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Solubility Enhancement of Ketoconazole by different techniques and its comparison study

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ABSTRACT

Enhancement of the solubility is an important physicochemical parameter which affects the absorption of drug and its therapeutic activity. The poor aqueous solubility of drug affects the lack of formulation development. In this study the antifungal drug ketoconazole were prepared with β-cyclodextrin and PEG-6000 by four different methods with an intention to improve its dissolution properties. Solubility of ketoconazole was prepared by sonocrystallization, solid dispersion, hydrotropy and Inclusion complex formation technique. In vitro release profile were evaluated and compared with standard ketoconazole. Solubility by the hydrotropy method was found to be 12.159 fold increases while by inclusion complex, solid dispersion, and melt sonocrystallization method was found to be 9.644, 7.349, and 5.517 fold respectively. Dissolution profile of all four formulations (aqueous suspension), it was found that the formulation prepared by the hydrotropy method showed the best release profile that is 83.16%. Investigations of the properties of the dispersions were performed using release studies with analytical studies, Differential scanning calorimetery (DSC), and Fourier transform infrared (FTIR). FT-IR spectra revealed no chemical incompatibility between drug and ß-cyclodextrin. Interaction of Drug-polymer was investigated using differential scanning calorimetry (DSC).

Keywords: Ketoconazole, β-cyclodextrin, PEG-6000, Solubility enhancement, Hydrotropy.

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INTRODUCTION

There are many types of fungal germs (fungi) live harmlessly in the soil, on food, on our skin and in other places in the environment. However, some types of fungi can thrive and multiply on the surface of the body, to cause infection of the skin, nails, mouth or vagina. There are several different antifungal preparations that are used to treat various fungal infections. They are as creams, shampoos, pessaries, tablet, and injections. Ketoconazole (KZ) is a dibasic imidazole antifungal synthetic agent. It is developed for the first choice treatment of human mycotic infections[1]. It is poorly soluble in water having molecular weight 531.44; it is administered either topically or by mouth[2].

Ketoconazole is classified in the Biopharmaceutics Classification Scheme (BCS) as a class II drug, since it has a high permeability and its solubility in aqueous media is not sufficient for the whole dose to be dissolved in the GI fluids under normal conditions literatures are reported that poor water solubility and wettability of the drug can cause problems with drug release and bioavailability in various pharmaceutical forms[3-4]. The common oligosaccharides of cyclodextrins which can form inclusion complexes with large organic molecules thus improving their dissolution rate. The most common cyclodextrins are the α-, β-, and γ-cyclodextrins[5]. There have been continuous efforts to improve the solubility and dissolution of drugs. These include, reducing the particle size, formation of inclusion complexes with cyclodextrins, solubilization in surfactant systems, melt sonocrystallization, hydrotrophy using pro-drugs and drug derivatization and formation of solid dispersions[6]. The formulation of solid dispersions is one of the most popular ones but some of the products are in market of this concept[7-10].

The aim of present study was to prepare and characterize different method such as melt sonocrystallization, solid dispersion, hydrotrophy and inclusion complex formation with PEG and cyclodextrins, these methods are efficient for enhancing the water solubility of hydrophobic or poorly water soluble Ketoconazole. Solubility enhancement study, we have used four different techniques (melt sonocrystallization, solid dispersion, hydrotropy and inclusion complex formation) for enhancing the solubility of ketoconazole. The solubility of the final formulations can be checked in dichloromethane (DCM) as it shows the highest solubility of ketoconazole rather than other solvents. Solubilised concentration can be determined by UV spectrophotometer (Schimadzu, Japan) and it is also characterize by the Infrared spectroscopical study. The solubility enhancement of the ketoconazole can be further proved by the in-vitro release profile of the ketoconazole (Structure-1).

*****(Structure-1: Ketoconazole, β-cyclodextrin)**

MATERIALS AND METHOD

Material and Reagents

Ketoconazole standards were obtained as gift sample from Ranbaxy laboratories Ltd. (Gurgaon, India); melting point varies from 146 to148°C. β-cyclodextrin were obtained from Sigma-Aldrich (Bangalore, India) Phosphate buffer, and acetonitrile (HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). PEG 6000, 4000, octanol, methanol, and ethyl alccohol was purchased from S.D. Fine Chemicals, Ltd**.** (Mumbai, India) and Dichloromethane was procured from Central Drug House, New Delhi, India. All the materials used in the study were of analytical grades. In order to evaluate the effect of carriers on Ketoconazole, melt sonocrystallization, solid dispersion, hydrotropy and inclusion complex formation studies were performed. Physical analysis based on UV (Shimadzu UV-1601,Tokyo, Japan), FTIR (8400S, Shimadzu, Tokyo- Japan) and DSC (Perkin Elmer, Massachusetts, USA) was performed to evaluate the structure of the dispersions and to detect the possible drug-carrier interactions.

METHOD

Melt Sonocrystallization technique

Drug was melted in a china dish on a paraffin oil bath at 148°C (the melting point of ketoconazole), the molten mass was poured in a beaker containing deionized water at 60°C and the content was sonicated for 15 minutes with a frequency of 1.5 MHz using bath ultrasonicator. The solidified dispersed droplets were separated by filtration and dried at room temperature[11].

