

Formulation and Spectroscopic Studies and Dissolution Behavior of Levofloxacin- β Cyclodextrins Inclusion Complex

Mukesh Chandra Sharma^{1*}, Smita Sharma²

¹School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P)-452001, India

²Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalaya, Bhind (M.P) - 477001 India

*Corres.author: mukesh2206@rediffmail.com

Abstract: Enhance solubility and dissolution rate of Levofloxacin by complexation with β -cyclodextrin (β -CD) and subsequent dispersion with water-soluble polymers. The water-soluble polymers used were Hydroxypropyl methylcellulose, polyvinyl pyrrolidone K30, Polyethylene glycol 6000 and Croscarmellose sodium. The Levofloxacin - β -CD complex was prepared at the concentration of 1:2 molar ratios by co-precipitation method and the polymers were added at the concentration of 5%w/w to the complex by kneading. The binary system was characterized by differential scanning calorimetry, IR spectroscopy and dissolution rate. Phase solubility studies revealed that the complexation with β -CD increases the solubility of drug.

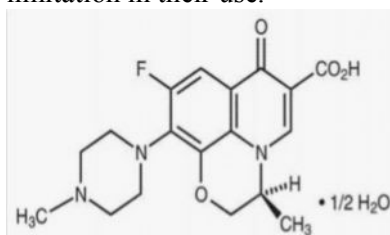
Keywords: P^H, Levofloxacin, Dissolution rate, β -Cyclodextrins.

Introduction

The approach of complexation with β -Cyclodextrins has been frequently used to increase the aqueous solubility and dissolution rate of water insoluble and slightly soluble drugs in an effort to increase oral bioavailability [1]. However, in certain instances, this approach is also used to increase drug stability [2], control drug release rate, improve organoleptic properties and maximize the gastrointestinal tolerance [3]. Generally speaking, β -Cyclodextrins are potential carriers for achieving such objectives but for a variety of reasons including cost, production capability and toxicity, the amount of Cyclodextrins incorporated into a drug formulation is limited [4-5]. It is, therefore, important to develop methods, which can be applied in order to enhance the efficiency of drug-Cyclodextrins complexation [6]. The complexation efficiency and

solubilising effect of β -Cyclodextrins in aqueous solutions have been increased by addition of water-soluble polymers [7]. This might be a useful strategy to decrease the amount of β -Cyclodextrins needed in oral dosage forms and to increase the pharmaceutical usefulness of β -Cyclodextrins in solid oral dosage forms. Consequently, the rationale of this study was to improve the therapeutic efficacy of Levofloxacin utilizing the approach of inclusion complexation with β -Cyclodextrin (β -CD). Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α -CD), seven (β -CD) or eight (γ -CD) α -1, 4-linked glucopyranose units with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity in the centre. The hydrophilic exterior of the CD molecules can make them water soluble, but the hydrophobic cavity provides an environment for appropriate sized non-polar molecules. In aqueous solution CDs are capable

of forming inclusion complex with many molecules by taking up a whole molecule or some part of it, into the cavity. These non-covalent complexes offer a variety of physicochemical advantages over uncomplexed molecules including increased water solubility and stability. Levofloxacin, (-)-(-)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl) - 7-oxo-7H-pyrido (1, 2, 3-de)- 1, 4-benzoxazine-6-carboxylic acid hemihydrate is a quinolone anti-microbial agent which exhibits broad spectrum in-vitro bactericidal activities against gram positive and gram negative aerobes. Levofloxacin is also marketed worldwide for oral and IV use, as well as used in ophthalmic solutions. Levofloxacin is a chiral fluorinated carboxyquinolone. Investigation of ofloxacin, an older drug that is the racemic mixture, found that the l form [the (-)-(-)-S enantiomer] is more active. This specific component is Levofloxacin [8, 9]. Levofloxacin interacts with a number of other drugs, as well as a number of herbal and natural supplements. Such interactions increase the risk of cardio toxicity and arrhythmias, anticoagulation, the formation of non-absorbable complexes, as well as increasing the risk of toxicity [10]. Levofloxacin is associated with a number of serious and life-threatening adverse reactions as well as spontaneous tendon ruptures and irreversible peripheral neuropathy. Such reactions may manifest long after therapy had been completed and in severe cases may result in life long. The peculiar arrangement of the glucose units imparts the molecule a cone like structure, which makes the exterior of the cone hydrophilic and interior of the cone hydrophobic in nature. This characteristic of the polymer enables encapsulation of the drug in the cavity resulting in the improvement in the solubility, drug release as well as taste masking. They have hydrophobic central cavity and a hydrophilic outer surface [11]. CDs have been found to be very useful in enhancing the solubility of poorly water-soluble drugs owing to the formation of inclusion complex of the drug in its hydrophobic cavity [12-17]. The most common natural CDs are α Cyclodextrins, β Cyclodextrins and γ Cyclodextrins, which are formed by six, seven, and eight glucose units, respectively. Apart from these naturally occurring CDs, various derivatives are also available [18-19]. which may produce better solubility when a complex [18, 21] but cost and toxicity factors poses limitation in their use.



