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RESEARCH ARTICLE

Enhancement of Solubility of Itraconazole by Complexation with β Cyclodextrin Derivatives

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Enhancement of Solubility of Itraconazole by Complexation with β Cyclodextrin Derivatives

SUMMARY

The purpose of this study was to increase the solubility of itraconazole (IT) with inclusion complexes. For this aim, different types of IT-cyclodexrin complexes were prepared by using beta cyclodextrin (βCD), hydroxypropyl beta cyclodextrin (HPβCD) and randomized methylated beta cyclodextrin (RAMEB). The phase solubility studies were made in order to determine the molar ratios of complexes and for βCD, BS type, for HPβCD and RAMEB, AL type solubility diagrams were revealed. Since the BS type diagrams indicate the complexes with limited solubility, the studies were continued with HPβCD and RAMEB. 1:1 and 1:2 molar ratio of IT:HPβCD and IT:RAMEB complexes were prepared by using physical mixture, kneading and coprecipitation method. Inclusion complexes were confirmed by the results from the studies of differential scanning calorimetry (DSC). When the solubility of complexes determined in pH 1.2, it was seen that the solubility of IT which is 4.5 µg/ml, increased to 12.39 µg/ml with HPβCD and to 14.05 µg/ml with RAMEB by using kneading method and 1:2 IT:CD molar ratio. Due to the solubility values and the stability constants which show the stability of the complexes (for HPβCD, Kc= 3 M-1, for RAMEB, Kc= 75 M-1) it was decided to prepare complexes with RAMEB in formulation studies. A water soluble polymer, polyethylene glycol 4000 (PEG 4000) were added to RAMEB complexes as solubility enhancer and it was seen that the solubility increased to 28.72 µg/ml.

Key Words: *Itraconazole, β cyclodextrin derivatives, physical mixture, kneading method, coprecipitation method, DSC*

İtrakonazolun Çözünürlüğünün ve Çözünme Hızının, β Siklodektrin ve Türevleriyle Kompleks Oluşturularak Artırılması

ÖZET

Bu çalışmada, mide ortamındaki çözünürlüğü çok düşük olduğu bilinen itrakonazolün (IT), siklodekstrinlerle kompleksleri hazırlanarak çözünürlüğünün artırılması amaçlanmıştır. Bu amaçla etkin maddenin farklı siklodekstrin tipleri (beta siklodekstrin (βCD), hidroksipropil beta siklodekstrin (HPβCD) ve randomize metillenmiş beta siklodekstrin (RAMEB)) ile kompleksleri oluşturulmuştur. Komplekslerin hangi molar oranlarda hazırlanacağını tespit etmek için faz-çözünürlük çalışmaları yapılmış ve βCD ile BS tipi, HPβCD ve RAMEB ile AL tipi çözünürlük diyagramları elde edilmiştir. BS tipi diyagramlar sınırlı çözünürlüğe sahip kompleks oluşumunun göstergesi olduğundan, çalışmalara HPβCD ve RAMEB ile devam edilmiştir. İtrakonazolün HPβCD ve RAMEB ile 1:1 ve 1:2 oranında, önce fiziksel karışımları daha sonra örme ve birlikte çöktürme yöntemleri kullanılarak kompleksleri hazırlanmıştır. Komplekslerin pH 1.2 ortamında çözünürlük tayinleri yapıldığında 4.5 µg/ml olan itrakonazolün çözünürlüğünün, en yüksek örme yöntemiyle ve 1:2 molar oranda IT:CD ile hazırlanan komplekslerde elde edildiği ve HPβCD kullanıldığında 12.39 µg/ml, RAMEB kullanıldığında 14.05 µg/ml olduğu saptanmıştır. Çözünürlük değerleri ve oluşan kompleksin stabilitesini gösteren stabilite sabitlerinden yola çıkarak (HPβCD için Kc= 3 M-1, RAMEB için Kc= 75 M-1) formülasyon çalışmalarına RAMEB kullanarak hazırlanan komplekslerle devam edilmesi kararlaştırılmıştır. Suda çözünen polimerlerin çözünürlük artırıcı etkileri bilindiğinden RAMEB'e % 0.5 polietilen glikol 4000 (PEG 4000) ilavesiyle kompleksler hazırlanmış ve çözünürlüğün 28.72 µg/ml'e yükseldiği saptanmıştır. Kompleks oluşumunu ve oluşan komplekslerin özelliklerini belirlemek üzere DSC termogramları alınmıştır.

