Connors (1965). [14] An excess amount of leflunomide was added to 10 ml of aqueous solution containing various concentrations of HP-β-CD (0, 2, 4, 6, 8 and 10 mM) into the glass vials. The contents were shaken for 48 hours at room temperature on rotary flask shaker. After equilibrium, the samples were withdrawn, filtered through a Whatmanpaper and diluted appropriately. A portion of samples were analyzed by UV spectrophotometer at 257 nm. The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram using the following equation:

$$K\,1{:}1 = \frac{\text{Slope}}{S\,(1-\text{Slope})}\ m\ -1$$

Where,
S = Solubility of drug without cyclodextrin
M = Molar concentration
K = Apparent stability constant
Slope is calculated from regression equation

Preparation of Solid Inclusion Complexes

The following solid inclusion complexes of leflunomide with HP-β-CD were prepared in both 1:1 and 1:2 molar ratios.

Physical Mixture

The physical mixtures of leflunomide with HP-β-CD in 1:1 and 1:2 molar ratios were prepared by simply mixing the powders with a spatula.

Co-grinding Method

The co-grinding mixture of Leflunomide with HP-β-CD in1:1 and 1:2 molar ratios were prepared by triturating the powder in glass mortar pestle for 20 minutes and passed through sieve no. 85.

Freeze-drying Method

The required 1:1 and 1:2 stoichiometric quantity of leflunomide was dissolved in methanol and added to an aqueous solution of HP-β-CD. The resulting solutions were frozen at 20 C for 3 hrs and lyophilized in freeze dryer (Benchtop 4.0k L Freeze Dryer, Virtis, USA) maintained at freezing temperature of (-70 to -75 C), vaccum applied in dried chamber: 75 to 80 mT, at ambient temperature (16 to 18.5 C). The lyophilized powder was passed through a sieve no. 60 and stored in a sealed glass vial.

Differential Scanning Calorimetry

The DSC thermograms of pure drug, HP-β-CD, physical mixture, co-grinding complex, and freeze dried complex with HP-β-CD were recorded. The samples were separately sealed in aluminum cells and set in Perkin Elmer (Pyris 1) DSC. The thermal analysis was performed in a nitrogen atmosphere over a temperature range of 50 C to 250 C.

X-Ray Diffraction Spectroscopy

The X-ray diffraction pattern of pure drug, HP-β-CD, physical mixture, co-grinding complex and freeze dried complex with HP-β-CD were recorded from 5 to 80 at an angle 2θ using diffractometer system.

In-vitro Dissolution Studies

The prepared complexes (n = 3) were subjected to release studies using USP-II (Paddle) dissolution apparatus at 100 rpm. Dissolution medium was used simulated gastric fluid (SGF pH 1.2, 900 ml) and simulated intestinal fluid (SIF pH 7.4, 900 ml) maintained at 37±0.5 C. The samples were withdrawn (10 ml) at different time intervals and replaced with an equivalent amount of fresh medium and analyzed immediately by using UV Visible spectrophotometer. The concentration of leflunomide released was determined by measuring the absorbance at 257 nm. After analyzing the drug content in the dissolution samples, corrections were made for the volume replacement and the graph of percentage of drug release versus time was plotted.

Result and discussion

The phase solubility diagram of the complex formation between leflunomide with HP-β-CD is shown in Fig. No.1. This plot showed that the aqueous solubility of drug increases linearly as the function of HP-β-CD concentration. The phase solubility diagram can be classified as type A_L, according to Higuchi and Connors. It is assumed that the increase in solubility observed was due to the formation of a 1:1 M inclusion complex. The stability constant (Ks) of leflunomide with HP-β-CD complex was calculated as 224.3 M^{-1} from the linear plot of the phase solubility diagram.

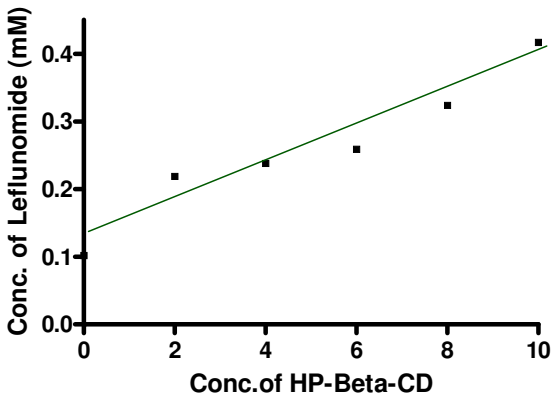

Figure 1: Phase Solubility Studies of Leflunomide with HP-β-CD

Differential Scanning Calorimetry (DSC)

The thermal behavior of leflunomide with HP-β-CD solid complex was studied using DSC in order to confirm the formation of solid complexes. The DSC curve of leflunomide and solid inclusion complexes are shown in Figure No. 2-9.