Levofloxacin

Materials and Method

Levofloxacin was obtained as a complimentary sample from Laboratories Ltd, Hyderabad. Commercial tablet of levofloxacin were procured from the market. All other chemical used were of analytical grade. All other chemicals were of analytical grade and used without further purifications. Measurements of pH were performed using a calibrated Elico pH meter. Levofloxacin concentrations were determined at 266.5 nm using Shimadzu UVspectrophotometer. As a starting point for this study, the solubility of levofloxacin as a function of pH was studied. A series of buffer solutions from pH range 4.5 to 9.3 were prepared and Levofloxacin was added in sufficient quantity to saturate each solution. To avoid change in concentration due to evaporation, the solutions were kept in vials sealed with Teflon lined screw caps and wrapped with paraffin. All solutions were then placed on a test tube rotator for mixing. They were checked daily for the saturation and pH was adjusted as necessary.

Phase solubility study of Levofloxacin using β -CD

Solubility measurements were carried out according to the Higuchi and Connors (1965) method [22-23]. An excess of levofloxacin was added to phosphate buffer solutions (pH 8.3) containing different concentrations of β -CD. The suspensions were shaken at 28°C for 72h and then filtered through a millipore filter (0.45mm). An aliquot portion of the filtrate was analyzed for its drug content by measuring its extinction at 242.1 nm against blank solution containing the same concentration of β -CD.

Preparation of Levofloxacin - β -CD complex

Inclusion complexation of levofloxacin with β -CD was prepared by the liquid/liquid co-precipitation method [24]. Levofloxacin and β -CD equivalent to its 1:2 molar ratios were selected. An accurate quantity of drug was dissolved in acetone and stirred to obtain a clear solution. In another beaker weighed amount of β -CD in water at 75° was stirred for one hour to obtain clear solution. Absolute ethanol solution of Levofloxacin was added drop wise to aqueous solution of β -CD with continuous stirring at 40° until precipitate was formed. The precipitate formed was filtered and complex was dried under vacuum at 75° for 2h. The formed complex was collected and stored for the further studies.

Determination of Stability Constant (K)

Complexation studies were performed according to the method reported by Higuchi. An excess amount of levofloxacin was added to the aqueous solution of various concentrations (0.01-0.050 ml) of β -CD

solution (molecular weight = 1135). The contents were stirred for 45 hours at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After equilibrium, the samples were filtered and absorbance was measured at 266.5 nm (UV/ VIS spectrophotometer, Japan). The apparent stability constant was calculated for this complex using the equation 4.5-6.3.

Preparation of Levofloxacin - β -CD-Polymer ternary systems

Ternary systems consisting of levofloxacin - β -CD and a water-soluble polymer were prepared by kneading method. Five water soluble polymers viz., HPMC, PVP, PEG-6000, Avicel, and CRS, in a concentration of 15% to the weight of inclusion complex, were used. The complex and the polymer were kneaded thoroughly with least amount of water. The paste formed was dried under vacuum at 60° for 12h; it was collected and stored in desiccators for the further studies.

