ORIGINAL ARTICLE

Large Ring Cyclodextrin and Cosolvency-based Techniques to Improve Solubility of Antifungal Drug

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Abstract

Objective: Posaconazole is used to treat oral candidiasis in patients who are severely immunocompromised. Posaconazole is categorized under BCS Class-II drug, i.e., low solubility and high permeability. Posaconazole has very poor water solubility. The current study aimed to improve water solubility of posaconazole using different approaches. **Materials and Methods:** To enhance the solubility of drug, two methods, inclusion complex and cosolvency approach, were performed. In inclusion complex method, beta-cyclodextrin (β-CD) and hydroxypropyl β-CD (HP-β-CD) were used for the preparation of inclusion complex. In cosolvency method, two different solvents polyethylene glycol-400 (PEG-400) and propylene glycol (PG) were used. **Results:** The inclusion complex of HP-β-CD was prepared by physical mixing, kneading, and solvent evaporation methods by taking 1:0.5, 1:1, and 1:2 molar ratio of Drug: HP-β-CD. From the results of dissolution study of complex, it was observed that there was no significant improvement in solubility of drug. In cosolvency approach, maximum solubility of drug was found in a ratio of 0:100% v/v for both solvents. The solubility of drug in Water: PEG- 400 and Water: PG solvents were found to be 20.228 ± 0.0169 mg/mL and 17.204 ± 0.0178 mg/mL, respectively. **Conclusion:** In cosolvency method, it was observed that drug has more solubility in PEG- 400 compared to PG. Hence, PEG-400 solvent was selected for drug solubilization.

Key words: Posaconazole, solubility enhancement, complexation, co-solvency

INTRODUCTION

Posaconazole is a triazole antifungal medication that exhibits broad-spectrum activity against various fungal species, including Aspergillus and Candida.[1-3] Drug solubility is a critical factor in developing an efficient drug delivery system, as the drug must have sufficient solubility at the absorption site to be effective.[4,5] Drugs that are poorly soluble in water may have reduced absorption rates, resulting in lower-than-desired drug concentrations in the bloodstream and diminished pharmacological responses. Inclusion complexes can be used to overcome the limitations of low solubility and bioavailability.[6,7]

Beta-cyclodextrin (β-CD) and hydroxypropyl-β-CD (HPBCD) are cyclic oligosaccharides that have been widely studied for their ability to enhance the solubility of poorly water-soluble drugs. The mechanism of action of these cyclodextrins involves the formation of inclusion complexes with the drug molecules. The hydrophobic drug molecules are encapsulated within the hydrophobic cavity of the cyclodextrin molecule, shielding them from the aqueous environment and reducing their tendency to aggregate or precipitate. This increases the solubility and bioavailability of the drug.[8,9]

β-CD is a naturally occurring product obtained from the enzymatic degradation of starch. Its small cavity size limits its ability to encapsulate larger molecules. In contrast, HPBCD is a modified β-CD molecule that has a larger cavity size and can accommodate a wider range of drug molecules. In addition to improving solubility, β-CD and HPBCD can also enhance the stability, permeability, and pharmacokinetic properties of drugs. They have been approved by regulatory agencies such as the US FDA and are generally considered safe for use in pharmaceutical applications.[10,11]

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Co-solvency is a technique used in the pharmaceutical industry to enhance the solubility of poorly soluble drugs. It involves the addition of one or more co-solvents to a solvent system to increase the solubility of the drug in the solvent. Cosolvents are typically small, water-miscible organic molecules that can interact with both the drug and the solvent to form a stable, homogeneous solution.[12]

The role of cosolvency in solubility enhancement is to alter the thermodynamic properties of the solvent system to increase the solubility of the drug. Cosolvents can achieve this by reducing the intermolecular forces between the drug molecules and the solvent molecules, which allows the drug to dissolve more readily in the solvent system. In addition, cosolvents can also act as a stabilizer, preventing the drug from precipitating out of solution. The selection of cosolvent is based on various factors, such as the physicochemical properties of the drug and the solvent, the desired solubility, and the intended route of administration. Common cosolvents used in the pharmaceutical industry include propylene glycol (PG), polyethylene glycol (PEG), ethanol, and glycerol.[13]