Solid Dispersion technique

Carrier (PEG 6000) was dissolved in 1:1 methanol and dichloromethane using magnetic stirrer to which the drug was added and allowed to dissolve. The resulting mixture was transferred into petridish and evaporation of the solvents was carried out by keeping the petridish at room temperature. The mass obtained was crushed with the blunt end of a glass rod and passed

through 44 mesh sieve[12].

Hydrotropy technique

Hydrotrophy technique of ketoconazole and solution was prepared in evaporable organic solvent (ethyl acetate) previously saturated with distilled water in a separating funnel to which hydro trope(tri-sodium citrate) solution was added. Then, the separating funnel was sealed and immersed in a constant temperature bath and kept overnight for equilibration. After this, the aqueous layer was transferred into a beaker[13].

Inclusion Complex Formation technique

Solid inclusion complex of ketoconazole and β cyclodextrin were prepared by the co-evaporation method. This technique ketoconazole and β cyclodextrin were taken in stoichiometric ratio and kneaded thoroughly with minimum amount of water to obtain a paste. This paste was dried under vacuum in vacuum pump at room temperature using phosphorus pentoxide as a drying agent[14].

Solubility Study

Solubility of ketoconazole was performed in various solvents that is 200 mg of the drug was accurately weighed over butter paper from which pinch by pinch was added to 2 ml of different solvents viz, acetone, chloroform, dichloromethane, distilled water and ethanol, till a saturated solution was obtained. Then the amount of drug remaining was weighed and subtracted from the original weight to obtain the solubility of the drug in that particular solvent (Table-1).

Table-1 Solubility of the ketoconazole was found to be in different solvent systems:

S. No.	Solvent	Concentration (mg/ml)
1	Dichloromethane(DCM)	5.091
2	Distilled Water	1.88
3	Octanol	3.864

Ketoconazole showed highest solubility in DCM (5.091 mg/ml). This was agreement with the I.P 2007[15-16].

Dissolution Study

Dissolution rate studies were performed with USP Type-I In-vitro dissolution apparatus using distilled water as dissolution medium. This method were described as 1 ml of formulation was taken in dialysis bag and it was kept inside the basket type of dissolution apparatus (USP Type 1 dissolution apparatus). Volume of dissolution media (distilled water) 900 ml, Temperature was kept 37± 0.5°C and speed of the basket was 100 rpm. Sampling was done at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs by withdrawing 2 ml of the sample and replacing them with 2ml of solvent (Dissolution medium). The analysis of sample was done by taking absorbance with UV spectrophotometer. Concentration of the drug in sample was calculated from the calibration

curve prepared in distilled water, by the extrapolation method.

***In vitro* release study**

A 2% aqueous suspension of ketoconazole was prepared for the *in vitro* release study. Release study was performed in distilled water by dialysis membrane method. 1ml of the formulation was taken in dialysis bag and it was kept inside the basket type of dissolution apparatus (USP Type 1 dissolution apparatus). Volume of dissolution media (distilled water) 900 ml, Temperature was kept 37± 0. 5^0C and speed of the basket was 100 rpm. Sampling was done at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs by withdrawing 2 ml of the sample and replacing with 2ml of solvent (Dissolution medium). The analysis of sample was done by taking absorbance with UV spectrophotometer. Concentration of the drug in sample was calculated from the calibration curve prepared in distilled water, by the extrapolation method.

IR Spectroscopy Study

Infrared spectrum of any compound or drugs gives information about the groups present in that particular compound. A spectrophotometer for recording the spectra in the infrared region consists of an optical system capable of providing the monochromatic light in the region of 4000 to 400 cm^{-1} (about 2.5 to 16 µm) and the means of measuring the quotient of the intensity of the transmitted light and the incident light. 1 mg of the sample and 300 mg of potassium bromide were taken in a mortar and triturated with the help of pastle. A small amount of triturated sample was taken in a pellet maker and was compressed at 10kg/ cm^2. The pellet was kept on the sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1}. The infra-red spectrum of drug sample was obtained using FTIR- 8400S, Shimadzu (Tokyo- Japan) [Figure-1 & 2]. This IR spectrum was compared with the IR of ketoconazole reported in the official monograph of IP 1996[17].

Differential scanning calorimetry study

Differential scanning calorimetry studies were performed using Pyris 6 DSC instrument (Perkin Elmer, Massachusetts, USA). 3-4 mg samples were sealed in pierced aluminium pans of 40µL and were measured at scanning speed of 10^0C/min over a temperature range from 50-300^0C. The empty pan used as reference [Figure-3].

UV Visible Spectrophotometry (scans in different medium) study

U.V. Analysis was done by dissolving 10 mg of ketoconazole in methanol and volume was made up to 100 ml with the same. 1 ml of the above solution was diluted with distilled water, DCM, and Octanol to make up the volume up to 10 ml respectively. Then solutions were scanned between 200-400 nm by Shimadzu UV-1601 (Tokyo, Japan) spectrophotometer respectively. The λmax of ketoconazole was found to be 244 nm (In distilled water), 244.2 nm (In DCM), and

244.4 (In Octanol).

