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A PHARMACEUTICAL STUDY OF VARIOUS ADDITIVES ON ITRACONAZOLE AS SOLUBILIZED SYSTEMS FOR OCULAR DELIVERY

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ABSTRACT

Itraconazole, a triazole antifungal agent, present significant challenges to the formulator for providing a suitable effective system for ocular delivery. The effect of different cosolvents and/or surfactants on the solubility of itraconazole shows various effects on the enhancement of itraconazole solubility. PEG 400 has the greatest solubilizing power for itraconazole and the micellar solubilization show that the aqueous solubility of itraconazole increased in a linear relationship with the concentrations of Cremophor® RH 40 shows the utmost micellar solubilization comparing with others. The effect of PEG 400-Cremophor® RH 40 combined system shows insignificant effect on improving itraconazole solubility. Phase-solubility techniques were used to assess the effect of cosolvent and/or surfactant on itraconazole complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). The data suggested Ap-type solubility relationships, indicating higher order complexation at higher HP-β-CD concentrations. The itraconazole-HP-β-CD complex was formed even in the presence of 10% w/v PEG 400 and/or 5% w/v Cremophor® RH 40. Although the cosolvent-surfactant combined system made the interaction of itraconazole with HP-β-CD weaker due to the competitive inclusion. The 10% w/v PEG 400-5% w/v Cremophor® RH 40 decrease the inclusion strength of HP-β-CD that upon dilution gives a larger free fraction of drug which is favorable for ocular absorption. The combination of using appropriate cosolvent and/or surfactant with the drug will be particularly useful for design of the cyclodextin-based pharmaceutical ocular formulations.

KEYWORDS: Itraconazole; Cosolvent; Surfactant; Hydroxypropyl-β-cyclodextrin; Solubilization.

1. INTRODUCTION

Ocular fungal infections, or ophthalmic mycoses, are being increasingly recognized as an important cause of morbidity and blindness; certain types of ophthalmic mycoses may even be life-threatening.[1] Fungal infections of the cornea (mycotic or fungal keratitis, keratomycosis) present as suppurative, usually ulcerative, lesions.[2] Keratitis (corneal infection) is the most frequent presentation, but the orbit, lids, lacrimal apparatus, conjunctiva, sclera, and intraocular structures may also be involved. Such a corneal infection poses a challenge to the ophthalmologist because of its tendency to mimic other types of stromal inflammation, and because its management is restricted by the availability of effective antifungal agents and the extent to which they can penetrate into corneal tissue. [3]

Itraconazole is a useful broad spectrum triazole antifungal agent that inhibits most human fungal pathogens. Itraconazole is the first marketed orally bioavailable antifungal agent to be useful in both the treatment of *Candida sp*. and *Aspergillus sp*., the two most commonly occurring fungal pathogens.[4] Physicochemically, itraconazole present significant challenges to the formulator. The compound can be characterized as a very poorly water soluble, weak base with an aqueous solubility estimated at approximately 1 ng/ml at neutral pH and ~6 µg/ml at pH 1. The pKa was determined to be 4 with other relevant data including the calculated log P was 6.2. Itraconazole is practically insoluble in ocular humor at physiological pH conditions and soluble only under extremely acidic media, leading to a poor ocular bioavailability with large individual variations. This information as well as permeability meaning that dissolution rate or solubility improvement will optimize the dosage form for ocular delivery. [5-7]

A number of formulation approaches have been assessed in order to improve the pharmaceutical performance of itraconazole including the use of cosolvent and surfactant. [8,9] To improve the formulation, especially for use in systemic fungal infections, two aqueous formulations for itraconazole (an oral solution and i.v. product) were developed through the use of cyclodextrin complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) chosen as the functional excipient. The selected cyclodextrin, HP-β-CD, is a safe and well-tolerated material and effects drug solubilization through the formation of dynamic (non-covalent) complex formation.[10]

The purpose of the present study was to investigate the effect of various cosolvents and/or surfactants on the solubility of itraconazole. In addition, we deal with the inclusion complexation of itraconazole with HP-β-CD mainly in the presence of cosolvent and/or surfactant solution, in order to confirm the complexation in the solubilized system to gain insight into the solubilization behaviour of the drug.

2. MATERIALS AND METHODS

2.1. Materials

Itrachonazole was purchased from Medicorp Technologies India Ltd. (Telangana, India). Tween® 80 (polysorbate 80) and Tween® 20 (polysorbate 20) were purchased from ICI Americas (Wilmington, DE, USA). Pluronic® F-127 (Poloxamer 188), Pluronic® F-68 (Poloxamer 407) and Cremophor® RH 40 were purchased from BASF SE group (Limburgerhof, Germany). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) was supplied from Wacker Chemie AG (München, Germany). Polyethylene glycol 400 (PEG 400) and propylene glycol were purchased from Acino Pharma AG (Liesberg, Switzerland). All other chemicals were of analytical grade and commercially available.

2.2. Solubility studies of itraconazole

2.2.1. Effect of cosolvents

The excess amount (20 mg) of itraconazole was added in the screw capped vials containing 5 ml of glycerol, propylene glycol and PEG 400 at various concentrations (5, 10, 25, 50 and 75% w/v). The vials were shaken at 150 strokes/minutes in a thermostated shaking water bath (GFL GmbH, Burgwedel, Germany) at 25 ± 0.5 °C. After equilibrium was attained (approx. 3 days) the saturated solutions were filtered through 0.45µm Millipore membrane filter (Millipore corporation, Bedford, USA). The first 3 ml of the filtrate was discarded to saturate the adsorption sites for the drug on the filter. Two milliliters of the filtrate of each sample were diluted with deionized water/acetonitrile/DMSO (50:40:10 v/v) solution and analyzed spectrophotometrically at λmax 262 nm by using UV-visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.2.2. Effect of surfactants

The excess amount (20 mg) of itraconazole was added in the screw capped vials containing 5 ml of Tween® 80, Tween® 20, Pluronic® F-127, Pluronic® F-68 and Cremophor® RH 40 at different concentrations (0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0% w/v). The vials were

shaken at 150 strokes/minutes in a thermostated shaking water bath at 25 ± 0.5 °C. After equilibrium was attained (approx. 3 days) the saturated solutions were filtered through 0.45µm Millipore membrane filter. Two milliliters of the filtrate of each sample were diluted and analyzed spectrophotometrically at λmax 262 nm, as previously described.

2.2.3. Effect of cosolvent-surfactant combined system

The excess amount (20 mg) of itraconazole was added in the screw capped vials containing 5 ml of solvent-surfactant combined system at different concentrations. Tween® 80, Tween® 20, Pluronic® F-127 and Cremophor® RH 40 were used in 0.5, 1.0, 2.0 and 5.0% w/v, while glycerol, propylene glycol and PEG 400 were used in 5, 10 and 20% w/v. The vials were shaken at 150 strokes/minutes in a thermostated shaking water bath at 25 ± 0.5 °C. After equilibrium was attained (approx. 3 days) the saturated solutions were filtered, diluted and analyzed spectrophotometrically at λmax 262 nm, as previously described.

2.3. Investigation of potentiality of complex formation between itraconazole and HP-β-CD

The solubility method was employed according to the method of Higuchi and Connors.[11] Accurately weighed sample of itraconazole in quantities exceeding its aqueous solubility were shaken at room temperature (25 ± 0.5 °C) with aqueous solutions of HP-β-CDs in increasing concentrations (10-130 mM), for a period of 3 days until equilibrium was established. Aliquots volume of the solutions was withdrawn, filtered through 0.45µm Millipore filter, diluted and analyzed spectrophotometrically at the maximum absorbance for itraconazole (262 nm). The apparent stability constant was calculated from the phase-solubility diagram, using the following equation.