Anahtar Kelimeler: *İtrakonazol, β siklodekstrin türevleri, fiziksel karışım, örme yöntemi, birlikte çöktürme yöntemi, DSC*

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INTRODUCTION

Itraconazole (IT) is a broad spectrum triazole antifungal agent. The drug is primarily fungistatic at clinically achievable serum concentrations and acts by impairing the synthesis of ergosterol, an essential component of the fungal cell membrane (Koks et al., 2002). It is absorbed from the stomach and the upper part of the small intestine. Due to its low solubility and high permeability, itraconazole is classified as a Class II pharmaceutical compound in Biopharmaceutical Classification System (BCS) (Barrett et al., 2008). In this study, it was intended to prepare the inclusion complexes of IT with cyclodextrins in order to improve the solubility and bioavailability.

Cyclodextrins are molecules with a polar hydrophilic outside, and an apolar hydrophobic cavity, which provides a guest-host relation to hydrophobic drugs in hydophilic media which is called "inclusion complex" (Del Valle, 2004; Carrier et al., 2007).

In this complexes, the drug (guest molecule) is entrapped in the cavity of the cyclodextrin (CD) (host). After the complexation, some physical properties of the guest molecule can be changed. For instance, the strength against oxidation, hydrolysis and photochemical reactions can increase, the evaporation speed of volatile substances can significantly decrease. Also when poor soluble drugs are in complex form, the solubility increases. For this reason, in order to enhance the water solubility of poor soluble substances, complex forming with CD's are widely used (Kurkov & Loftsson, 2013; Salustio et al., 2011).

The inclusion complexes can be prepared by several methods such as, coprecipitation method, neutralization method, lyophilization method, kneading method etc. (Hirayama & Uekama, 1987).

In this study, the complexes were prepared by using phyical mixture, coprecipitation and kneading method.

MATERIALS AND METHODS

Materials

Itraconazole (IT), β cyclodexrin (β CD) and randomized methylated β cyclodextrin (RAMEB) were gift from Nobel Pharmaceuticals. Hydroxypropyl β cyclodexrin (HPβCD) was supplied from Cyclolab and polyethylene glycol 4000 (PEG 4000) was supplied from Merck.

Apparatus

For the experiments, ultraviolet (UV) spectrophotometer (Shimadzu, UV mini 1240), differential scanning calorimeter (DSC) (TA Instruments DSC Q100) were used.

Phase Solubility Studies

To determine the phase solubility diagrams of IT with CD's, solutions containing various concentrations of βCD, HPβCD ve RAMEB ranging from $0.57x10^{-3}$-$200x10^{-3}$ M in pH 1.2 buffer solution were prepared. An excess amount of IT was added to these closed flask containing 10 mL of the solutions and mixed in 37°C shaking water bath at 50 rpm (Memmert WB 22). The liquid phase was filtered through 0.45 μm filters and the UV absorbance was measured. The stability constant of the soluble complex was calculated according to the following equation:

$$K_c = \frac{S_t - S_0}{S_0 (L_t - S_t + S_0)}$$

where S_t is the total concentration of dissolved IT, S_0 is the equilibrium solubility of IT in the presence of βCD, HPβCD or RAMEB, and L_t is the total concentration of CD used (Higuchi & Connors, 1965).

The Preparation of Complexes

The complexes of IT with HPβCD and RAMEB were prepared by using the following three different methods.

Physical mixture

The pysical mixture was prepared by a simple dry mixing of IT:HPβCD and IT:RAMEB in 1:1 and 1:2 molar ratios in a mortar for 10 minutes.