Here, an attempt has been made to enhance the solubility of drug using various approaches such as complexation and cosolvency. Different complexes were prepared using β-CD and HPBCD in different ratio using various methods. Prepared complexes were analyzed for solubility and dissolution study. Cosolvency study was also performed to check the effect of different cosolvents commonly used in pharmaceuticals, such as PEG 400 and Propylene Glycol affect the solubility of posaconazole in water.

MATERIALS AND METHODS

Posaconazole was received as a gift sample by Torrent Pharmaceuticals Ltd. India. β-CD and HPBCD were purchased from Chemdyes Corporation, Rajkot. PEG 400, PG were purchased from Suvidhinath Laboratories, India. All other chemicals were of analytical reagent grade, and freshly prepared distilled water was used throughout the study.

EXPERIMENTAL

Solubility study

The saturation solubility of posaconazole was determined using the shake flask method. Excess posaconazole was added to 10 mL of water and phosphate buffer pH 6.8, and the mixture was shaken overnight to ensure complete dissolution. The resulting solution was then filtered through Whatman® filter paper, and the filtrate was appropriately diluted with a suitable vehicle if required. The absorbance of the solution was measured at a wavelength of 251 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).[14] This method is commonly used to determine the maximum amount of drug that can be dissolved in a specific solvent under defined conditions of temperature, pH, and agitation. The data obtained from this study can be used to formulate drug delivery systems and determine appropriate dosages for therapeutic applications.

Table 1: Cosolvent mixture

Water (% v/v)	PEG-400 (% v/v) or propylene glycol
0	100
10	90
20	80
30	70
40	60
50	50
60	40
70	30
80	20
90	10
100	0

PEG: Polyethylene glycol

Table 2: Solubility of posaconazole in solvents

Solvents	Solubility (mg/mL)±SD	Description
Water	0.0001±0.00002	Poorly soluble
Phosphate buffer pH 6.8	0.0003±0.0001	Poorly soluble

SD: Standard deviation

Analytical method development for solubility study

To prepare a stock solution of posaconazole, 10 mg of the drug was dissolved in a small amount of methanol, and the solution was diluted to a final volume of 10 mL with methanol to obtain a concentration of 1000 µg/mL. From the stock solution, 5 mL was pipetted out and diluted to 50 mL with phosphate buffer pH 6.8 to obtain a working stock solution of 100 µg/mL. The working stock solution was then serially diluted with phosphate buffer pH 6.8 to obtain a range of solutions in the concentration range of 5–30 µg/mL. The λmax of the solution was determined by measuring the absorbance of the different diluted solutions using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) at a wavelength of 251 nm.[15] Finally, a calibration curve was plotted for drug concentration (µg/mL) versus absorbance. The regression equation and regression coefficient (R^2) were reported. This method is commonly used to quantify the concentration of a drug in a solution and to determine its purity. The information obtained from this study is useful for drug development and quality control purposes.

Solubility enhancement of drug by inclusion complex

By using β-CD

Phase solubility study (according to the model proposed by Higuchi and Connors) was performed by dissolving 10 mg of drug with solution of β-CD. β-CD solutions were prepared of different concentrations (0.003–0.015 M) and were added to conical flask. Flask was covered and stirred for 72 h by placing it in Orbital Shaker. Temperature was maintained 37 ± 0.5°C. Excess of drug was separated by centrifugation and supernatant was analyzed by UV Spectrophotometer.[16]

By using HP-β-CD

Similarly, phase solubility study was also performed using HP-β-CD solutions at different molar concentrations (0.003–0.015 M).[17]

Preparation of inclusion complex

Inclusion complexes using β-CD and HP-β-CD were prepared by physical mixture, kneading, and solvent evaporation method.[18]

Physical mixture

Drug and β-CD as well as drug and HP-β-CD were blended in mortar in different molar ratio (1:0.5, 1:1, and 1:2) for 30 min. The mixture was passed through sieve no. 44 and stored in airtight container.