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept}\,(1-\text{Slope})} \qquad (1)$$

2.4. Elucidation of the stoichiometric ratio of itraconazole-HP-β-CD complex

The relationship between HP-β-CD and itraconazole was analyzed using the phase-solubility approach described by Higuchi and Connors[11]. In this approach, the total concentration of cyclodextrin is the sum of the free cyclodextrin concentration plus all cyclodextrin associated with drug complexes such that for 1:1, 1:2, and 1:3 complexes, $[CD]_t$ is, respectively,

$$[CD]_t = [CD] + S_oK_{1:1}[CD] \qquad (2)$$

$$[CD]_t = [CD] + S_oK_{1:1}[CD] + 2S_oK_{1:1}K_{1:2}[CD]^2 \qquad (3)$$

$$[CD]_t = [CD] + S_oK_{1:1}[CD] + 2S_oK_{1:1}K_{1:2}[CD]^2 ..... + 3S_oK_{1:1}K_{1:2}K_{1:3}[CD]^3 \qquad (4)$$

The total drug concentration for the three respective drug complexes is given by the following three expressions.

$$S_t = S_o(1 + K_{1:1}[CD]) \quad (5)$$

$$S_t = S_o(1 + K_{1:1}[CD] + K_{1:1}K_{1:2}[CD]^2) \quad (6)$$

$$S_t = S_o(1 + K_{1:1}[CD] + K_{1:1}K_{1:2}[CD]^2 + K_{1:1}K_{1:2}K_{1:3}[CD]^3 \quad (7)$$

Where $K_{1:1}$, $K_{1:2}$, and $K_{1:3}$ are the stability constants associated with drug–cyclodextrin complex, the drug–2(cyclodextrin), and drug– 3(cyclodextrin) species, and S_o is the solubility of the drug in the absence of cyclodextrin. The calculation of the free [CD] concentration is straightforward for 1:1 complexes and can be derived from the quadratic relationship developed by Higuchi and Kristiansen for 1:2 complexes.[12] For 1:3 and higher order systems, a simplex optimization procedure was applied.[13] In the approach used, trial values of the stability constants (Ks) are first obtained by numerically fitting a third-order polynomial using the total HP-β-CD concentration as the independent variable. The rough estimates are then used to calculate the solubility of itraconazole as a function of the HP-β-CD concentration as well as the concentration of free cyclodextrin at each solubility point using an exact solution to the equation.

2.5. Effect of cosolvent and/or surfactant on the itraconazole-HP-β-CD complex

An excess of itraconazole in various concentrations of aqueous HP-β-CD ranging from 10 to 130 mM were prepared by sonication for 10 min in the presence of 10% v/v PEG 400 and/or 5% w/v Cremophor® RH 40 in the solution. The systems were shaken at 150 strokes/minutes in a thermostated shaking water bath at 25 ± 0.5 °C for 3 days. After equilibrium was attained the solutions were filtered, diluted and analyzed spectrophotometrically at λmax 262 nm, as previously described.

2.6. Statistical analysis

All the data represent the mean ± SEM. The differences were considered to be significant at a level of $p < 0.05$, using paired T test.

3. RESULTS AND DISCUSSION

3.1. Solubility studies of itraconazole

3.1.1 Effect of cosolvents

Cosolvent addition is a highly efficient technique for enhancement of solubility of poorly water-soluble drugs.[14-16] Cosolvents increase the aqueous solubility of poorly water-soluble

drugs by disrupting the intermolecular hydrogen bonding networks that are present in aqueous systems. This leads to a decrease in the polarity of the solvent and the creation of an environment with physicochemical properties that are more similar to that of the drug.[17]

Table 1 shows the solubility of itraconazole in the presence of glycerol, propylene glycol and PEG 400. An exponential increase in drug aqueous solubility at 25 °C with increase in cosolvent concentration up to 25% w/v. At 50% w/v of cosolvents resulted in an increase in itraconazole solubility 120-times, 910-times and 1885-times higher for glycerol, propylene glycol and PEG 400, respectively. The increment of itraconazole solubility at 75% w/v of cosolvent was 2496-times, 34072-times and 81500-times higher for glycerol, propylene glycol and PEG 400, respectively.

Table 1: Effect of cosolvents on the solubility of itraconazole

Cosolvent Concentration % (w/v)	Solubility of itraconazole (mg/L)		
	Glycerol	Propylene glycol	PEG 400
5	ND	ND	ND
10	ND	ND	ND
25	ND	0.14±0.01	0.21±0.06
50	0.08±0.00	0.64±0.02	1.33±0.31
75	0.17±0.00	24.02±1.07	57.51±1.78

Each data represents the mean ± SEM (n = 3).

ND: Not Determined.

Unlike PEG 400, glycerol showed very little increase in solubility. The difference in solubilizing power of glycerol and PEG 400 is due to the fact that PEG 400 is less polar than glycerol, making it possible for hydrogen bonding interactions in water molecules to be effectively disrupted. This in turn determines the ability of PEG 400-water system to squeeze out itraconazole molecules.[18] In addition, the higher solubility of itraconazole in PEG 400 more than propylene glycol may be due to the ability of itraconazole to form a hydrogen bond with polyoxyethylene groups in PEG 400.[19]

Equation (8) describes the relationship between the solubility of a solute in a binary water-cosolvent system (S_{mix}), where S_w is the molar solubility of the solute in water, f_c is the volume fraction of the cosolvent, and (σ) is the solubilization power of the cosolvent.

$$\text{Log } S_{mix} = \log S_w + \sigma f_c \qquad (8)$$

The result of log-linear solubility relationship of itrarconazole in cosolvents-water mixtures shows that rank order in solubilization power is PEG 400 > propylene glycol > glycerol, which reflect the higher solubility of itraconazole in PEG 400-water mixture.[14]

3.1.2. Effect of surfactants

The solubility of poorly water-soluble drugs can likewise be raised by the surfactants. The enhancement in solubility is mainly due to encapsulation of hydrophobic solute by the micelles formed by the surfactants. A number of nonionic surfactants are common in drug delivery owing to their better safety, superior capacity to solubilize nonpolar solutes at lower concentrations, and good compatibility with other pharmaceutical excipients.[20]

The effect of cosolvents PEG 400, propylene glycol and glycerol shows that PEG 400 has the highest solubilizing power for itraconazole. However, the addition of cosolvents generally did not contribute much to improving the solubility of itraconazole except in high concentration of cosolvent (which are not suitable due to safety concerns).

Solubilization of a drug by a surfactant is given by the following equation.

$$S_t = S_o + k\,(C_{Surfactant} - CMC) \qquad (9)$$

Where S_t is the total concentration of solubilized drug, S_o is the solubility of the drug in the absence of the surfactant, $C_{Surfactant}$ is the concentration of surfactant, CMC is the critical micellar concentration and k is defined as.

$$k = S_{Micelle} / C_{Micelle}$$

Where $S_{Micelle}$ is the drug concentration within the micelle and $C_{Micelle}$ is the molar concentration of surfactant and k is the molar solubilization capacity.

If the CMC is significantly lower than the concentration of surfactant used, the solubility relationship simplifies to.[21]

$$S_t = S_o + k\,C_{Surfactant} \qquad (10)$$

Table 2 and Fig. 1 show the effect of surfactants on the solubility of itraconazole. A linear relationship was observed when surfactant concentration (Cremophor® RH 40: r^2=0.9826; Tween® 20: r^2=0.9891; Tween® 80: r^2=0.9602; Pluronic® F-127: r^2=0.9972) was plotted vs. itraconazole solubility.