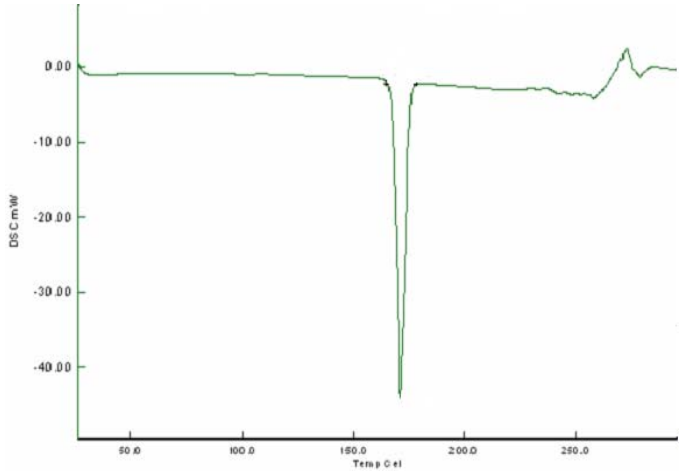

Figure 2 DSC of Leflunomide

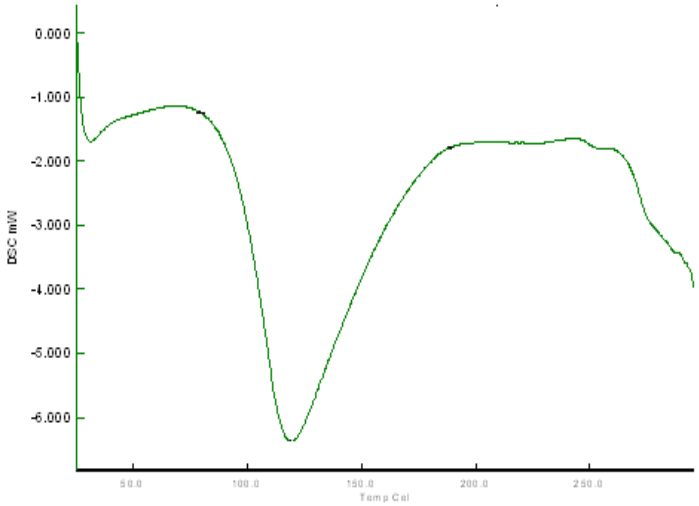

Figure 3 DSC of HP-β-CD

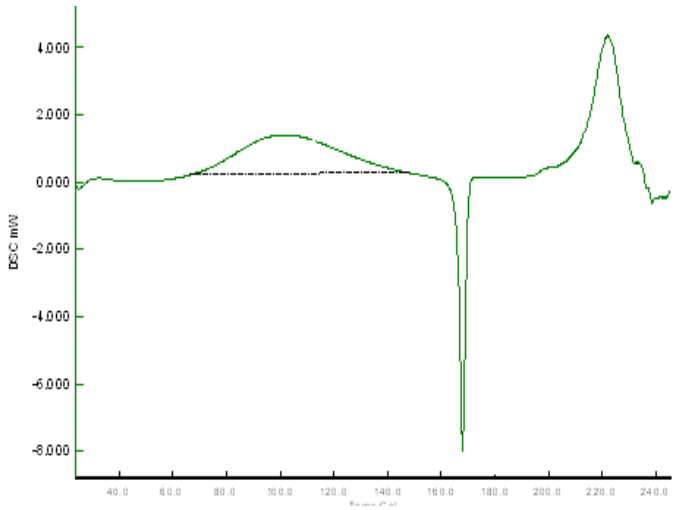

Figure 4 DSC of Physical Mixture of Leflunomide with HP-β-CD in the ratio of (1:1)