RESULTS AND DISCUSSION

Drug polymer compatibility study by IR

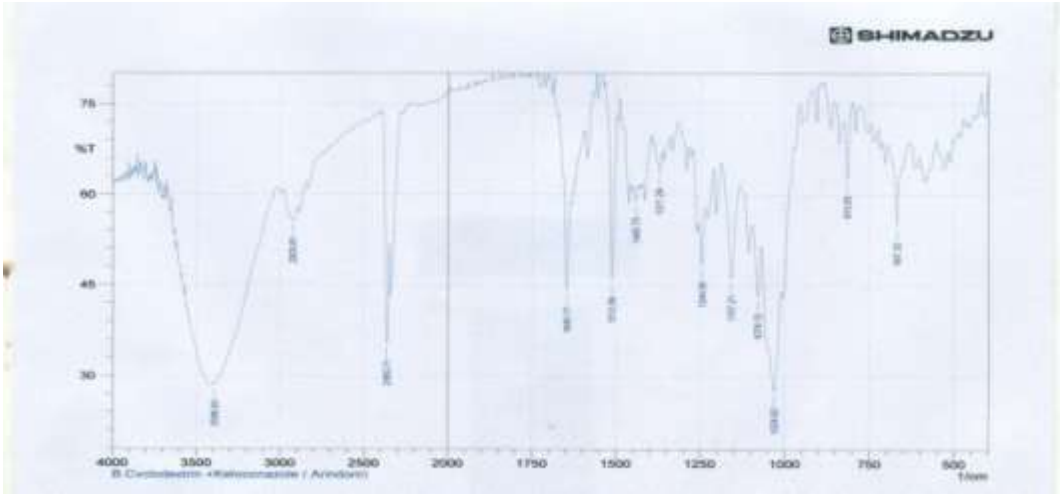

Figure-1: IR Spectrum of mixture of Ketoconazole and β-cyclodextrin

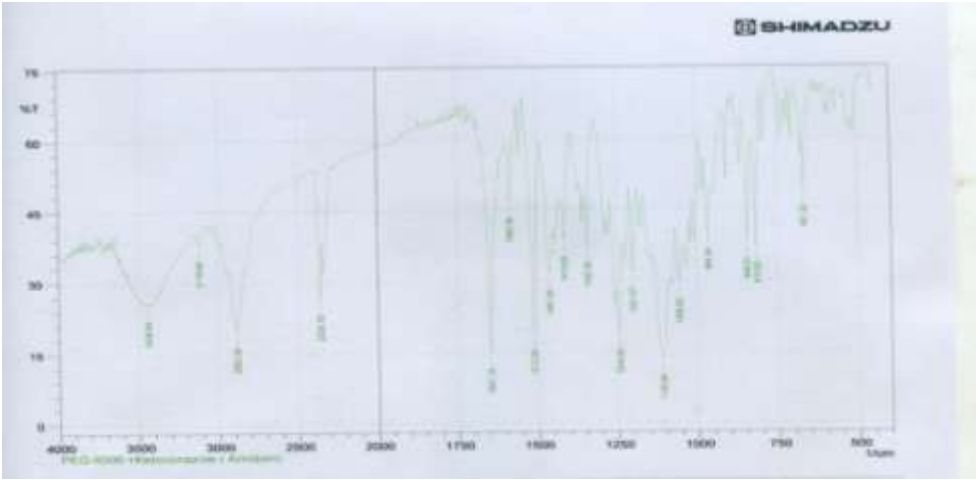

Figure-2: IR Spectrum of mixture of Ketoconazole and PEG-6000.

The infra-red spectrum of pure drug sample was found to be in agreement with the reported reference spectra of the IR (IP 1996). CH2 , C-H, C=O, C-N, C=C functional group showed 1465, 3000-2850, 1680-1630, 1350-1000 cm^{-1} in official data where as it is found as 1460, 2964, 1647, 1201 in sample which showed in agreement with official data. If the drug and the polymer would interact the functional group in IR spectra would show band shifts and broadening of the peaks as compared the spectra of the standard and polymer. The spectral study showed that there was no chemical interaction of the drug with polymer even in the amorphous state when the granules were prepared by the solid dispersion method. When increase the polymer content in formulation also they did not initiate any drug polymer interactions.

Differential scanning calorimetry (DSC) Study

Differential scanning calorimetry curves obtained for pure Ketoconazole, β-cyclodextrin and PEG-6000 has shown in Figure-3. Pure powdered Ketoconazole and PEG-6000 showed a sharp melting endothermic peak (Tm) at 151.40°C and 61.30°C respectively. Solid dispersion prepared of β-cyclodextrin showed reduced and a slightly broad peak of drug at 118.42°C and peak of ketoconazole and PEG-6000 slightly broad and shifted 81.70°C. This implies from DSC data that the drug is present in crystalline form in β-cyclodextrin solid dispersion but the crystallinity has been reduced as compared to pure drug while drug is present in completely amorphous form in PEG-6000 solid dispersion.