Table 2: Effect of surfactants on the solubility of itraconazole

Surfactant Concentration % (w/v)	Solubility of itraconazole (mg/L)				
	Cremophor® RH 40	Tween® 20	Tween® 80	Pluronic® F-127	Pluronic® F-68
0.05	0.40±0.06	0.20±0.08	0.32±0.05	ND	ND
0.1	0.60±0.04	0.30±0.11	0.45±0.11	ND	ND
0.5	1.10±0.12	0.50±0.18	0.91±0.13	ND	ND
1.0	1.70±0.15	1.20±0.17	1.50±0.15	0.27±0.02	ND
1.5	2.73±0.23	2.00±0.24	2.01±0.38	0.36±0.03	ND
2.0	3.32±0.34	2.80±0.14	3.10±0.25	0.44±0.03	ND
2.5	4.46±0.21	3.30±0.27	4.20±0.36	0.56±0.04	ND
3.0	6.00±0.18	4.20±0.13	5.30±0.33	0.70±0.04	ND
5.0	9.84±.15	7.81±0.17	8.88±.18	1.16±0.06	ND

Each data represents the mean ± SEM (n = 3).

ND: Not Determined.

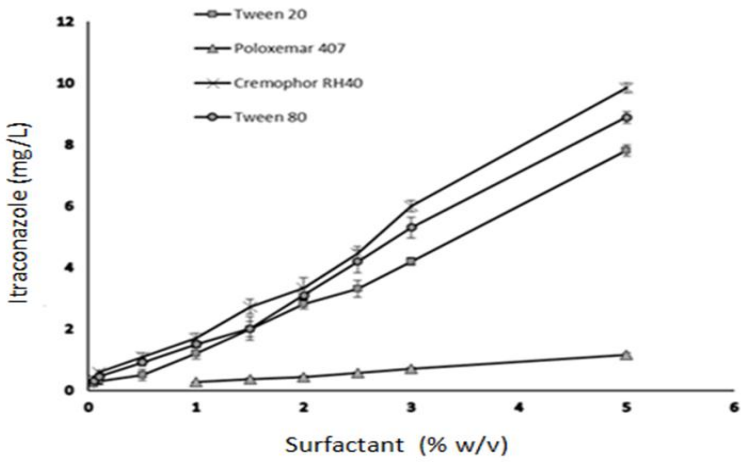

Fig. 1. Solubility of itraconazole in various concentration of surfactants. Each data point represents mean ± SEM (n=3).

The molar solubilization capacity (k value) of used surfactant in micellar solubilization of itraconazole was 8×10^{-4} for Cremophor® RH 40, 3×10^{-4} for Tween® 20, 4×10^{-4} for Tween® 80, and 4×10^{-4} for Pluronic® F-127. The rank order of solubility improvement at 5 % w/v of surfactant is as follows, Cremophor® RH 40 (9.846 mg/L) > Tween® 80 (8.884mg/L) > Tween® 20 (7.813 mg/L 11082) > Pluronic® F-127 (1.164 mg/L) > Pluronic® F-68 (not determined).

The CMC of Cremophor® RH 40, Tween® 80, Tween® 20, and Pluronic® F-127 was 0.02% w/v, 0.0013% w/v, 0.0073 % w/v, and 0.0013% w/v, respectively. As well the surfactant

concentrations used in this study were all well above the CMC. Cremophor® RH 40 shows the best micellar solubilization comparing with Tween® 80, Tween® 20, Pluronic® F-127 and Pluronic® F-68. Pluronic® F-68 has a non-applicable effect on itraconazole solubility in the concentrations used.

In class of polyoxyl castor oil, Cremophor® RH 40 HLB value between 14 to 16, the main composition of which is polyoxyethylene hydrogenated castor oil was found to improve solubility to the greatest extent. Whereas, Pluronic® F-127 gives lowest solubility improvement that was expected since it has got higher (HLB value 22) and very short hydrophobic portion. From the class of polysorbate, Tween® 80 (HLB value 15) with longer hydrocarbon chain length, was found to be more efficient solubilizer than Tween® 20 (HLB value 16.7), having a shorter hydrocarbon chain length.

The solubilizing power of polysorbates was directly related to the alkyl chain lengths, with an increase in the chain length corresponding to an increase in the solubilizing capacity. Tween® 80 with a long hydrocarbon chain was shown to be more effective as a solubilizer than Tween® 20.[22] The solubilization effects of micelle forming agents increased as the hydrophobic chain length of the surfactant is increased. This is due to an increase in the volume of the hydrocarbon in the micelle interior. In addition, the increase in the alkyl chain length increases the size of the surfactant micelle which accommodates more quantity of nonpolar solute and hence increases its solubilization effect.

Pluronic® F-127 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol. Addition of Pluronic® F-127 in pharmaceutical formulations leads to enhance the solubilization of poorly water-soluble drugs.[23,24] In our study, Pluronic® F-127 with longer PPO has greater influence on drug solubility comparing with Pluronic® F-68 with shorter PPO which has no significant effect on drug solubility up to 5 % w/v. The solubilization of hydrophobic drugs in aqueous solutions of block copolymers is related to the formation of micelles stabilized by a corona of PEO-blocks with the hydrophobic cores being the major site of a solubilized hydrophobic drug.

The CMC values of Pluronics were influenced by the hydrophobic PPO chain length, not by molecular weight or HLB value of the polymer.[25] Previous studies showed that Pluronics PEO-blocks making only a minor contribution to the solubilization of hydrophobic drugs. In

addition, the ability of solubilization increases with increasing the length of the PPO hydrophobic chain of the Pluronics which is approved with our results.[26]

3.1.3. Effect of cosolvent-surfactant combined system

Cosolvency aims to reduce the polarity of an aqueous medium to improve the solubility of a hydrophobic drug. Cosolvents may also be used in combination with surfactants which impact not only the solution properties of the drug, but also the efficiency of micellar solubilization.[27] Many attempts have been made where the solubilization capacity of the cosolvents is not sufficient in many cases by combination with other solubilization techniques, such as pH adjustment, surfactant, and cyclodextrin.[28-30]

Understanding the combination of cosolvents with surfactants is complicated, because the characteristics of the carrier, i.e. micelles, are significantly affected by the addition of cosolvent.[29,31] In addition, cosolvents significantly change the solution conditions, such as dielectric constants and polarity, which affect the interaction between surfactant molecules.[32,33] In general, the CMC values increase with increasing solvent polarity and decrease with increasing dielectric constant. Increasing the solubilizing powers of a surfactant has been termed a synergistic effect and decreasing them an antigistic effect.

Despite the negative effect of cosolvents on micellar solubilization increases the drug solubility in bulk solution. As such, the net impact on drug solubilization is difficult to predict a priori and reflects the relative efficiencies of cosolvency versus micellar solubilization on drug solubility.[27,29,31] Thus, the cosolvent-surfactant combined system may decrease in micellar drug solubilization, particularly when the drug shows high affinity for the surfactant core.[34] However, the cosolvent-surfactant combinations on total solubilization has significantly reduced the potential risk of drug precipitation on dilution of cosolvent formulations.[35,36]

Table 3 shows the combined effect Tween® 80 with the various cosolvents on the itraconazole solubility. The results show a decrease in the drug solubility capacity of Tween® 80 with increasing the percentage of cosolvent in aqueous mixture. The concentration of itraconazole decreases to 6.274 mg/L at 20% w/v PEG 400 combined with 5% w/v of Tween® 80 while the solubility of itraconazole in 5% Tween® 80 alone was 8.884 mg/L.