Kneading method

Drug and complexing agents were moistened with small quantity of methanol until damp mass was formed. Mixing was carried out for 15 min using mortar and pestle to complete the process of complexation. Prepared slug was passed through sieve, air dried, and stored.

Solvent evaporation

Accurately weighed quantity of drug and complexing agents were dissolved in methanol. Solvent was allowed to evaporate completely while kept stirring. Residue was collected, dried completely, and sieved to store.

CHARACTERIZATION OF PREPARED COMPLEX[19]

U.V. absorbance of prepared complex

Accurately weighed amount of complex was dissolved in 10 mL of water using magnetic stirrer for 24 h. The solution was centrifuged at 3000 rpm for 15 min. Separated supernatant was analyzed at 250 nm by UV spectrophotometer.

Drug content

Accurately weighed amount of complex equivalent to 5 mg of drug was dissolved in water and UV absorbance was measured at 250 nm. Further.

Weighed complex equivalent to 5 mg drug was transferred to 100 mL standard flask. Made up the solution with phosphate buffer of pH 7.4 and after UV absorbance was measured at 339.2 nm using UV-visible spectrophotometer.

In vitro dissolution studies

In vitro dissolution studies were carried out for pure Embelin and the inclusion of CD complexes to compare the solubility of complexes prepared by different methods. US Pharmacopeia Type I dissolution test apparatus was used for the study. CD complexes equivalent to contain 10 mg Embelin were placed in dissolution vessels containing 900 mL of phosphate buffer of pH 7.4 kept at 37 ± 0.5°C and stirred at 50 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. 10 mL was withdrawn and filtered through Whatman filter paper. Absorbance was read at 339.2 nm against a blank.

Solubility enhancement of drug by cosolvency approach[20]

Water and PEG 400

Distilled water and cosolvent (PEG 400) were mixed volumetrically in different ratios to form mixtures containing 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (total 10 mL solvent system). Excess amount of drug was added directly into each mixture. Each mixture was vortexed by using vortex shaker at room temperature (25 ± 1°C) for 1 h. After vertexing, all the tubes containing mixture were allowed to keep aside for 24 h to attain the equilibrium. After 24 h, all the mixtures were subjected to centrifugation at 3000 rpm for 15 min. Supernatant was withdrawn and diluted suitably. These samples were analyzed using UV-VIS spectrophotometer at 250 nm wavelength and solubility of the drug in each mixture was calculated.

Water and PG

PG is used as a cosolvent in this method. Water and PG were proportionately mixed in different ratios ranging from 0 to 100% (Table 1). Drug was added to prepare saturated solution. Maximum amount of drug was dissolved by subjecting the mixture to vortex. All the samples were kept aside for 24 h to attain the maximum solubilization. Prepared samples were subjected to centrifugation and supernatant was separated and analyzed subsequently using UV-VIS spectrophotometer at 250 nm wavelength. The solubility of drug from each sample was calculated.

RESULTS AND DISCUSSION

Solubility study

Posaconazole was found to be poorly soluble in water and phosphate buffer pH 6.8 (Table 2).

Analytical method development

The calibration curve for posaconazole was prepared using phosphate buffer pH 6.8 as a solvent. To prepare the calibration curve, six different concentrations of the drug were made and the absorbance was measured using a UV spectrophotometer. The λmax of the drug in phosphate buffer pH 6.8 was identified as 251 nm. The linearity of the calibration curve was observed within the drug concentration range of 5–30 µg/mL (Figure 1).