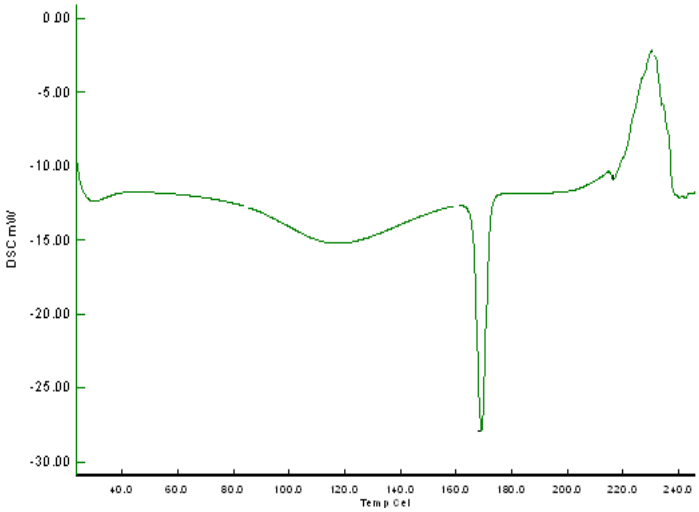

Figure 5 DSC of Physical Mixture of Leflunomide with HP-β-CD in the ratio of (1:2)

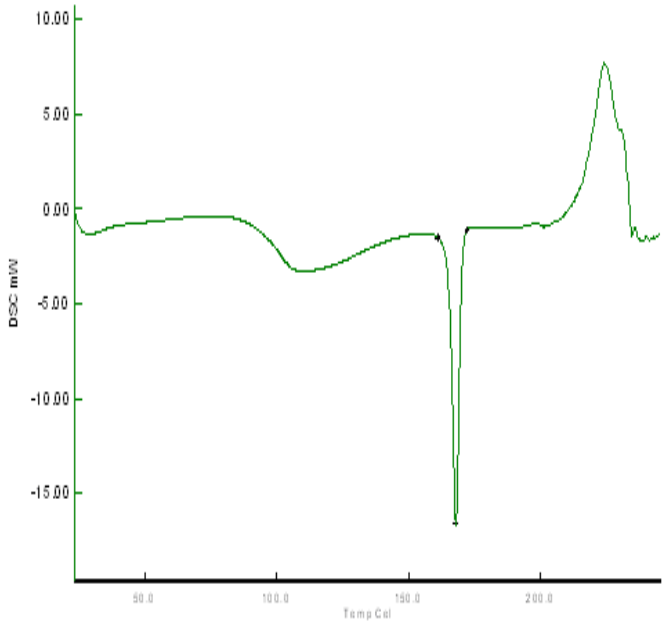

Figure 6 DSC of Co-grinding Complex of Leflunomide with HP-β-CD in the ratio of (1:1)

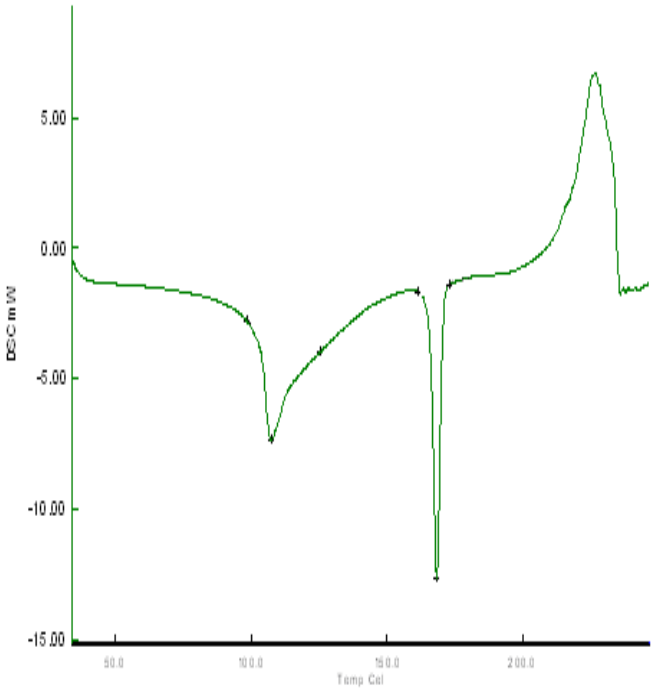

Figure 7 DSC of Co-grinding Complex of Leflunomide with HP-β-CD in the ratio of (1:2)