Assay of inclusion complex and ternary systems

The binary complex and all the ternary systems were assayed for drug content. An accurate weight of preparation equivalent to 5 mg of levofloxacin was dissolved in 20mL of methanol in 100mL, the volume was made to the mark with phosphate buffer solution pH 7.4 and the solution was filtered through whatman filter paper No. 41. The above solution was further diluted with phosphate buffer solution pH 7.4. The absorbance of the above solution was read at the wavelength 226nm using UV Spectrophotometer and the % purity was determined as,

$$\frac{\text{Concentration in mg/mL} = \text{Concentration} \times \text{Dilution} \times 100}{\text{in } \mu\text{g/mL} \quad \text{factor}}$$

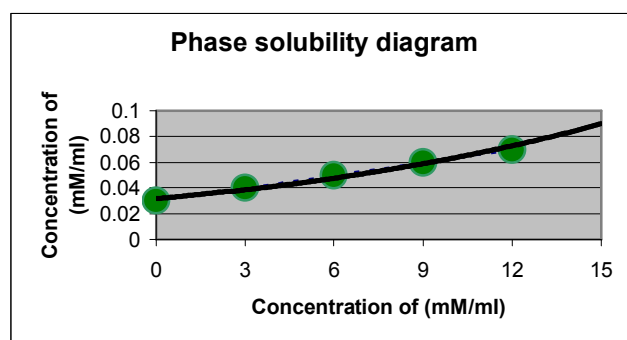
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Evaluation of Levofloxacin - β -Cyclodextrins

Phase solubility study-

Phase-solubility studies were performed by the method of Higuchi and Connors[23]. Levofloxacin in constant amounts (5 mg) that exceeded its solubility, was transferred to screw capped vials containing 25 ml of

aqueous solution of β -CD or at various molar concentrations (0, 5, 7, 8, 10.0, and 13.0 μM). The contents were stirred on rotary shaker for 48 hrs. At $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ and 2000 rpm. The time duration was fixed based on pilot experiment and found to be sufficient to achieve equilibrium of mixture. After reaching equilibrium, samples were filtered through a 100 μm membrane filter, suitably diluted and analyzed spectrophotometrically for drug content at 266.5 nm UV/Visible spectrophotometer.



Preformulation studies

Preformulation studies were performed on free drug and complexes to assess the suitability of the complexes for capsule dosage forms. Bulk density, Tapped density, percentage compressibility, angle of repose of the drug and the complex were found out. Thermo grams of the pure drug, BCD and 1:2 complexes were recorded by analyzing the samples by differential thermal analysis. Solubility of the drug and the complex in phosphate buffer pH 7.4 were determined.

Infrared spectroscopy

I.R. spectra of Levofloxacin powder, physical mixture of Levofloxacin and β -CD, inclusion complex and ternary systems were monitored as KBr disc prepared at a pressure of 150 to 200 kgcm^{-2} . All the samples were scanned at the resolution of 4 cm^{-1} over the wave number region $4000\text{-}200 \text{ cm}^{-1}$ using a Shimadzu 8400S IR spectrophotometer.

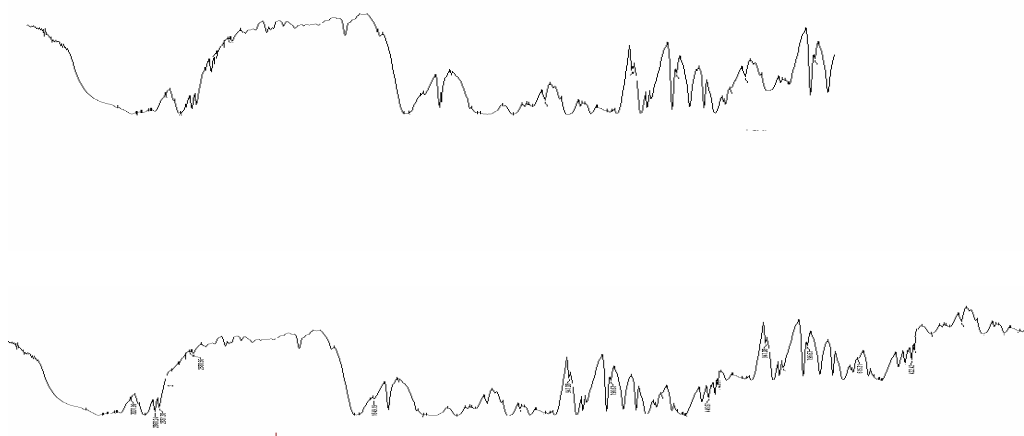


Figure-IR spectrum of Levofloxacin and Levofloxacin - β -cyclodextrin inclusion complex