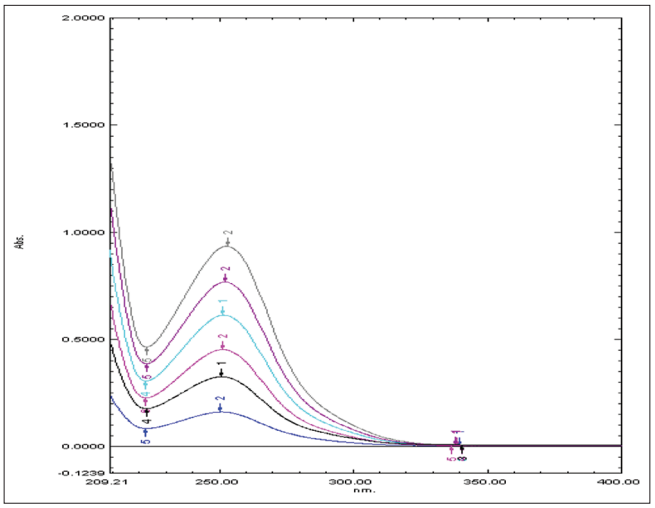

Figure 1: Overlay spectra of posaconazole standard calibration in phosphate buffer pH 6.8

Solubility enhancement of drug by inclusion complex

Phase solubility study (according to the model proposed by Higuchi and Connors)

a. By using β-CD

From the phase solubility study data for β-CD, it was observed that with increasing concentration of β-CD (3–12 mmol), the concentration of drug solubilized also increased (0.0005–0.0052 mg). The maximum concentration of drug found to be solubilized is 0.0052 mg at 12 mmol conc. of β-CD which is approximately 5 times higher than intrinsic solubility of drug (Figure 2).

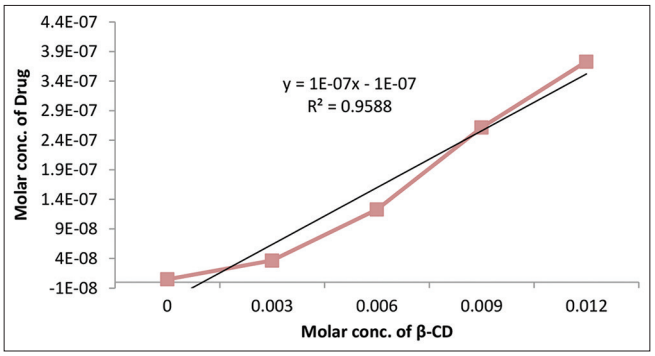

Figure 2: Phase solubility of posaconazole using β-CD

From the obtained result, graph of molar conc. of drug v/s molar conc. of β-CD was plotted. From straight-line equation, stability constant for prepared complex (drug and β-CD) was calculated.

$$Stability\ Constant \tan(K) = \frac{Slope}{So\ (1 - Slope)} \quad (1)$$

Where,
So = Molar concentration of drug in 20 mL of water
Stability constant (K)= 20120.72 M

From the above equation, stability constant for prepared complex of drug and β-CD was found to be 20120.72 M. Such a high value of stability constant indicated that prepared complex is stable due to complete inclusion in hydrophobic cavity of cyclodextrin.

b. By using HP-β-CD

Phase solubility study [Figure 3] of HP-β-CD showed that prepared inclusion complex exhibits maximum solubility at

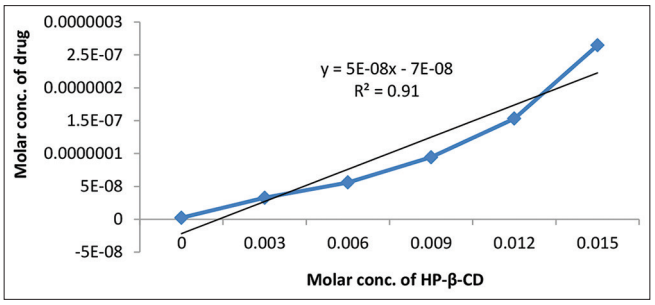

Figure 3: Phase solubility of posaconazole using HP-β-CD

15 mmol concentration. Proportionate increase in solubility was found to be 8 times higher than solubility in water.