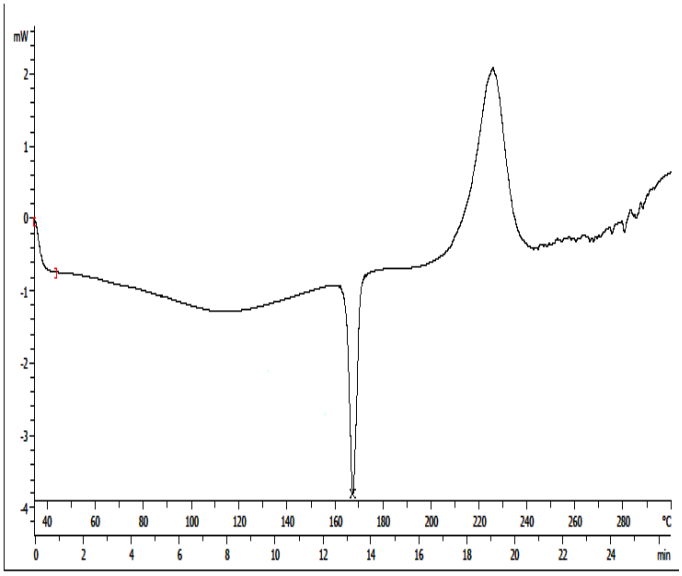

Figure 8 DSC of Freeze Dried Complex of Leflunomide with HP-β-CD in the ratio of (1:1)

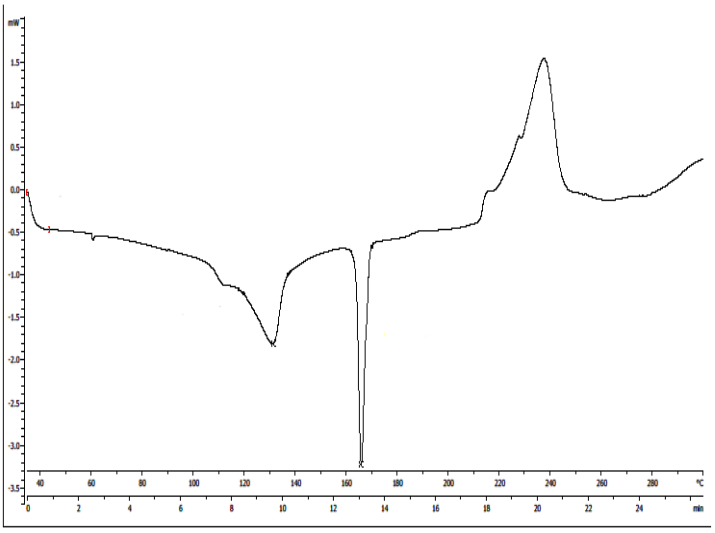

Figure 9 DSC of Freeze Dried Complex of Leflunomide with HP-β-CD in the ratio of (1:2)

The DSC curve of leflunomide exhibited a sharp endothermic peak at 171.2 C corresponding of its melting point. The broad endothermic peak at 118.9 C was observed for amorphous HP-β-CD, was related to loss of water molecules i.e. dehydration process. In the thermogram of leflunomide with HP-β-CD the height or intensity of endothermic peak of drug was reduced and also in inclusion complexes less heat is required during melting process as compared to the leflunomide. [15, 16]

The enthalpy values of the drug, HP-β-CD, physical mixture, co-grinding complex and freeze dried complex in 1:1 and 1:2 molar ratios are shown in following Table No 1.

Table 1: Enthalpy Values of Different Complexes

Sr. No.	Chemicals and Complexes	Enthalpy (mJ/mg)
1.	Leflunomide	114.46
2.	HP-β-CD	134.99
3.	Physical mixture of leflunomide HP-β-CD in the ratio (1:1)	42.82
4.	Physical mixture of leflunomide HP-β-CD in the ratio (1:2)	35.83
5.	Co-grinding complex of leflunomide HP-β-CD in the ratio (1:1)	29.42
6.	Co-grinding complex of leflunomide HP-β-CD in the ratio (1:2)	20.33
7.	Freeze dried complex of leflunomide HP-β-CD in the ratio (1:1)	(-) 31.34
8.	Freeze dried complex of leflunomide HP-β-CD in the ratio (1:2)	(-) 22.04

Solid inclusion complex formation was suggested by a decrease in enthalpy of fusion when compared to the pure drug and physical mixture. As the bound water molecules of cyclodextrin cavity are replaced with the drug molecules during complexation, the resultant fusion enthalpy is relatively less. It indicates the crystallinity of drug is decreases. The similar result was reported by other authors. [17, 18]

The freeze dried complex in 1:2 molar ratios showed relatively much less enthalpy fusion value (H -22.04) as compared to pure drug and other inclusion complexes due to higher inclusion formation, which is in conformation with the differential solubility technique.