Table 3: Effect of various cosolvents-Tween® 80 combination on the solubility of itraconazole.

Tween® 80 Concentration % (w/v)	Solubility of itraconazole (mg/L)								
	Glycerol			Propylene glycol			PEG 400		
	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)
0.5	0.75±0.01	0.65±0.03	0.60±0.07	0.77±0.08	0.68±0.04	0.64±0.04	0.86±0.06	0.89±0.04	0.83±0.04
1.0	0.97±0.07	0.92±0.12	0.87±0.17	1.11±0.16	0.96±0.05	0.90±0.04	1.42±0.06	1.39±0.21	1.33±0.16
2.0	2.24±0.27	1.70±0.16	1.85±0.12	2.41±0.10	1.73±0.08	1.58±0.13	2.88±0.15	2.82±0.12	2.64±0.11
5.0	5.74±0.39	4.39±0.29	3.09±0.30	6.21±0.43	4.44±0.23	3.11±0.14	7.99±0.44	7.55±0.43	6.27±0.43

Each data represents the mean ± SEM (n = 3).

It is appeared that the decrease in solubility on the addition of PEG 400 may be explained in terms of a change in the micelle characteristics.[37] Cosolvents enhance the solvation of Tween® 80 by reducing the polarity of the solution, giving rise to increases in CMC and decreases in micelle size. This effect of PEG 400 may be due to its hydrophobicity and its increased ability to alter the polarity of the solvent. The change in the dielectric constant affects the interaction between surfactant molecules, resulting in the change in the micelle morphology such as its shape and aggregation number.[31] The hydration state of the head group of the surfactant may be affected by replacing bonded water with another solvent. In the cases of PEG, (penetration of cosolvent, change in the packing characteristics) can be regarded as possible explanations for the change in the polarity inside the micelles. Therefore, addition of PEG 400 increase the polarity inside the micelles, consequently, the change in the polarity inside micelles may enhance the penetration of cosolvents into micelles and a change in the packing characteristics of the micelles that affects the penetration of drugs into the micelle core.[37]

Propylene glycol-Tween® 80 combined systems as shown in Table 3, it is obviously appears at 20% w/v propylene glycol water mixture in the presence of 5% w/v Tween® 80 the solubility of itraconazole decreases to 3.115 mg/L comparing with a solubility of drug in 5% w/v Tween® 80 alone (8.884 mg/L). Propylene glycol reduces the polarity of the solution, enhances the solvation of surfactant, increases the CMC, and decreases in micelle size. This effect of propylene glycol may be due to its hydrophobicity and its increased ability to alter the polarity of the solvent.[18] The decrease in solubility of itraconazole may be explained by assuming that the small-sized nonpolar hydrocarbon portions of propylene glycol had more affinity for the interiors of micelles of the surfactant than the bulky nonpolar portions of Itraconazole.

In the case of glycerol-Tween® 80 combined systems the drug solubility capacity of Tween® 80 is decreased with increasing the glycerol concentration (Table 3). At 20% w/v glycerol water mixture in the presence of 5% w/v Tween® 80 the solubility of itraconazole decreases to 3.0978 mg/L comparing with solubility in 5% w/v Tween® 80 in the absence of glycerol. Our results matched with the previous study, which investigated the solubilization behavior of a poorly soluble model drug, phenytoin, under combined use of surfactants Tween® 80 and glycerol that reported an increase of CMC value of Tween® 80 in the presence of 10% glycerol.[37]

Table 4 shows the effect of cosolvents-Tween® 20 combined system on itraconazole solubility. The results show a decrease in the drug solubility capacity of Tween® 20 with increasing the concentration of cosolvent in aqueous mixture. The solubility of itraconazole in 5% w/v of Tween® 20 with 20 w/v PEG 400 was decreased to 5.45 mg/L, while the solubility of itraconazole in 5% w/v Tween® 20 was 7.813 mg/L.

Table 4: Effect of various cosolvents-Tween® 20 combination on the solubility of itraconazole.

Tween® 20 Concentration % (w/v)	Solubility of itraconazole (mg/L)								
	Glycerol			Propylene glycol			PEG 400		
	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)
0.5	0.17±0.02	0.14±0.01	0.12±0.00	0.18±0.01	0.15±0.02	0.14±0.02	0.19±0.02	0.17±0.02	0.15±0.02
1.0	0.98±0.03	0.84±0.05	0.72±0.04	1.05±0.04	0.88±0.03	0.84±0.03	1.10±0.03	0.96±0.04	0.87±0.05
2.0	2.27±0.39	2.04±0.33	1.66±0.09	2.32±0.12	1.98±0.09	1.88±0.47	2.57±0.08	2.32±0.10	2.12±0.13
5.0	6.09±0.30	5.46±0.20	4.79±0.16	6.25±0.27	4.68±0.23	4.05±0.18	7.03±0.18	6.40±0.29	5.45±0.37

Each data represents the mean ± SEM (n = 3).

This effect of PEG 400 may be due to its hydrophobicity and its increased ability to alter the polarity of the solvent.Tween® 20 and PEG 400 combination reduce the polarity of the solution, enhance the solvation of Tween® 20, giving rise to increases in CMC and decreases in micelle size. The micellization process of Tween® 20 is less favorable in the ethylene glycol -water mixture and worsens as the cosolvent content increases. This effect has been attributed to the structure-breaking ability of EG and the interaction of the cosolvent with the polyoxyethylene groups of the surfactant. In addition, the presence of the cosolvent produces a reduction in the micellar aggregation number and an increase in the whole micellar solvation.[38]

Along with propylene glycol the drug solubility capacity of Tween® 20 by increasing the percentage of cosolvent in aqueous mixture. The solubility of itraconazole decreases to 4.05 mg/L (about 48% decrease) in 20% w/v of propylene glycol-5% w/v Tween® 20 comparing with a solubility of itraconazole in 5% w/v Tween® 20 only (7.813 mg/L). Previous results reported that the effect of propylene glycol on the solubility of the hydrophobic drug model was decreased in propylene glycol-Tween mixture[18]. The reduction in the drug solubility could be explained by assuming that the small-sized nonpolar hydrocarbon portions of propylene glycol had more affinity for the interiors of micelles of the surfactant than the bulky nonpolar portions of itraconazole.

As well as the addition of glycerol with Tween® 20 has a negative effect on itraconazole solubility. The solubility of itraconazole was 7.813 mg/L in 5% w/v of Tween® 20 decreased up to 4.79 mg/L in addition of 20% of w/v of glycerol. D'Errico et al. have studied the effect of glycerol on the micellization of the ethoxylated nonionic surfactant who determined that the CMC significantly increased at high glycerol concentrations (20% w/v). Besides, glycerol induces a lowering of the aggregation number of nonionic surfactant that which may be interpreted in terms of a salting-out effect, according to which glycerol competes with the surfactant for water molecules, causing a dehydration of the surfactant ethoxylic headgroup.[39]

Consistent with these data, a wide range of pharmaceutical cosolvents (e.g., ethanol, propylene glycol, polyethylene glycol, DMA, and glycerol) have been shown to increase the CMC of aqueous solutions of polyoxyethylene surfactants[29,37,39] and the block copolymer surfactants.[40-42] Obviously, the solubilization capacity of micelles is much larger than that of the cosolvents. Therefore, a change in the micelle characteristics should have a greater impact on the solubility rather than the contribution of the cosolvents to the solubilization, notably when the solubilization capacity of the cosolvents is moderate. Thus, the addition of the cosolvents to the surfactant solutions generally offered only a small advantage from the viewpoint of improving solubility because of the decrease in the solubilization capacity of the micelles.