Stability constant (K) = 22123.89 M

Stability constant for the complex of posaconazole and HP-β-CD was found to be 22123.89 M which was calculated as shown in Equation 1 which is having comparative high value than drug and β-CD complex. Hence, due to increased solubility of drug with HP-β-CD compared to β-CD, HP-β-CD was selected for complex preparation. Posaconazole and HP-β-CD complex was

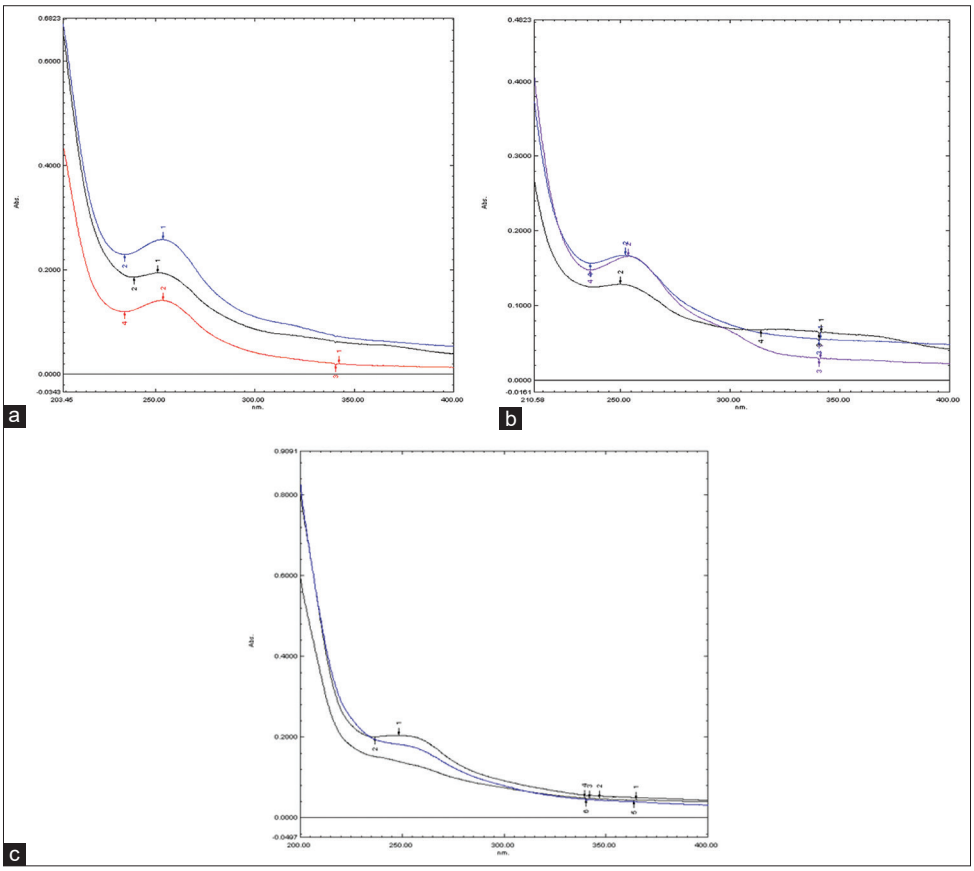

Figure 4: UV spectrum of complex prepared by (a) physical mixture, (b) kneading, (c) solvent evaporation

prepared using three different methods. Three different stoichiometry ratios were selected to prepare complex 1:0.5, 1:1, and 1:2.