X-Ray Diffraction studies

The X-ray diffraction patterns of leflunomide with HP-β-CD in solid complexes are shown in Figure No.10-17.

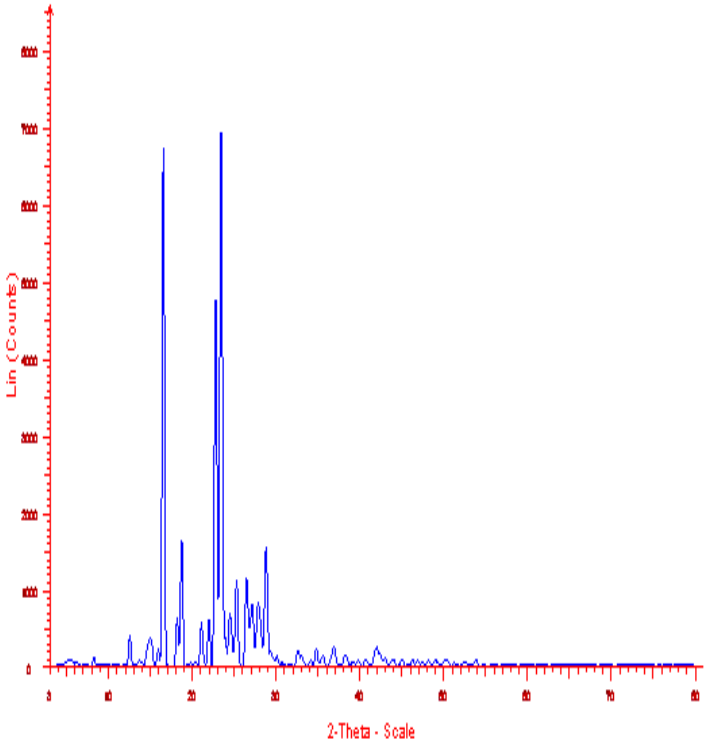

Figure 10 X-RD of Leflunomide

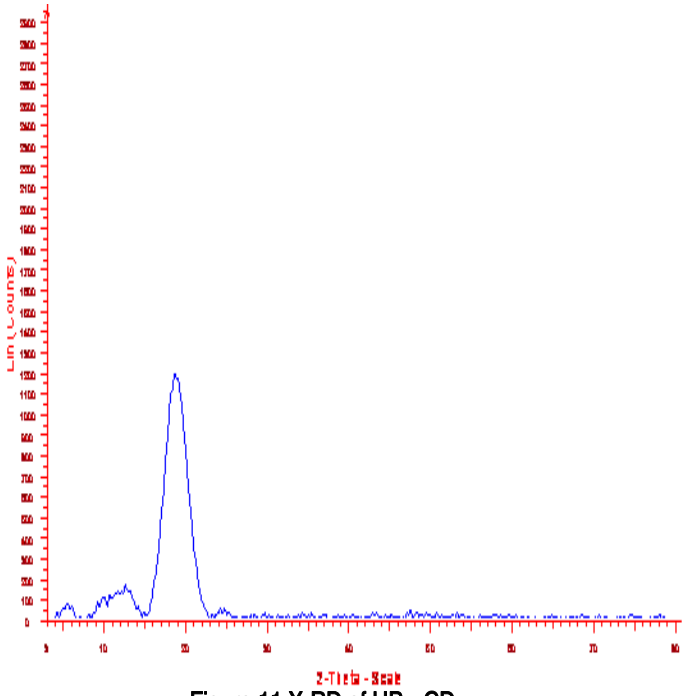

Figure 11 X-RD of HP- -CD

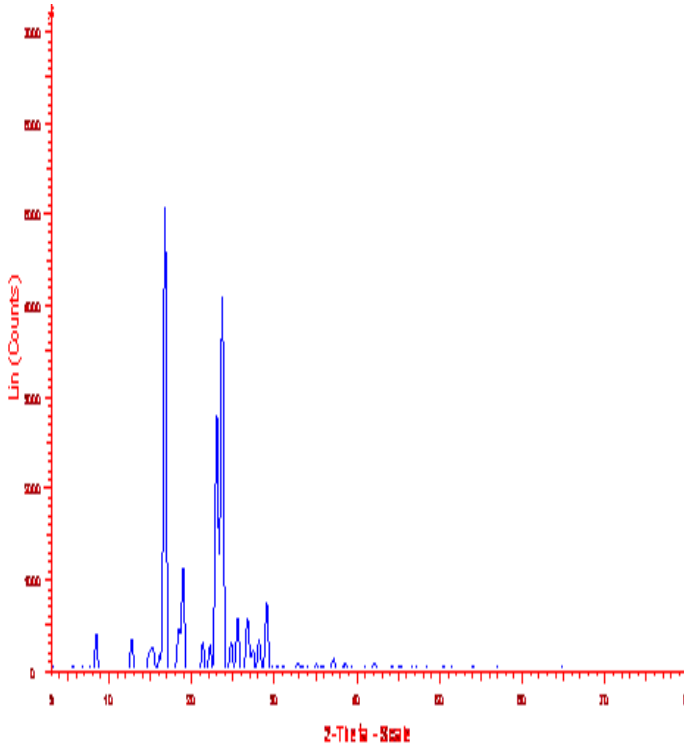