The effect of cosolvent-Pluronic® F-127 combined mixture on the itraconazole solubility is shown in Table 5. The results show an increase in the drug solubility capacity of Pluronic® F-127 with increasing the percentage of cosolvent in aqueous mixture. At 5% w/v Pluronic® F-

127 the solubility of itraconazole (1.164 mg/L) was increased approx. 2.2-times (2.565 mg/L) by the addition of 20 %w/v PEG 400.

Table 5: Effect of various cosolvents-Pluronic® F-127 combination on the solubility of itraconazole.

Pluronic® F-127 Concentration % (w/v)	Solubility of itraconazole (mg/L)								
	Glycerol			Propylene glycol			PEG 400		
	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)
0.5	0.20±0.01	0.29±0.01	0.35±0.02	ND	0.23±0.01	0.24±0.00	0.21±0.00	0.27±0.01	0.30±0.00
1.0	0.50±0.03	0.53±0.04	0.62±0.05	0.29±0.01	0.31±0.01	0.35±0.01	0.47±0.03	0.50±0.03	0.54±0.04
2.0	0.68±0.06	0.78±0.07	1.19±0.11	0.52±0.01	0.58±0.01	0.66±0.01	0.65±0.01	0.71±0.01	0.83±0.01
5.0	1.84±0.05	2.22±0.07	2.56±0.05	1.42±0.08	1.60±0.06	1.76±0.02	1.71±0.06	2.01±0.06	2.56±0.05

Each data represents the mean ± SEM (n = 3).

ND: Not Determined.

The solubility of hydrophobic drugs increase in aqueous solutions of Pluronic® F-127 on addition of different PEG to a value approaching double that of a solution of Pluronic® F-127 alone.[43] The synergetic effect of combination on drug solubility may be due to a consequence of the association of the EO-chains of PEG with the PEO-blocks of Pluronic® F-127 micelle corona thus providing an expanded region of reduced polarity for drug solubilization.

Our results show an increase in the drug solubility capacity of Pluronic® F-127 with increasing the percentage of cosolvent in aqueous mixture. The solubility of itraconazole in 5% w/v Pluronic® F-127 in water was 1.164 mg/L but this was increased to 1.767 mg/L (about 1.52 fold increase) by the addition of 20 %w/v PEG 400.

Propylene glycol is preferably located in the relatively apolar PPO rich domains and at the interface, and keep the micelle curvature constant. Although propylene glycol is a water-miscible polar solvents, it may have a preference to locate in the relatively apolar PPO rich domains and/or to participate together with the block copolymer in the formation of the interface between the PEO-rich and the PPO-rich domains. Therefore, the interfacial area per PEO-block, part of propylene glycol participates in the formation of the interface presumably by the swelling of both PEO and PPO blocks of the copolymer.[40]

Glycerol-Pluronic® F-127 mixture shows an increase in the drug solubility capacity of Pluronic® F-127 with increasing the percentage of cosolvent in aqueous mixtures (Table 5).

The solubility of itraconazole in 5% w/v Pluronic® F-127 in water was 1.164 mg/L but this was increased to 2.565 mg/L (about 2.2 fold increase) by the addition of 20 %w/v glycerol. Glycerol has been decreased the CMC of the block copolymer, increase the radius of the apolar (PPO-rich) micelle core and compact (i.e. dessolvate) the polar (PEOrich) micellar corona.[40] Cosolvent effects on the poloxamer liquid crystalline structure where the lattice spacing is invariant in the case of glycerol, but it decreases with increasing content of propylene glycol. Therefore, cosolvents, which have a smaller influence on the lattice spacing (here, glycerol and propylene glycol) can maintain the liquid crystalline microstructures up to higher solvent/(solvent+water) volume fraction ratios. Hence, the increase of the interfacial area with increasing the propylene glycol content is related to the swelling of both PEO and PPO blocks by the solvent. Thus, water-miscible polar solvents may act differently: glycerol is a cosolvent, while propylene glycol acts as cosurfactants. In this sequence, the relative polarity of cosolvent/surfactant increases and simultaneously their ability to promote structures with less positive curvature decreases.[40]

The Pluronic® F-127 (Poloxamer 407; poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer) has a molecular weight of 12,600 and 70 wt.% PEO content and can be represented as EO100- PO70-EO100 directions. The effects that the different cosolvents or surfactants exhibit on the Pluronic® F-127 phase behavior are interpreted in terms of the preference of the cosolvent/surfactant molecules to locate in different domains of the PEO–PPO–PEO block copolymer self-assembly. Organic solvents, depending on their relative polarities, locate preferably in the PEO-rich or the PPO-rich domains of the microstructure.[40] The block copolymer-solvent interactions can lead to either suppressing or facilitating the formation of micelles in solution. The different outcome of the polymer interaction with different solvents can be related to the different solvent effects on a molecular level and to the solvent preferences to locate in different domains of the block copolymer assembly.

Table 6 shows the effect of the of various cosolvents-Cremophor® RH 40 combination on the itraconazole solubility. A small decrease in the drug solubility capacity of Cremophor® RH 40 with increasing the percentage of PEG 400 in aqueous mixture. The solubility of itraconazole in 5% w/v Cremophor® RH 40 in water was 9.85 mg/L that decreased to 8.28 mg/L on the addition of 20% w/v PEG 400. The negative effect of cosolvent on micellar

solubilization by Cremophor® RH 40 decrease with decreasing the PEG 400 percent and appears to be insignificant at low cosolvent concentration.

Table 6: Effect of various cosolvents-Cremophor® RH 40 combination on the solubility of itraconazole.

Cremophor® RH 40 Concentration % (w/v)	Solubility of itraconazole (mg/L)								
	Glycerol			Propylene glycol			PEG 400		
	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)	5.0 % (w/v)	10.0 % (w/v)	20.0 % (w/v)
0.5	1.13±0.05	1.08±0.06	1.17±0.03	1.10±0.02	1.06±0.04	0.89±0.04	1.09±0.04	1.13±0.01	1.08±0.03
1.0	1.72±0.06	1.76±0.05	1.81±0.05	1.66±0.03	1.64±0.06	1.44±0.03	1.73±0.04	1.68±0.07	1.64±0.09
2.0	3.28±0.08	3.45±0.08	3.52±0.09	3.24±0.07	3.12±0.05	3.06±0.06	3.24±0.06	3.43±0.06	3.50±0.08
5.0	9.96±0.15	10.23±0.12	10.47±0.19	9.45±0.04	8.46±0.04	7.73±0.07	9.34±0.10	8.96±0.11	8.28±0.11

Each data represents the mean ± SEM (n = 3).

Insignificant decrease in the drug solubility capacity of Cremophor® RH 40 with increasing the percentage of propylene glycol in an aqueous mixture where the solubility of itraconazole in 5% w/v Cremophor® RH 40 was decreased from 9.85 mg/L to 7.73 mg/L on the addition of 20% w/v propylene glycol. These data reflect the change in the micelle characterization upon addition of propylene glycol (Table 6).

Table 6 shows that the addition of glycerol to the Cremophor® RH 40 solutions had a minor impact on the solubility. Insignificant increase in the drug solubility capacity of Cremophor® RH 40 with increasing the percentage of glycerol in aqueous mixture. The solubility of itraconazole in 5% w/v Cremophor® RH 40 was 9.85 mg/L which increased to 10.478 mg/L in combination with 20% w/v glycerol. This positive effect of glycerol on micellar solubilization by Cremophor® RH 40 decreased with decreasing the cosolvent percent and appears to be insignificant at low cosolvent concentration. It seems that that by reducing the polarity of the solution, cosolvents enhance the solvation of Cremophor® RH 40 surfactants, giving rise to increases in CMC and decreases in micelle size.[16]

3.2. Investigation of potentiality of complex formation between itraconazole and HP-β-CD

The solubility method is useful for investigating an inclusion complexation of poorly water-soluble drugs with cyclodextrins in water, because it gives not only the solubilizing ability of host molecules but also the stability constant of the complexes by analyzing the solubility

curve. Table 7 and Fig. 2 show the phase solubility diagram of itraconazole with HP-β-CD at 25 °C.