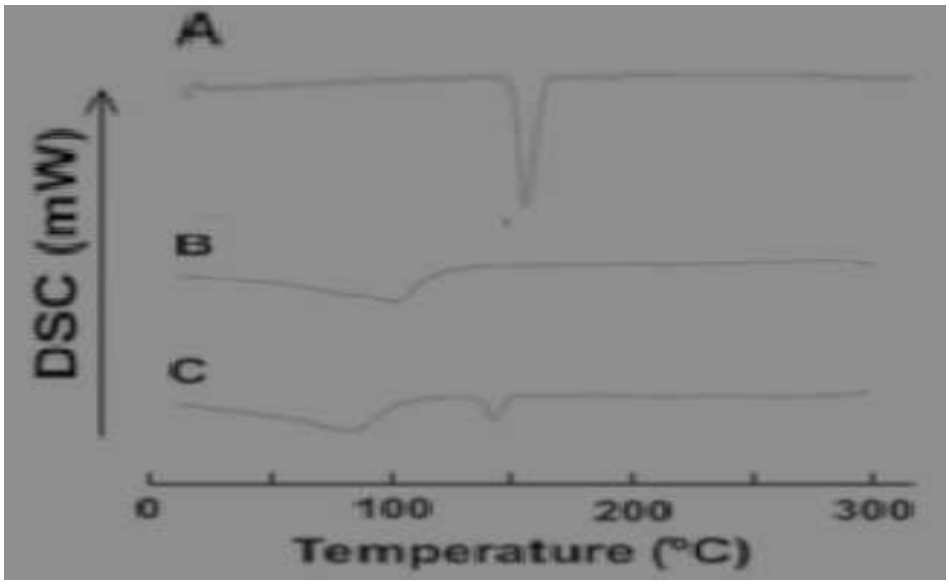

Figure-3: Differential Scanning Calorimeter scans of mixture of Ketoconazole, PEG-6000, and β-cyclodextrin.

UV Visible Spectrophotometry study

Preparation of calibration curve in dichloromethane (DCM)

Table-2: Absorbance of ketoconazole solution in dichloromethane at λ max 244.2 nm.

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.174
3	15	0.285
4	20	0.391
5	25	0.489
6	30	0.585
7	35	0.733
8	40	0.814

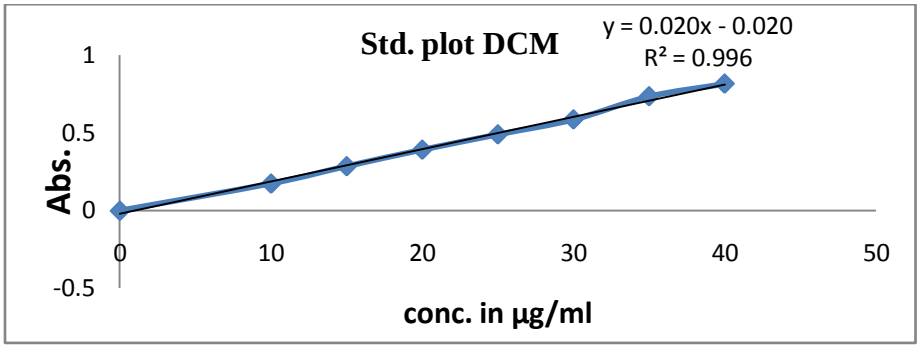

Figure-4: Standard Graph of Ketoconazole in dichloromethane (DCM) at λmax 244.2 nm

Preparation of calibration curve in distilled water

Table-3: Absorbance of ketoconazole solution in distilled water at λ max 244 nm.

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.305
3	15	0.402
4	20	0.531
5	25	0.621
6	30	0.756
7	35	0.831
8	40	0.956

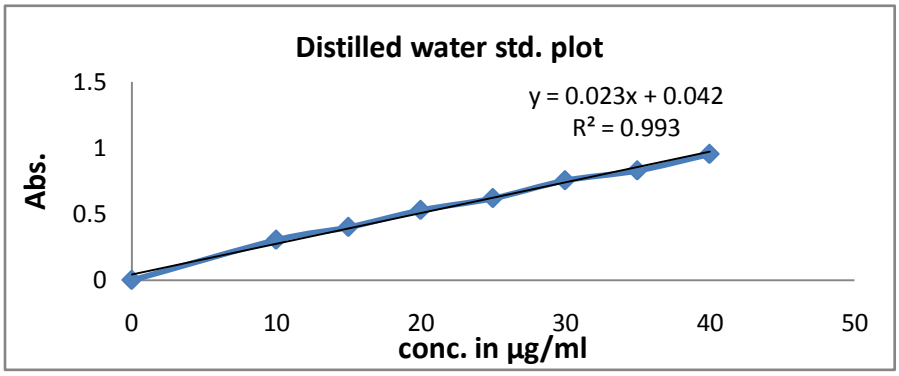

Figure-5: Standard Graph of Ketoconazole in distilled water at λmax 244nm.

Preparation of calibration curve in octanol

Table-4: Absorbance of ketoconazole solution in octanol at λ max 244.4 nm.