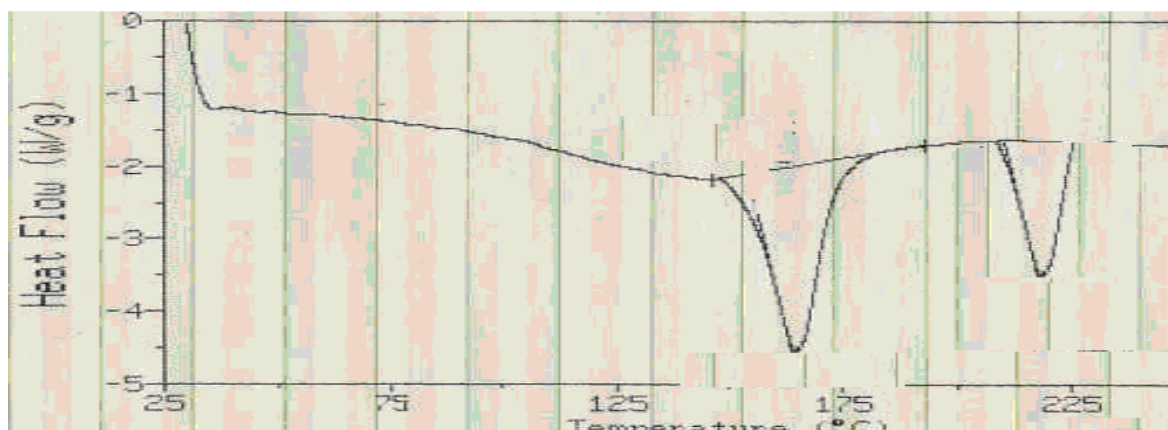


Figure- DSC measurements Levofloxacin - β -cyclodextrin inclusion complex

Differential scanning calorimetric (DSC) measurements

The stability and thermal behaviour of Levofloxacin and its physical mixture and complex with β -CD were traced on a Dupont DSC model. The instrument was calibrated with indium and zinc prior to analyzing the samples under nitrogen at the flow rate of 20mL/min. approximately 4mg of each sample was scanned in sealed aluminum pans at the heating rate of 25 $^{\circ}$ /min over the temperature range of room temperature to 110 $^{\circ}$.

Dissolution Studies

The dissolution profile was studied using USP dissolution rate test apparatus employing paddle stirrer. In 900 ml dissolution medium (2 hrs using 0.1 N HCl and the medium was replaced with phosphate buffer pH 7.4), a sample of 5 mg drug equivalent complex (1:1m, 1:2m) was placed and set rpm at 300 and temperature +37 $^{\circ}$ C. Aliquots of 5 ml was

withdrawn at 10 min intervals of time and replaced with the same medium and analyzed at 266.5 nm by using uv visible spectrophotometer.

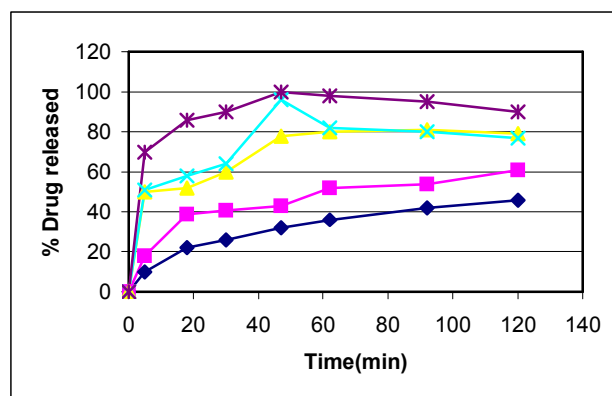


Figure. Dissolution Profiles of Levofloxacin and its complexes with β -Cyclodextrin in simulated gastric fluid without pepsin

Formulation studies-

Tablets containing 5 mg of Levofloxacin were prepared by direct compression using different excipients like Lactose monohydrate, colloidal silicon dioxide, and magnesium stearate. Tablets containing complexes (equivalent to 5 mg Levofloxacin) prepared by kneading and co evaporation method were also prepared similarly using less quantity of lactose. The blend was compressed on a six-station single rotary machine using round-shaped, flat punches to obtain tablets having thickness 3–5 mm and hardness 8–10 kg/cm². The tablets were studied in 6 replicates for release profile of Levofloxacin using the same method described in dissolution studies.