Coprecipitation Method

To prepare the complexes of IT:HPβCD and IT:RAMEB in 1:1 and 1:2 molar ratios by coprecipitation method, the solution of IT in chloroform was added to the aqueous solution of CD's, stirred both in ultasonic bath (Bandelin Sanorex RK 510H) for 20 minutes and in magnetic stirrer (Heidolph) for 24 hours and then filtered through 0.45 μm filters. The solvent was allowed to evaporate using rotavapor (Büchi R200) and then dried under room temperature (Özdemir & Erkin, 2012).

Kneading Method

To prepare the complexes of IT:HPβCD and IT:RAMEB in 1:1 and 1:2 molar ratios by kneading method, the calculated amount of IT is added to CD with one drop of water and mixed for 30 minutes until a creamy homogenous product was obtained. Then it was dried for 24 hours under room temprature. In this study, to investigate the effect of PEG's to solubility, 0.5 % PEG 4000 was added to IT and RAMEB mixture of

1:2 molar ratio and same process was applied before preparing the complexes (Loftsson & Fridriksdottir, 1998).

Chacterization of Complexes

Solubility Studies

Solubility of active material in complexes was studied at pH 1.2. To this aim, an excess amount of IT was added to a closed flask containing 20 mL buffer solution and mixed in 37°C water bath (Memmert WB 22). The liquid phase was filtered through 0.45 µm filters and the UV absorbance was measured. By calculating the concentrations in the equilibrium status, the solubility of IT in each complex was determined. All the solubility studies were performed in triplicate.

DSC Analyses

To this aim, 5 mg of 1:2 molar ratio of both physical mixture and complexes using kneading method of IT:RAMEB were taken in sealed aluminium pans and the DSC termograms were obtained with 50 ml/min nitrogen gas flow rate, at a constant speed of 10°C/min, between 25-300°C.

RESULTS AND DISCUSSION

Inclusion complexes were prepared using different types of cyclodextrins (HPβCD and RAMEB) to improve the solubility of the active substance. To determine the molar ratios of the substances in the complexes, phase solubility studies were made. With βCD, B_S type, for HPβCD and RAMEB, A_L type solubility diagrams were obtained respectively. A_L type curves indicate the formation of soluble complexes while B_S type suggests the formation of inclusion complexes with poor solubility (Szejtli, 1998).

The phase solubility diagrams of complexes by using active substance and βCD, HPβCD, RAMEB are shown in Figure 1, 2 and 3.