Figure 12 X-RD of Physical Mixture of Leflunomide with HP-β-CD in the ratio of (1:1)

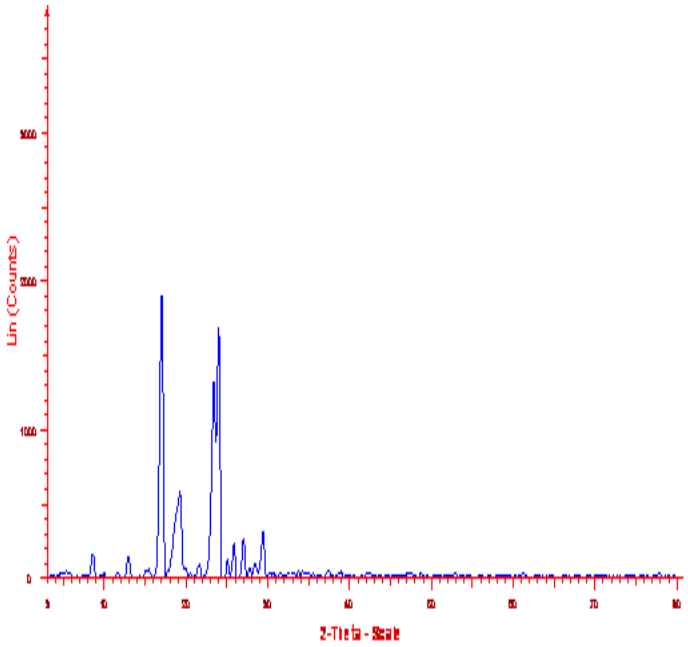

Figure 13 X-RD of Physical Mixture of Leflunomide with HP-β-CD in the ratio of (1:2)

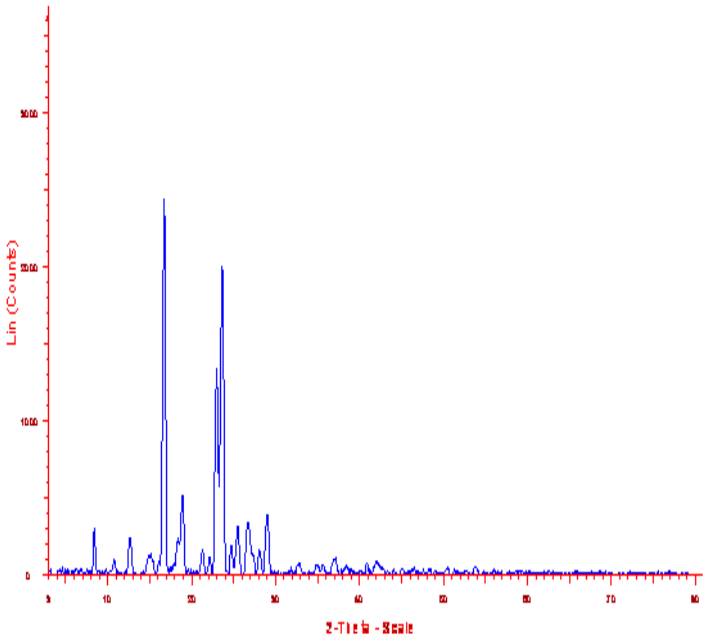

Figure 14 X-RD of Co-grinding Complex of Leflunomide with HP-β-CD in the ratio of (1:1)