Results and Discussion

Infrared spectra of the Levofloxacin and inclusion complex and physical mixture of Levofloxacin with β -CD are shown in Fig [a and b]. An infrared spectrum was used to evaluate the functional groups of Levofloxacin involved in the complexation. Infrared spectrum of Levofloxacin is characterized by identification of the carbonyl (C=O), methyl and fluorine group bands of carboxylic group. In the spectra of the inclusion complex, these bands were shifted towards higher frequencies and the asymmetrically vibration peak of C=O band was obtained as three increases intensity peaks, suggesting that after the formation of the inclusion complex. The IR spectrum of β -CD is characterized by intense bands at 3000–3600 cm⁻¹, associated with the absorption of the hydrogen bonded -OH groups of β -CD. The vibrations of the CH-CH groups appear in the 2897–3250cm⁻¹ region. Thus, as spectral changes always concern COOH, -CH₃ and CH groups of the β -CD. DSC is a fast and relatively inexpensive technique to examine and verify the drugs that form inclusion complex with β -CD and to confirm the absence of the drug melting endotherm. The results of DSC thermograms for given samples are shown in Fig. The DSC curve of Levofloxacin showed an endothermic reaction and its melting peak was at the onset temperature of 198.55°. The thermal behaviour of β -CD exhibited a sharp endothermic peak at 186.43° due to its melting. Physical mixture showed a sharp endothermic peak at 180.36°, Therefore it was concluded that some part of the Levofloxacin is complexes with β -CD but some part remained outside of the complex. The effect of β -CD on the solubility of Levofloxacin in phosphate buffer solutions pH7.4 was investigated at 37±0.5°. It is evident that the solubility of Levofloxacin was increased markedly by complexation with β -CD. Inclusion complexation of the drug in β -CD enhanced the dissolution rate of the drug to a marked extent; the

dissolution efficiency was increased up to 11-13 folds. This increase in the dissolution rate of the drug can be attributed to both improvements in drug wettability and formation of readily soluble complexes such as both inclusion and non-inclusion complexes by β -CD. In addition to this, a β -CD form water-soluble the effect of inclusion complexation of Levofloxacin in β -CD and in presence of different polymers on the dissolution profile of Levofloxacin is illustrated in Fig. The dissolution profiles of the ternary systems showed an increase in the dissolution rate of Levofloxacin compared to the binary system. The investigated polymers increased the dissolution rate of the drug in the order of Croscarmellose sodium, PEG 6000, HPMC, PVP K30. The increase in the dissolution rate of Levofloxacin might be related to the increase of complexation efficiency and solubilizing effect of β -CD in presence of water-soluble polymers. It is evident that the dissolution rate of the drug was relatively less using PVP K30. This might indicate a sort of interaction between this polymer where many cyclodextrin molecules are threaded onto a linear polymer. Such inclusion complex formation between β -CD and polymers will reduce the ability of β -CD to form complex with the drug. On the other hand, Avicel pH101 and HPMC showed more or less same effect and the dissolution rate of Levofloxacin is not much enhanced, while croscarmellose sodium and PEG 6000 enhanced markedly the dissolution rate of the drug. These results agree with the well-established formation of soluble complexes between the water soluble polymeric carriers and poorly water-soluble drugs. Increased solubility may be due to the improved wettability of the Levofloxacin particles in aqueous solution from polymers. The values of Gibbs free energy change are an indication of the process of transfer of Levofloxacin from pure water to aqueous solution of polymers. The solubility and dissolution rate of Levofloxacin can be enhanced by inclusion complexation with β -CD and subsequent dispersion with water-soluble polymers. Usage of water-soluble polymers has the great advantage of reducing the dose of the drug and the amount of cyclodextrin needed. Reduction of particle aggregation of the drug, absence of crystalline, increased wettability and dispersibility, and alteration of surface properties of the drug particles may be responsible for the enhanced solubility and dissolution rate of Levofloxacin.

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