Inclusion complex of drug and HP-β-CD was prepared using physical mixture, kneading, and solvent evaporation methods. In each of these methods, three molar ratios of drug: (Figure 4) complexing agent (1:0.5, 1:1, and 1:2) were taken. After preparation of complex, accurately weighed amount of complex from each ratio for all three methods was subjected to dissolution. After measuring the absorbance, it was observed that as the molar ratio of HP-β-CD increased, the absorbance was also increased. HP-β-CD has more ability to form a stable complex with drug compared to β-CD. As a result, solubility of drug was also increased. The highest solubility of drug was found in a ratio 1:2 of solvent evaporation method. However, significant improvement in the solubility of drug was not found in inclusion complex method.

In vitro dissolution of prepared complex

Impact of cyclodextrin on dissolution of posaconazole was studied by comparing the dissolution of drug before and after complexation with β-CD and HP-β-CD using different methods of complexation. Pure drug has shown <10% of release during 1st h of dissolution study due to poor water solubility. Comparative dissolution of inclusion complex exhibited better dissolution with complex prepared by solvent evaporation method [Figure 5].

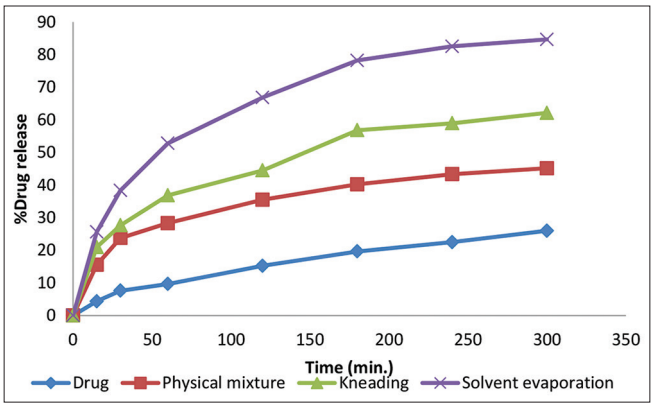

Figure 5: *In vitro* dissolution study of prepared complex by different methods

Cosolvency

In cosolvency method, PEG-400 and PG were used as a solvent for the solubilization of a drug. Different (% v/v) ratios of water: PEG-400 and water: PG solvent were taken. From the result [Figure 6], it was observed that the maximum solubility of drug was found in a ratio of 0:100% v/v for both

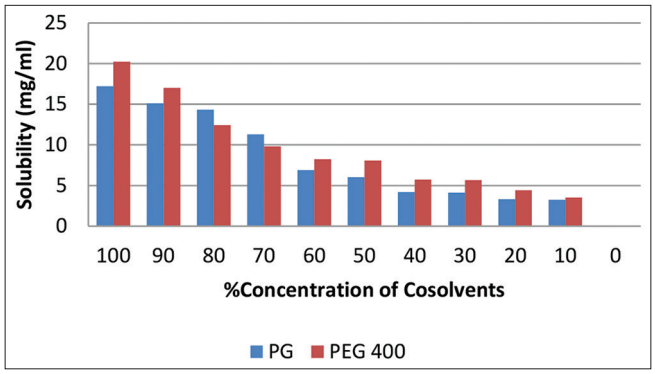

Figure 6: Solubility enhancement by cosolvency approach

cosolvent systems. The maximum drug conc. in water: PEG-400 and water: PG cosolvent systems were found to be 20.228 ± 0.0169 mg/mL and 17.204 ± 0.0178 mg/mL, respectively. From these results, it was concluded that maximum solubility of drug was found in PEG-400 (100%) solvent. Hence, PEG-400 was selected for drug solubilization.

CONCLUSION

Preparation of inclusion complex and cosolvency is the approach used for solubility enhancement. Inclusion complex was prepared by various methods such as physical mixture, solvent evaporation, and kneading. Dissolution study of complex prepared by solvent evaporation method exhibited more drug release compared to other. More satisfactory results for solubility enhancement of posaconazole were obtained by cosolvency approach. Different cosolvents, namely, PEG 400 and PG were used, from which PEG 400 has been an acceptable cosolvent using in terms of side-effect profile and most efficient solubilizing cosolvent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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