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.189
3	15	0.263
4	20	0.375
5	25	0.472
6	30	0.563
7	35	0.693
8	40	0.783

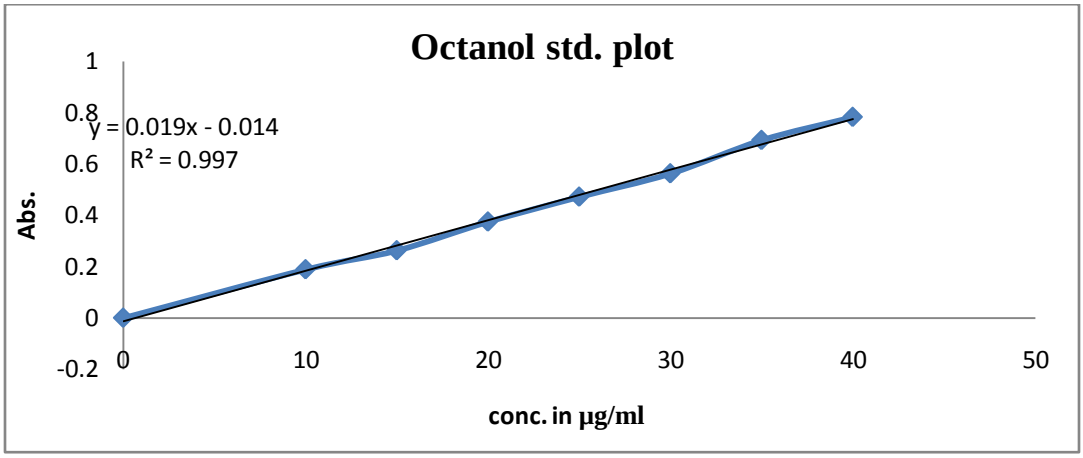

Figure-6: Standard Graph of Ketoconazole in octanol at λmax 244.4 nm.

Different Techniques Used For The Solubility Enhancement Of Ketoconazole and Their Results Obtained Were As Follows(Medium taken – Distilled water, and Absorbance at λmax 244)

Table-5: Fold increase in solubility by individual techniques

S. No.	Different techniques used	Solubility in dist. Water (mg/ml)		Fold increase in Solubility
		Initial	Final	
1	Melt Sonocrystallization	1.888	10.417	5.517
2	Solid Dispersion	1.888	13.875	7.349
3	Hydrotropy	1.888	22.958	12.159
4	Inclusion Complex Formation	1.888	18.208	9.644

Individual techniques were applied and the results obtained are given below in the graph**.**

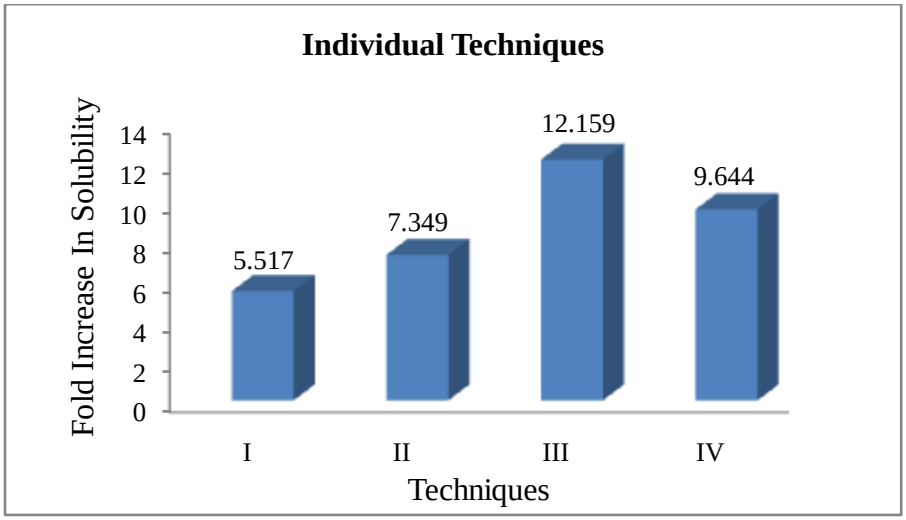

Figure-7: Fold increase in solubility by individual techniques

Where, I= Melt Sonocrystallization Technique,

II= Solid Dispersion Technique,

III= Hydrotropy Technique and

IV= Inclusion Complex Formation Technique.

EVALUATION OF SUSPENSION

Techniques for the evaluation of heterogeneous systems are generally complex and are far from being completely satisfactory sedimentation method employed for evaluation of suspension is discussed below:

Sedimentation volume:

It considers the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension as the suspension settles in a cylinder under standard conditions. The suspensions of formulations prepared from different techniques I, II, III and IV were filled in 100 ml graduated cylinder and kept undisturbed for 24 hour and initial height(Ho) was measured. The ultimate height (Hu) was measured at different time intervals. The Hu/Ho ratio is plotted versus time (Table-6).