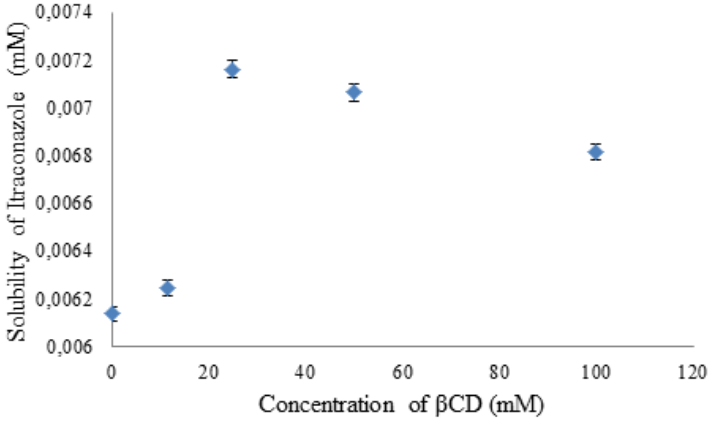

Figure 1. The phase solubility diagram of Itraconazole and βCD

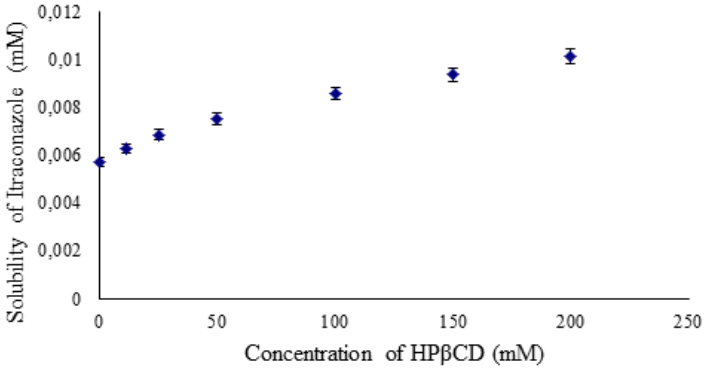

Figure 2. The phase solubility diagram of Itraconazole and HPβCD

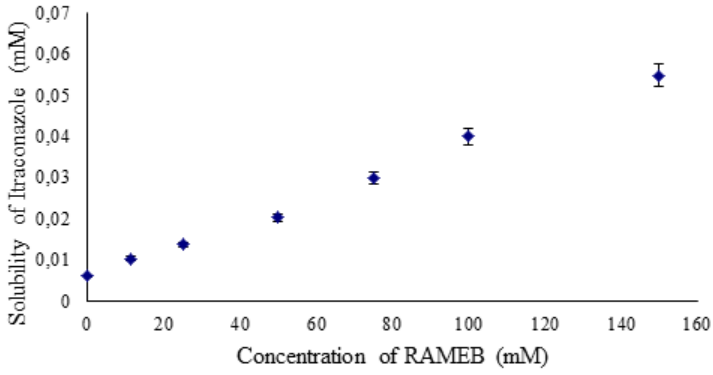

Figure 3. The phase solubility diagram of Itraconazole and RAMEB

As shown in Figure 1, with βCD, B_S type of solubility curve was obtained with a very small value of K_c which is 1.0 M^{-1}. A_L type of solubility curve was obtained with HPβCD and RAMEB, as shown in Figure 2 and 3. No significant increase in solubility occured and the stability constant of the complex was very small (K_c= 3.3 M^{-1}) with HPβCD. The stochiometric ratio of complex determined from the descending part of the diagram was found to be 1:2 (IT: RAMEB) and stability constant K_c was calculated as 75 M^{-1} .

The inclusion complexes of IT with HPβCD and RAMEB were prepared with the molar ratio of 1:1 and 1:2 (Lee et al., 2008). The solubility of active material from these complexes prepared by physical mixture, kneading and coprecipitation method are given in Table 1.

Analytic validation studies of IT were done in pH 1.2. The slope, interception and correlation coefficient values of the calibration equation were 26.62±0.0098, -0.0052 ±0.0028 and 0.9996 respectively. For the accuracy test, three different concentrations (12.5 µg/mL, 25 µg/mL, 32.5µg/mL) of IT were analyzed and recovery % values were determined as 99.02±0.75, 99.51±0.64 ve 99.62±0.62, respectively. LOD and LOQ values were 0.359 µg/mL ve 1.089 µg/mL, respectively.

Table 1. The solubility of IT from the complexes in pH 1.2 buffer solution (n=3)

	(Solubility of pure IT in pH 1.2 = **4.5 ± 0.3 µg/ml**)				
	Solubility (µg/ml) **Mean ± SD**				
Method	**IT:HP**βCD (1:1)	**IT:HP**βCD (1:2)	**IT:RAMEB (1:1)**	**IT:RAMEB (1:2)**	**IT:RAMEB +PEG4000 (1:2)**
Kneading	10.08±0.12	12.39±0.10	12.44±0.08	14.05±0.10	28.72±0.03
Coprecipitation	7.17±0.14	8.44±0.13	7.64±0.14	13.14±0.09	-----
Physical Mixture	6.12±0.16	7.03±0.15	7.26±0.15	9.13±0.12	-----

It was determined that, in pH 1.2, the solubility of IT increased by using kneading method with the molar ratio of 1:2 (from 4.5 µg/ml to 12.39 µg/ml with HPβCD and to 14.05 µg/ml with RAMEB respectively). However high stability constants of the complexes indicates high stability, very high values complicates the solubility of the drug. Compairing the solubility values and the stability constants, (for HPβCD, K_c= 3 M^{-1}, for RAMEB, K_c= 75 M^{-1}), it was decided to use RAMEB in the complexes in further studies. As the water soluble polymers are known as solubility enhancers, PEG 4000 with the concentration of 0.5 % was added to RAMEB complexes. The solubility of IT increased to 28.72 µg/ml from 14.05 µg/ml (Table 1.) (Miyake et al., 1999; Özdemir & Ordu, 1997).