Table 7: Effect of HP-β-CD on the solubility of itraconazole in water at room temperature (25 ^{o}C±0.5).

HP-β-CD concentration (mM)	Solubility of itraconazole (mM)	Increase of solubility (S_t/S_o)
10	0.001±0.0001	-
12	0.002±0.0002	2.00
14	0.003±0.0001	3.00
18	0.005±0.0001	5.00
20	0.006±0.0003	6.00
30	0.018±0.0007	18.00
35	0.027±0.0003	27.00
40	0.034±0.0019	34.00
45	0.040±0.0056	40.00
50	0.042±0.0091	42.00
70	0.093±0.0068	93.00
90	0.175±0.0056	175.00
110	0.318±0.0063	318.00
130	0.437±0.0075	437.00

Each data represents the mean ± SEM (n = 3).

S_o: solubility of itraconazole in water. (mM), S_t: solubility of itraconazole-HP-β-CD solution. (mM).

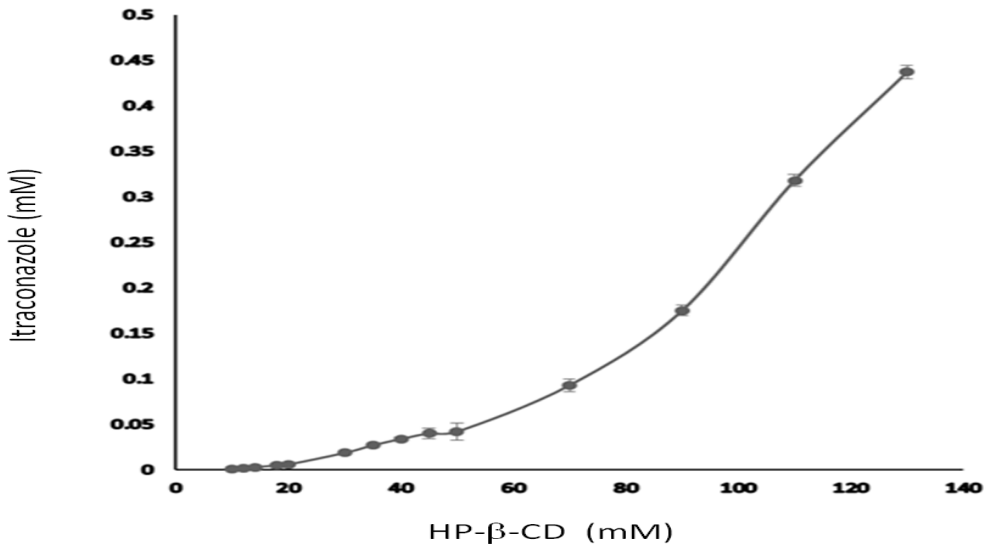

Fig. 2. The phase-solubility diagram of itraconazole in aqueous HP-β-CD solution at room temperature (25^{o}C±0.5). Each data point represents mean ± SEM (n=3).

There is a positive deviation from linearity with an increase in the solubility of itraconazole with increasing concentration of HP-β-CD, and no precipitation of the complex is detected. The solubility curve can be generally classified as Ap type, since the slope of the diagram is more than 1, the complex stoichiometry was assumed to be 1:1, 1:2, and 1:3.[11] A significant effect of HP-β-CD on the solubility of itraconazole such that the drug concentration increases to 13.2 mg/L at 30 mM of HP-β-CD and 308 mg/L at 130 mM of HP-β-CD (Table 7).

3.3. Elucidation of the stoichiometric ratio of itraconazole-HP-β-CD complex

The relationship between solubilizer and the drug was analyzed using the phase-solubility approach described by Higuchi and Connors.[11] In this approach, the total concentration of cyclodextrin $[CD]_t$ is the sum of the free cyclodextrin concentration [CD] plus all cyclodextrin associated with drug complexes such that for 1:1, 1:2, and 1:3 complexes described by eqs. 2, 3, and 4, respectively. The total drug concentration S_t for the three respective drug complexes is given by eqs. 5, 6, and 7, respectively.

However, this model assumes that the total $[CD]_t$ concentration is estimated by the free [CD] (i.e., uncomplexed) concentration. Higuchi and Kristiansen calculated free ligand concentrations with a modified quadratic equation based on known values of the total drug and total ligand concentration.[12] For 1:3 interactions, a third-order curve-fitting is appropriate. To account for the concentration of bound cyclodextrin in these cubic relationships, Peeters et al. suggested a non-linear optimization technique based on an iterative Nelder-Mead approach.[44] Additionally, the fact that it is difficult to identify an initial linear segment of the curve may also suggest non-inclusion complex related phenomena, including cyclodextrin aggregation or surfactant-based effects.[45] Therefore, the upward curvatures were quantitatively analyzed according to the optimization technique to obtain the stability constants of higher order complexes ($K_{1:n}$).[12]

$$St = So + K_{1:1}\, S_o[CD] + K_{1:1}\, K_{1:2}\, S_o[CD]^2 + K_{1:1}\, K_{1:2}\, K_{1:3}\, S_o[CD]^3 + \ldots + K_{1:1}\, K_{1:2}\, K_{1:3} \ldots\ldots K_{1:(n-1)}\, K_{1:n}\, S_o[CD]^n \quad (11)$$

$$[CD]_t = [CD] + K_{1:1}\, S_o[CD] + 2K_{1:1}\, K_{1:2}\, S_o[CD]^2 + 3\, K_{1:1}\, K_{1:2}\, K_{1:3}\, S_o[CD]^3 + \ldots + n\, K_{1:1}\, K_{1:2}\, K_{1:3} \ldots\ldots K_{1:(n-1)}\, K_{1:n}\, S_o[CD]^n \quad (12)$$

Where $K_{1:1}$, $K_{1:2}$, $K_{1:3}$…..,$K_{1:n}$ are the stability constants of the complexes with the stoichiometry of 1:1, 1:2, 1:3, …, 1:n (guest:host), respectively. S_o and S_t represent the solubility and the total concentration of the drug, respectively. [CD] and $[CD]_t$ stand for the free and total concentrations of HP-β-CD, respectively.