Table-6: Rare of sedimentation

Time (Hr)	Type I			Type II			Type III			Type IV		
	Ho (cm)	Hu (cm)	Hu/Ho	Ho (cm)	Hu (cm)	Hu/Ho	Ho (cm)	Hu (cm)	Hu/Ho	Ho (cm)	Hu (cm)	Hu/Ho
0	20	20	1	20	20	1	20	20	1	20	20	1
0.5	20	20	1	20	19.8	0.99	20	20	1	20	20	1
1	20	20	1	20	19.8	0.99	20	20	1	20	20	1
2	20	20	1	20	19.6	0.98	20	19.4	0.97	20	20	1
4	20	19.8	0.99	20	19.4	0.97	20	19.4	0.97	20	19.6	0.98
8	20	19.8	0.99	20	19.2	0.96	20	18.8	0.94	20	19.4	0.97
10	20	19.4	0.97	20	19	0.95	20	18.6	0.93	20	19.4	0.97
12	20	19.4	0.97	20	19	0.95	20	18.4	0.92	20	19.4	0.97
24	20	19.4	0.97	20	19	0.95	20	18.4	0.92	20	19.4	0.97

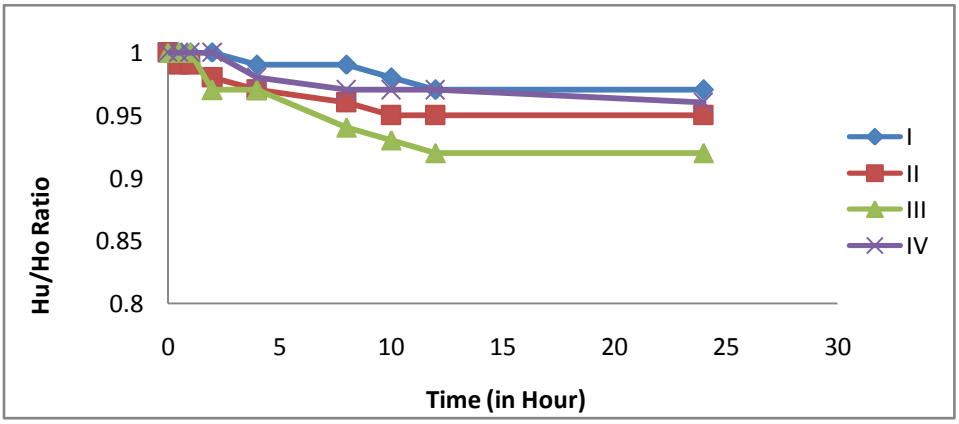

Figure-8: Plot of Hu/Ho ratio versus time for the evaluation of suspension indicating the stability of the suspensions

Where, I= Melt Sonocrystallization Technique, II= Solid Dispersion Technique, III= Hydrotropy Technique and IV= Inclusion Complex Formation Technique.

The sedimentation studies showed that all the suspension were stable indicated by the plot of Hu/Ho ratio versus time in figure-8. As shown in figure-8 the plot become horizontal after 4 hours for all the formulations. The Hu/Ho ratio for suspension of type I, II, III, and IV formulation after 24 hours were 0.97, .095, 0.92 and 0.97 respectively and all suspension passed the test of stability testing because of the acceptable Hu/Ho value.

***In Vitro* Release Study**

Four formulations prepared by four different solubility enhancement techniques (melt crystallization, solid dispersion, hydrotrophy, inclusion complex formation were checked for their dissolution profile to assess the best formulation.

A 2 % aqueous suspension of ketoconazole was prepared for the *in vitro* release study. Release study was performed in distilled water by dialysis membrane method. 1 ml of the formulation was taken in dialysis bag and it was kept inside the basket type of dissolution apparatus (USP Type 1 dissolution apparatus). Volume of dissolution media (distilled water) 900 ml, Temperature was kept 37$\pm$ 0.5^{o}C and speed of the basket was 100 rpm. Sampling was done at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs by withdrawing 2 ml of the sample and replacing with 2ml of solvent (dissolution medium). The analysis of sample was done by taking absorbance with UV spectrophotometer (Schimadzu, Japan) at λmax 244 nm. Concentration of the drug in sample was calculated from the calibration curve prepared in distilled water, by the extrapolation method (Table-7).

Table-7: Release profile of formulation prepared by hydrotrophy method.

Time (hrs)	Absorbance	Concentration	Cumulative drug release in µg/ml	% Drug release
0	0	0	0	0
0.5	0.12	8.33	7500	7.5
1	0.26	17.66	15900	15.9
2	0.41	27.26	24540	24.54
4	0.60	40.20	36180	36.18
6	0.82	54.53	49080	49.08
8	0.91	59.76	53784	53.78
12	1.01	67.20	60480	60.48
24	1.38	92.40	83160	83.16