Evidence of inclusion formation between IT and RAMEB, was provided by the analysis of the results obtained from DSC. DSC termograms of IT, RAMEB, PEG 4000 and the complexes are shown in Figure 4.

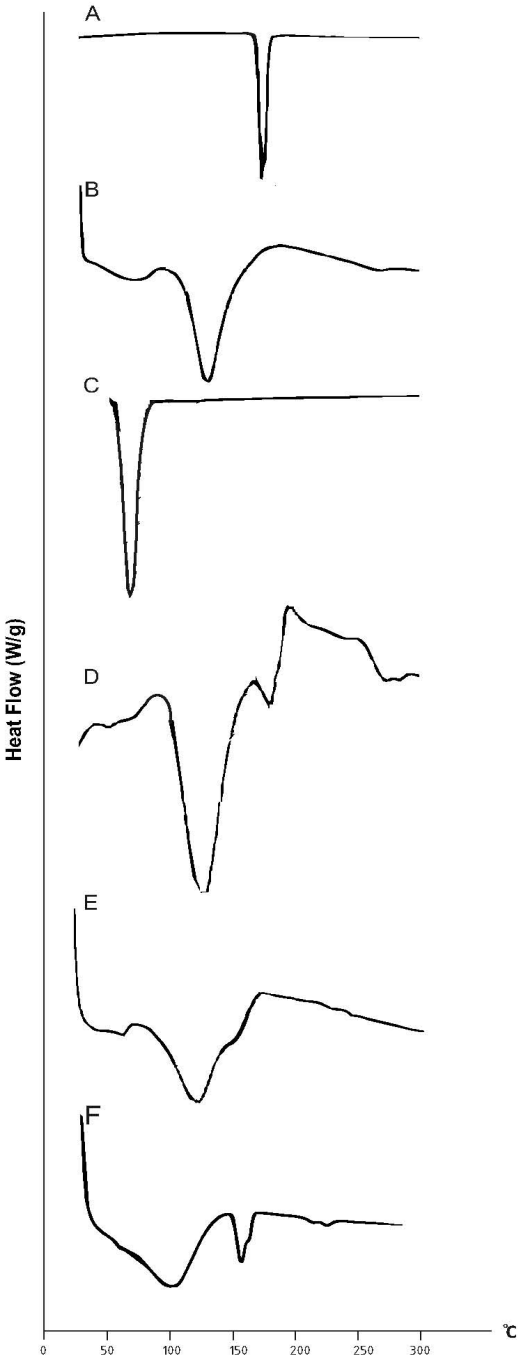

Figure 4. The DSC thermograms of (A) Itraconazole, (B) RAMEB, (C) PEG 4000, (D) 1:2 molar ratio of the physical mixture of IT:RAMEB, (E) 1:2 molar ratio of IT:RAMEB complex by kneading method, (F) 1:2 molar ratio of IT:RAMEB-PEG 4000 complex by kneading method.

Figure 4 shows the thermograms of IT, RAMEB and PEG 4000 alone and the complexes prepared in 1:2 molar ratio obtained from DSC measurements. In the termograms of the complexes, it can be seen that the violence of endothermic peaks of IT at 169ºC, RAMEB at 128ºC and PEG 4000 at 66ºC decreases, gets smoother and slides. These results can be considered as a strong indication for the formation of inclusion of the drug into the CD cavity.

CONCLUSION

In this study, to enhance the solubility of IT which is limited in the gastrointestinal area, inclusion complexes were developed. Thus, an improved absorbtion and bioavailability of IT was intended.

As a result of this study it may be concluded that the solubility of IT was significally enhanced by the complex formation with RAMEB. Furthermore the stable complexes were obtained. Preparation of inclusion complexes in the presence of polymers such as PEG 4000 increased the solubilizing effect of RAMEB. Ideal IT-RAMEB complexes were used to prepare floating dosage forms in order to increase *in vivo* bioavailability.

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