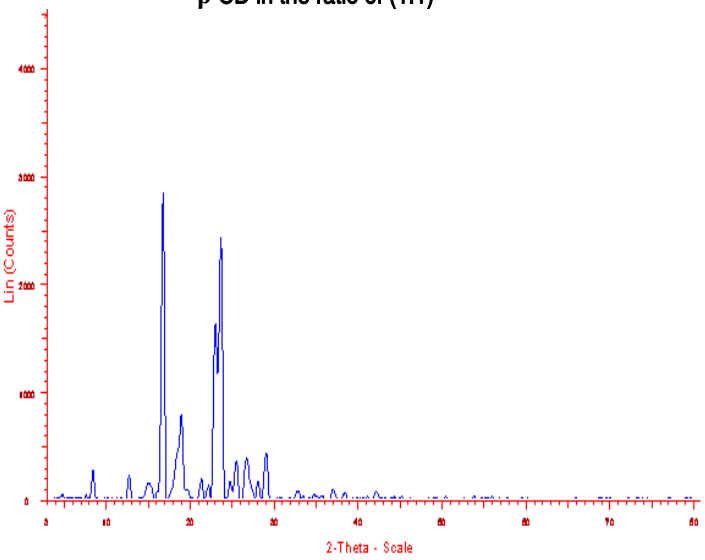

Figure 15 X-RD of Co-grinding Complex of Leflunomide with HP-β-CD in the ratio of (1:2)

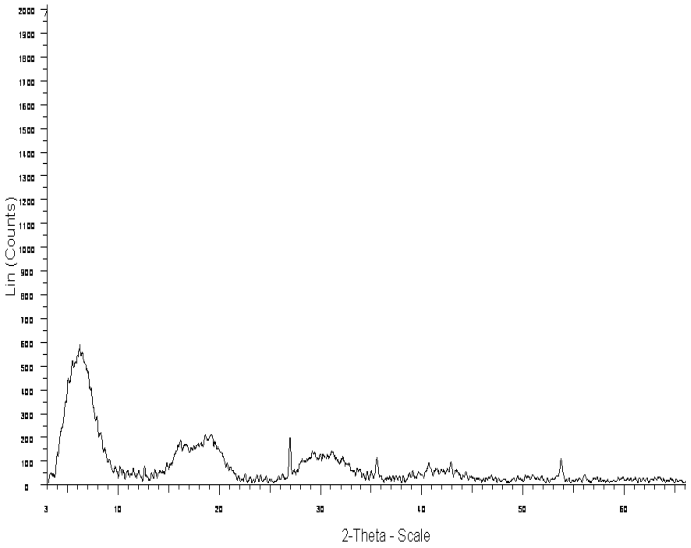

Figure 16 X-RD of Freeze Dried Complex of Leflunomide with HP-β-CD in the ratio of (1:1)

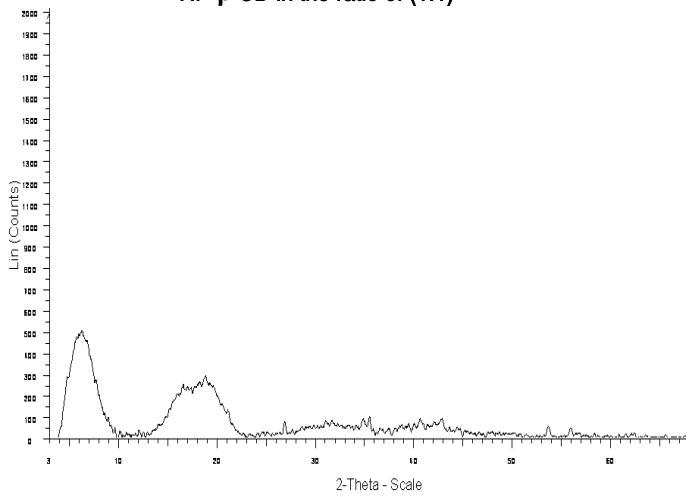

Figure 17 X-RD of Freeze Dried Complex of Leflunomide with HP-β-CD in the ratio of (1:2)

The X-ray diffractometry is a useful tool for the detection of cyclodextrin complexation in powder or microcrystalline states. [19] The diffractogram of leflunomide exhibits intense lines and characteristic peaks which are indicative of their crystallinity. (20) The results indicated that the most of peaks of physical mixture were the superposition of peaks of leflunomide and HP-β-CD. Partial peaks of leflunomide declined showing that the slight complexation may occur in physical mixture and in co-grinding complex. Whereas, the X-ray diffraction pattern of freeze dried complex of leflunomide with HP-β-CD was completely different from that of leflunomide or HP-β-CD, demonstrating that a new solid phase formed and showed a more diffuse pattern as compared to the other complexes.