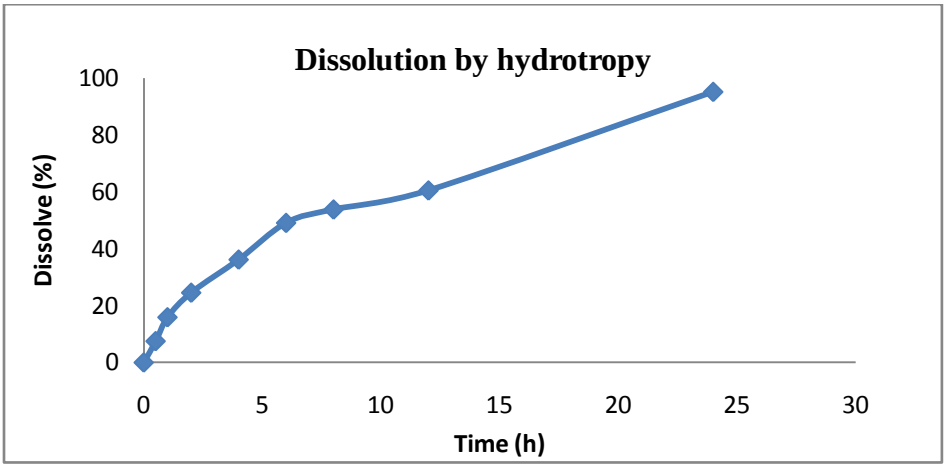

Figure-9: Release profile of formulation prepared by Melt Sonocrystallization method

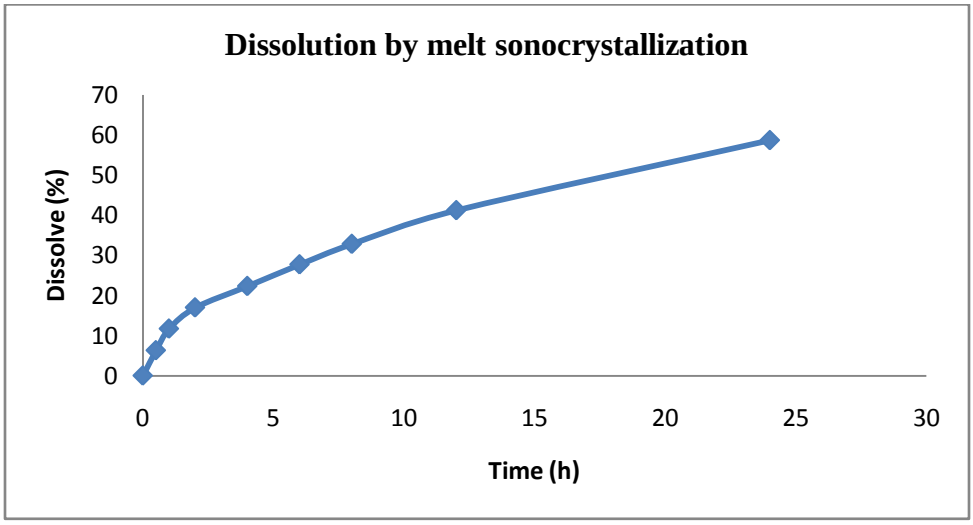

Figure-10: Release profile of formulation prepared by Melt Sonocrystallization method