For 1:3 and higher order systems, a simplex optimization procedure was applied. In the approach used, trial values of the stability constants are first obtained by numerically fitting a third-order polynomial using the total HP-β-CD concentration as the independent variable. By setting [CD] = $[CD]_t$ as a first approximation, Eq. 11 was analyzed by a nonlinear least-squares method to obtain each apparent stability constant. [CD] values were then calculated from Eq. 12 using the apparent stability constants. This procedure was repeated until each stability constant converged on a constant value. Qualitative assessment of relationships indicates a curvilinear dependence of solubility of itraconazole on HP-β-CD concentration indicating Ap type behavior for system studied. The results numerically are best fitting to third-order polynomial using the total HP-β-CD concentration as the independent variable indicating 1:3 complex formation between ITR and HP- β-CD. The apparent stability constants calculated was $K_{1:1}$ = 7042 M^{-1}, $K_{1:2}$ = 1490 M^{-1}, $K_{1:3}$= 5.95 M^{-1}. Previous results have reported that this strongly suggests the intervention of non-inclusion complex effects since coloumbic repulsion discourages higher order complexation based on charge interaction for this negatively charged cyclodextrin. All natural cyclodextrins self-associate in solution to form aggregates and micelles.[46,47] Chemically modified CDs (e.g., HP-β-CD) may also acquire surfactant-like properties after incorporation of a hydrophobic/lipophilic drug molecule.[46,48,49] The phase solubility profiles of the hydrophobic drug model with HP-β-CD have been reported to be A_L type with slopes of greater than 1, indicative of the formation of higher-order (e.g., 2:1) drug-CD complexes. Space-filling docking analysis of the 1:1 complex, however, suggested that the CD cavity in HP-β-CD was too small to accommodate an additional drug molecule, and the authors concluded that HP-β-CD solubilization of the drug was a combination of molecular complexation and drug solubilization via aggregates of the drug-CD complex. Moreover, the solubilizing potential of the cyclodextrins may be related to non-inclusion complex related phenomena such as cyclodextrin aggregation or surfactant-like properties inherent in these systems.[50-52]

3.4. Effect of cosolvent and/or surfactant on the itraconazole-HP-β-CD complex

Short-chain cosolvents such as low-molecular weight polyethylene glycols, and propylene glycol are often used to enhance drug solubility and may be also used in combination with other solubilizers (CDs). The use of propylene glycol to increase the solubilizing and stabilizing effects of cyclodextrins has been reported for other drugs.[53,54] However, propylene glycol employed as a solubilizing agent may work as a competing agent and thus hinder the inclusion complex formation of the drug with HP-β-CD in aqueous solution.[55]

The combined effects of complexation and cosolvents on drug solubilization can be of synergistic or antagonistic. Some of the factors that need to be considered when using combinations of cosolvent and complexing agents as a result of solubilization by cosolvency, the free drug concentration available for complexation may be higher, leading to a synergistic improvement in solubility. Formation of soluble drug–ligand–cosolvent ternary complexes leads to synergistic improvement in solubility. Competition between the drug and cosolvent molecules for complexation with the ligand leads to a decrease in drug solubility. Decrease in apparent binding constant for drug–ligand in cosolvent.[56]

Table 8 and Fig. 3 show that itraconazole-HP-β-CD complex solubility was decreased with the addition of PEG 400. At 2.5% w/v of HP-β-CD, addition of 10% w/v PEG 400 show insignificant increase in itraconazole solubility (4.269 mg/L) compared to 2.5% w/v of HP-β-CD (2.452 mg/L). However, at 5% w/v of HP-β-CD, addition of 10% w/v PEG 400 show negative effect on complexation of itraconazole solubility (7.275 mg/L) compared to 5% w/v of HP-β-CD (16.47 mg/L).

Moreover, at 20% w/v of HP-β-CD, the negative effect of 10% w/v PEG 400 significantly decrease the itraconazole solubility (72.02mg/L) compared to 20% w/v of HP-β-CD (308 mg/L). The apparent stability constants calculated was $K_{1:1}$ = 2000 M^{-1}, $K_{1:2}$ = 20.5 M^{-1}, $K_{1:3}$= 1.8 M^{-1}, which show a significant decrease comparing the system without the addition of 10% PEG 400 as previously described.

Table 8: Effect of PEG 400 and/or Cremophor® RH 40 on the solubility of itraconazole/ HP-β-CD complex.

HP-β-CD concentration % (w/v)	Solubility of itraconazole (mg/L)		
	+ 10% PEG 400 (w/v)	+ 5% Cremophor® RH 40 (w/v)	+ 10% PEG 400/ 5% Cremophor® RH 40 (w/v)
2.5	4.26±0.22	10.47±0.35	10.78±0.82
5.0	7.27±0.52	11.79±0.42	11.05±0.84
10.0	17.43±2.25	18.60±1.68	16.98±1.87
15.0	44.34±4.87	39.46±2.57	31.93±3.00
20.0	72.01±3.80	62.62±4.34	55.36±4.21

Each data represents the mean ± SEM (n = 3).

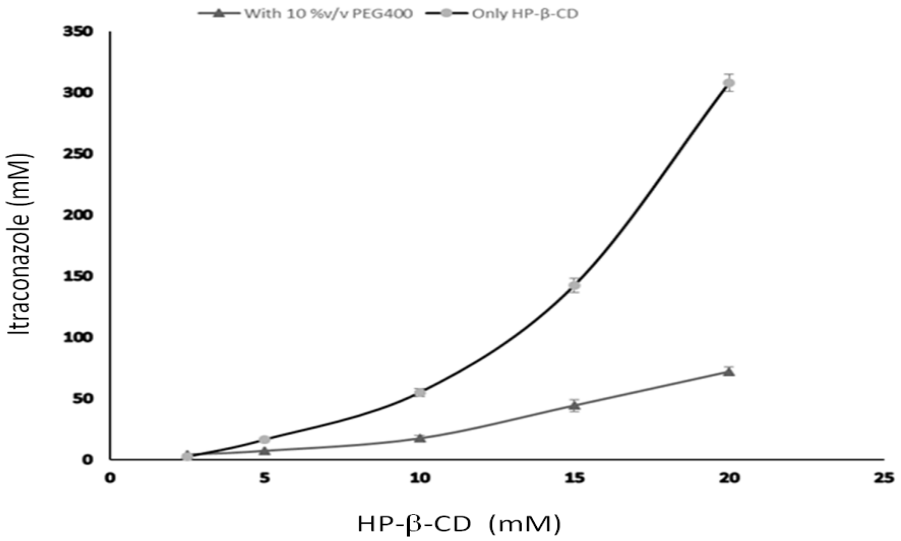

Fig. 3. The phase-solubility diagram of itraconazole /HP-β-CD complex with 10% w/v PEG 400 at room temperature (25°C±0.5). Each data point represents mean ± SEM (n=3).

These results consistent with that reported data show similar behavior for PG with Itraconazole -HP-β-CD complex.[55] However, cosolvent molecules promote the solubility of the non-complexed drug in free solution, they also compete with drug molecules for the CD hydrophobic cavity.[57] The potential for cosolvent to lower drug solubilization in the presence of low concentrations of CD likely reflects the competitive effects of the cosolvent for the hydrophobic cavity. As such, the drug-CD binding may be reduced.[58,59]

Several interactions can coexist in solubility experiments in aqueous solutions containing complexing agent, surfactant, and drug. They include competitive complexation of drug and surfactant monomer with the complexing agent, equilibria between monomer and micelle,

and solubilization of drug in the micelle.[56] Cyclodextrins form inclusion complexes with nonionic, leading to potential competition between drug and surfactant for CD binding sites.[60] Furthermore, the complexed surfactant fraction is unable to participate in micelle formation, reducing micellar solubilization. Thus, combinations of CDs and surfactants therefore often lead to a decrease in the concentration of solubilized drug.

Table 8 and Fig. 4 show that itraconazole-HP-β-CD complex solubility was decreased on addition of Cremophor® RH 40. At 2.5% w/v of HP-β-CD, addition of 5% w/v Cremophor® RH 40 insignificantly increase in itrachonazole solubility (10.478 mg/L) comparable to the effect of 2.5% w/v of HP-β-CD (2.452 mg/L). At 5% w/v of HP-β-CD, addition of 5% w/v Cremophor® RH 40 decrease the solubility of itraconazole-HP-β-CD complex (11.797 mg/L) comparable to the effect of 5% w/v of HP-β-CD (16.47 mg/L). Increasing the Cremophor® RH 40 concentration up to 5% w/v with 20% w/v of HP-β-CD significantly decreases the itraconazole solubility (62.621 mg/L) in comparison to the effect of 20% w/v of HP-β-CD (308 mg/L). The apparent stability constants were significantly decreased as follows for $K_{1:1}$ =10 M^{-1}, $K_{1:2}$ = 54 M^{-1}, $K_{1:3}$= 0.0055 M^{-1} in comparison to itraconazole-HP-β-CD.