Dissolution studies

The *in-vitro* dissolution profiles of drug and inclusion complexes in 1:1 and 1:2 molar ratios in dissolution mediums such as simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) are depicted in Figure No.18-19. The dissolution rate of complexes was higher as compared to the pure drug. It was observed that the co-grinding method and freeze-dried method in 1:2 molar ratios showed the increase in dissolution rate compared with pure drug and physical mixture. The dissolution profile of co-grinding complex in 1:2 molar ratio showed 56.63 % drug release in 60 min. in pH 1.2 and in pH 7.4 showed 35.18 % drug release in 20 min. corresponding to the pure drug. The dissolution profile of freeze-dried complex in 1:2 molar ratio showed 89.19 % drug release in 10 min. in pH 1.2 and in pH 7.4 showed 77.17 % drug release in 10 min. corresponding to the pure drug. The significant improved in dissolution characters in case of inclusion complex prepared by freeze-dried method with HP-β-CD may be due to formation of solid inclusion complex in dissolution media with better interaction of drug and HP-β-CD during freeze-dried method. And also marked increase in dissolution characters due to increase in drug wettability and reduction in crystallinity of drug.

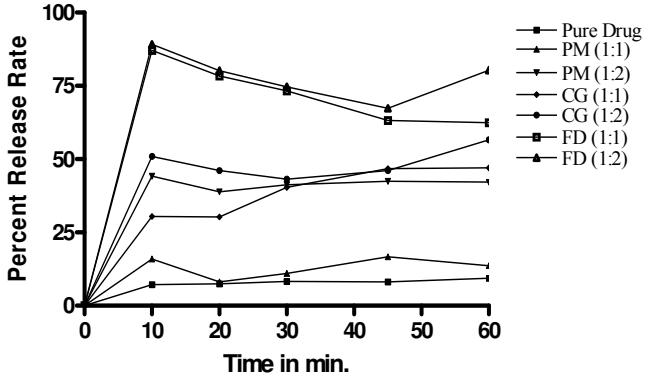

Figure 18 Results of Dissolution Profile of Pure Drug, PM, CG, FD (1:1) and (1:2) with HP- -CD in pH 1.2

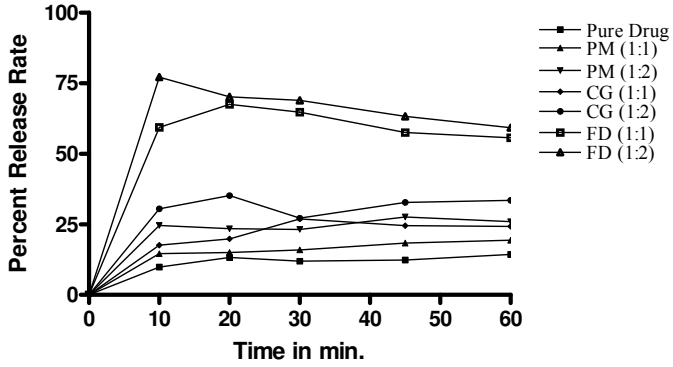

Figure 19 Results of Dissolution Profile of Pure Drug, PM, CG, FD (1:1) and (1:2) with HP- -CD in pH 7.4

Conclusion

The solubility and dissolution rate of leflunomide can be increased by inclusion complexation with HP-β-cyclodextrin. The phase

solubility studies indicated the formation of inclusion complexes with HP-β-CD at 1:1 stoichiometric ratio in solution with stability constant of 224.3 M^{-1}. The results obtained by different characterization techniques clearly indicated that freeze-dried method leads to formation of solid state complexes between leflunomide and HP-β-CD in pH 1.2. The complexation of leflunomide with HP-β-CD lends an ample credence for better therapeutic efficacy.

List of abbreviations

β-CD	-	Beta-cyclodextrin
HP-β-CD	-	Hydroxy Propyl Beta-cyclodextrin
DSC	-	Differential scanning calorimetry
FTIR	-	Fourier transform infrared spectroscopy
X-RD	-	X-ray diffraction

Author's contributions

Mrs. P.V. Bankar - Carried out experimental part of study
Dr. O. P. Mahatma and substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data, also involved in drafting or revising it critically for important intellectual content and given final approval of the version to be published.

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