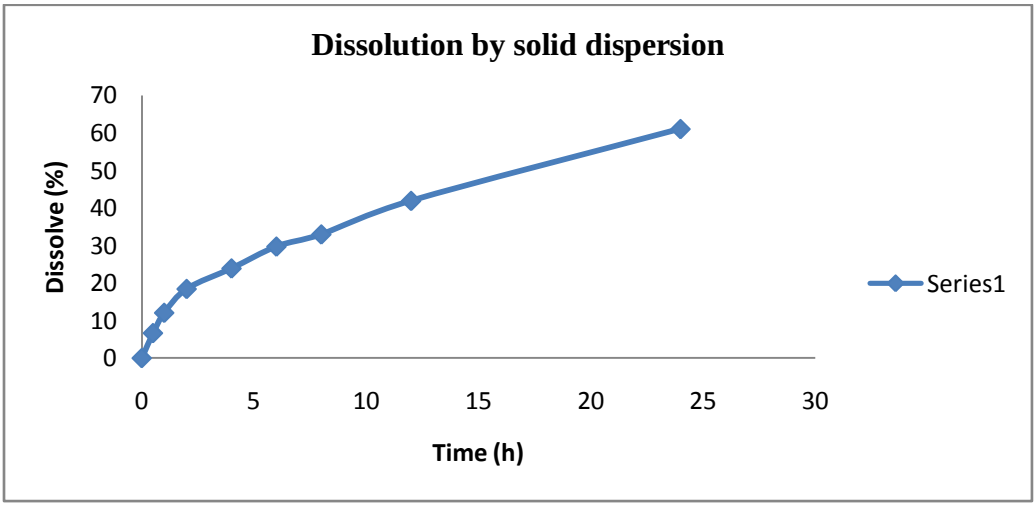

Figure-11: Release profile of formulation prepared by solid dispersion method

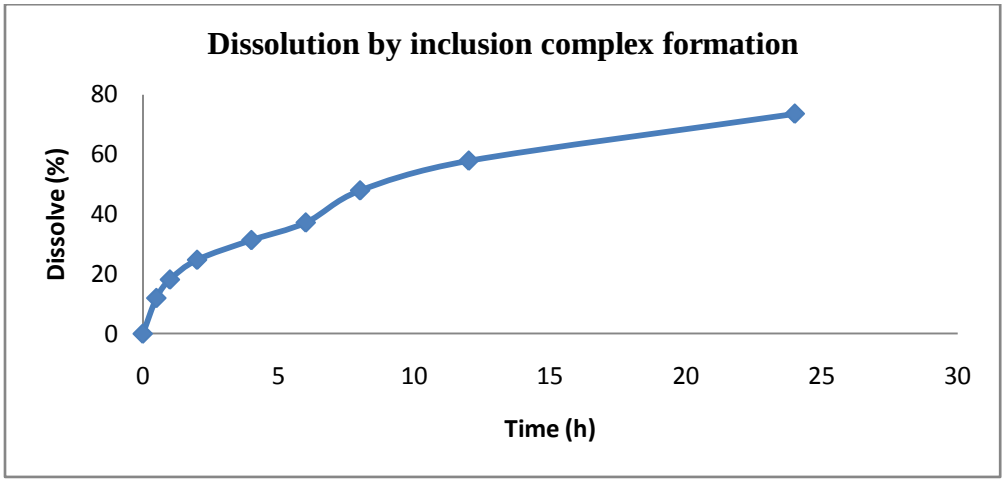

Figure-12: Release profile of formulation prepared by inclusion complex formation

COMPARISON OF DRUG RELEASE PROFILE OF FOUR SUSPENSIONS OF DIFFERENT TECHNIQUES OF SOLUBILITY ENHANCEMENT

Table-08: The comparison of drug release profile of four suspensions of different techniques of solubility enhancement

Times (hrs)	Hydrotrophy (% drug release)	Melt sonocry. (%drug release)	Solid Dispersion (% drug release)	Inclusion complex (% drug release)	Control (%drug release)
0	0	0	0	0	0
0.5	7.5	6.3	6.66	11.94	2.3
1	15.9	11.7	12.06	18.12	3.1
2	24.54	16.98	18.42	24.72	4.22
4	36.18	22.32	23.88	31.32	5.6
6	49.08	27.72	29.7	37.2	8.6
8	53.78	32.82	32.94	47.94	10.2
12	60.48	41.22	41.88	57.9	16.11
24	83.16	58.68	61.02	73.62	17.34

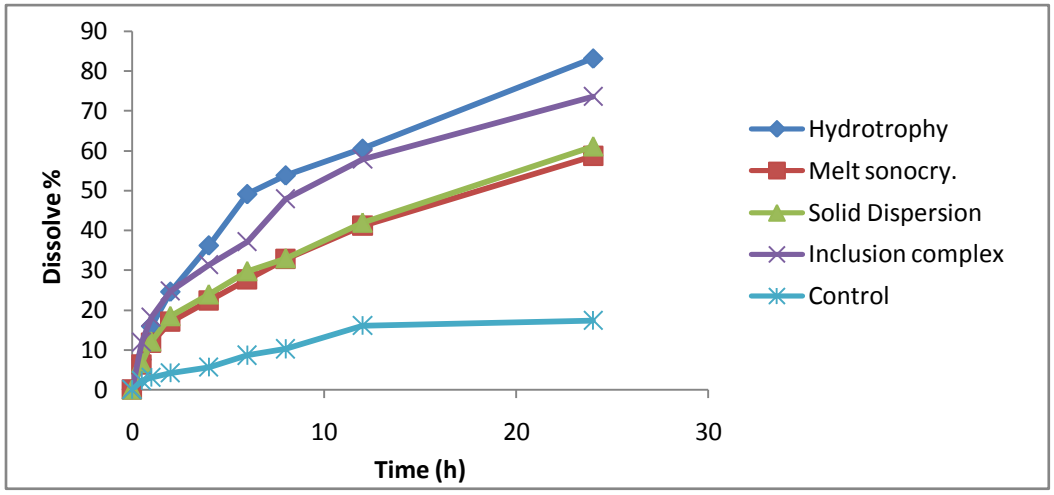

Figure-13: Comparative dissolution by different techniques

Result: On the basis of the dissolution study it was clear that the formulation which was prepared by the hydrotrophy method showed the best result for the solubility as well as for *In vitro* release study i.e. 12.159 fold increase in solubility and 83.16% release of the ketoconazole from its suspension was observed.

CONCLUSION

Four techniques namely melt sonocrystallization, solid dispersion, hydrotrophy and inclusion complex with cyclodextrin were used for the solubility enhancement. The order of the techniques for the solubility enhancement was found hydrotrophy>inclusion complex>solid dispersion>sonocrystallization method. Enhancement in the solubility by the hydrotrophy method was found to be 12.159 fold increases while by inclusion compound, solid dispersion, and melt sonocrystallization method was found to be 9.644, 7.349, and 5.517 fold respectively. Dissolution study of the four formulations prepared by the four different techniques was also performed. On the basis of data of drug dissolution profile of all four formulations (aqueous suspension) it was found that the formulation prepared by the hydrotropy method showed the best release profile that is 83.16%. While release from the suspension prepared by inclusion compound, solid dispersion, and melt sonocrystallization method were found to be 61, 73 and 58% respectively. Thus on the basis of this study we can say that the hydro trophy technique is the best technique for the solubility enhancement of the ketoconazole.

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