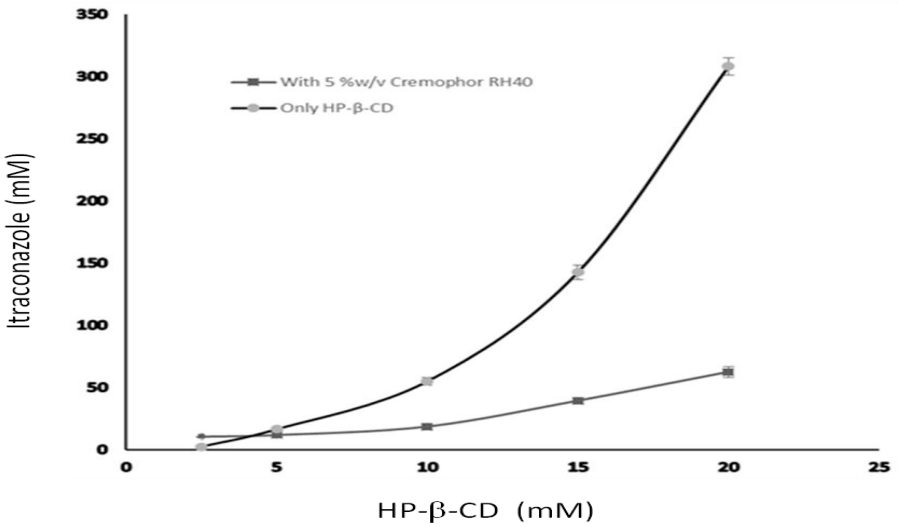

Fig. 4. The phase-solubility diagram of itraconazole /HP-β-CD complex with 5% w/v Cremophor® RH 40 at room temperature (25°C±0.5). Each data point represents mean ± SEM (n=3).

Yang et al. showed that the solubility of NSC-639829 in in aqueous solutions containing both sodium lauryl sulphate (SLS) and sulfobutyl-ether-beta-cyclodextrin (SBE)7M-β-CD is less than the sum of the solubility values in aqueous solutions containing SLS or (SBE)7M-β-CD.[61]

Table 8 and Fig. 5 show the effect of 5% w/v Cremophor® RH 40 and 10% PEG 400 on the itraconazole-HP-β-CD complex solubility. At 2.5% w/v of HP-β-CD, addition of 5% w/v Cremophor® RH 40 and 10% PEG 400 show minor increase in itraconazole solubility (10.78mg/L) in comparison to effect of 2.5% w/v of HP-β-CD alone (2.452 mg/L). Further increase of HP-β-CD concentration, the effect of 5% w/v Cremophor® RH 40 and 10% PEG 400 significantly decrease the effect of HP-β-CD on the solubility of itraconazole. At 20% w/v of HP-β-CD, the negative effect of 5% w/v Cremophor® RH 40 and 10% PEG 400 combination decreases the itraconazole solubility (55.368 mg/L) in comparison with 20% w/v of HP-β-CD (308 mg/L). The apparent stability constants were decreased as follows, $K_{1:1}$ = 0.5 M^{-1}, $K_{1:2}$ = 280 M^{-1}, $K_{1:3}$ = 13.9 M^{-1}, show a decrease in comparing with the system without the addition of the combined system as described previously.

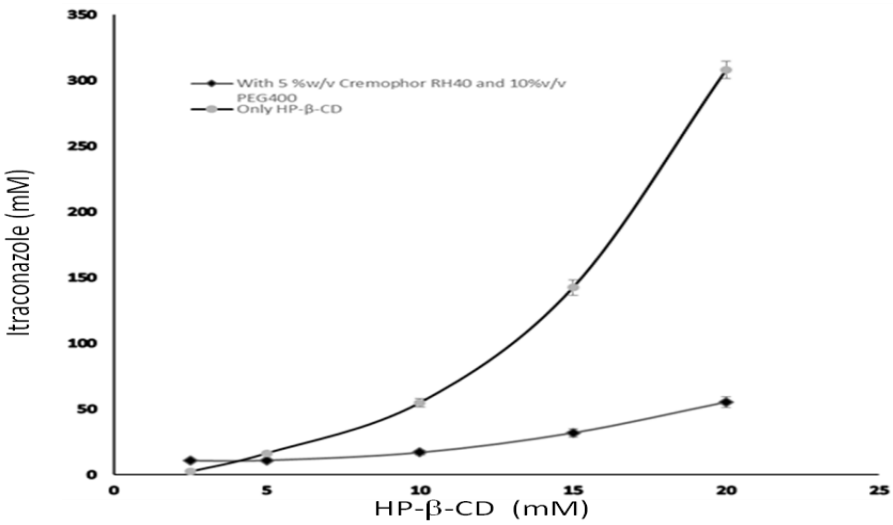

Fig. 5. The phase-solubility diagram of itraconazole /HP-β-CD complex with 5% w/v Cremophor® RH 40 and 10% w/v PEG 400 at room temperature (25°C±0.5). Each data point represents mean ± SEM (n=3).

Itraconazole solubility enhancement by the complexation with HP-β-CD was decreased by the addition of cosolvent and surfactant and the solubility curve was shifted to higher HP-β-CD concentrations. This may be attributed to the decrease in the effective CD concentration necessary for the complexation with itraconazole, due to the competitive inclusion of cosolvent and/or surfactant.[55,60,61]

The aforementioned results apparently indicate that itraconazole forms the inclusion complex with HP-β-CD in a various stoichiometry (1:1, 1:2, and 1:3) in water and 5% w/v

Cremophor® RH 40 and 10% PEG 400 aqueous solution.[44,55] The addition of Cremophor® RH 40 and/or PEG 400 made the inclusion strength of HP-β-CD weaker, because it works as a competing agent. After ocular administration, the primary driving force for dissociation of the complex is supposed to be simple dilution, although another factor such as competitive displacement of the drug by biological components.[62,63] After dilution the cosolvent and/or surfactant system gives a larger free fraction of drug which is favorable for ocular absorption. Knowledge of this kind will be particularly useful for design of the cyclodextin-based pharmaceutical ocular formulations.

4. CONCLUSION

This study investigates the enhancement of itraconazole solubility to provide a suitable effective system for ocular delivery. Effect of cosolvents shows that PEG 400 has the greatest solubilizing power for itraconazole. The micellar solubilization show that the aqueous solubility of itraconazole increased in a linear relationship with the concentrations of surfactants. Cremophor® RH 40 shows the utmost micellar solubilization comparing with others. The effect of cosolvent-surfactant combined system shows insignificant effect on improving itraconazole solubility. However, addition of cosolvents with or without surfactant generally did not contribute much to improving the solubility of itraconazole except in high concentration of cosolvent which is inconvenient for safety point. By investigating the interaction of itraconazole with HP-β-CD, the results show a positive deviation from linearity, and can be classified as type Ap suggesting the formation of a third-order complexes. The addition of Cremophor® RH 40 and/or PEG 400 made the inclusion strength of HP-β-CD weaker that upon dilution gives a larger free fraction of drug which is favorable for ocular absorption. The combination of using appropriate cosolvent and/or surfactant with the drug will be particularly useful for design of the cyclodextin-based pharmaceutical ocular formulations.

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