Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN 0976-9595
Research Article

FORMULATING β-CYCLODEXTRIN INCLUSION COMPLEXES FOR SOLUTION STABILITY ENHANCEMENT OF GRISEOFULVIN

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Received: 11-08-2022; Accepted: 09-09-2022; Published: 30-09-2022

 https://doi.org/10.55218/JASR.202213810

ABSTRACT

Griseofulvin is a poorly soluble antifungal antibiotic drug, the solubility of which can be enhanced by complexation with β-cyclodextrin. The inclusion complex was prepared by Physical and Kneading method in various molar ratios of 1:1, 1:2 and 2:1 of the drug and β-cyclodextrin, respectively. IR spectra of drug, polymer and a physical mixture of drug and polymer were obtained using FT-IR. The inclusion complex was characterized and evaluated by determination of aqueous solubility, determination of pH stability, estimation of griseofulvin in complexes and stability study. The aqueous solubility of the GCD inclusion complexes was determined using the Higuchi-Connor method. The results indicated that the use of kneading method for preparation of complexes was more effective than the physical mixture method. The inclusion complexes were on the other hand was able to prevent the degradation of griseofulvin at all the pH. The degradation of griseofulvin from the inclusion complexes was found to be prevented and around 20-35% drug degraded in the acidic buffer while 40-50% drug degraded in the neutral and alkaline buffers. It was very much evident from the stability data that the inclusion complexes were helpful in improving the solution stability of griseofulvin. Complex F5 was used as the complex of choice for stability analysis as it exhibited the highest aqueous solubility amongst all the inclusion complexes that were prepared. The results reveal that the kneading method was helpful in incorporating higher amount of griseofulvin in the complex, the inclusion complex of griseofulvin (F5) was subjected to short term accelerated stability testing by storing the complexes at room temperature and at 45°C. The samples were analyzed at an interval of one week, three weeks and six weeks for their physical appearance and drug content values. No appreciable changes observed with the above parameters.

Keywords: Griseofulvin, β-cyclodextrin, Physical and Kneading method, Higuchi-Connor method.

1. INTRODUCTION

Pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate, chemical stability, absorption, and bioavailability. Cyclodextrins have received increasing attention in the pharmaceutical field [1]. Cyclodextrins are cyclic malto oligosaccharides in which the glucose units are linked by α-1,4 glucosidic bonds [2]. Because of the particular arrangement of the glucose units, the molecule has a cone-like structure which makes the exterior of the cone hydrophobic in nature, leading to formation of inclusion complexes with various drugs into its cavity and resulting in improvement in solubility and drug release [3]. In the present work the interaction of β-cyclodextrin (β-CD) (which has larger cavity size [7.5 A] and better solubility than others) with Griseofulvin was investigated, with the aim of improving solution stability of griseofulvin by formulating as inclusion complexes.

2. MATERIAL AND METHODS

2.1. Material

Griseofulvin was purchased from Yarrow Pharmaceuticals and β-cyclodextrin was procured from Hi-media. All other reagents and chemicals were purchased from various sources and were of analytical grade. All the reagents, drug, polymers and chemicals were used without any purification. Distilled water was

prepared fresh in the laboratory at the time of used using distillation assembly.

2.2. Methods

2.2.1. Preformulation Studies [4]

2.2.1.1. Organoleptic characterization

A small quantity of pure griseofulvin powder was taken in a butter paper and viewed in well illuminated place to observe its color; the taste and odor were observed using tasting and smelling the drug.

2.2.1.2. Solubility

Solubility of griseofulvin was determined qualitatively in water, methanol and ethanol. Solubility studies were performed by shaking small amount of griseofulvin in test tubes containing the solvent and observing for undissolved particles (if any).

2.2.1.3. Melting point determination

The melting point of griseofulvin was determined by open capillary method by filling the drug in a capillary tube sealed at one end and placing it in the melting point apparatus to observe the temperature at which melting occured.

2.2.1.4. Drug polymer incompatibility Study

IR spectra of drug, polymer and a physical mixture of drug and polymer were obtained using FT-IR. The spectra were observed for physical and chemical incompatibility amongst the drug and the polymer under study.

2.2.1.5. Calibration curve of griseofulvin

Accurately weighed 10 mg of Griseofulvin was taken in 10 mL volumetric flask and dissolved in methanol to the mark resulting in a stock solution of 1000 µg/mL. 1 mL of the above stock solution was taken in another 10 mL volumetric and volume was made up with methanol to mark resulting in a solution of 100µg/mL. Aliquots of 1-6 mL of stock solution were taken into a series of 10 mL volumetric flask and volume was made up to the mark using methanol and were analyzed at 295 nm using UV spectrophotometer. A standard curve was constructed against absorbance and concentration.

2.2.2. pH solubility study of griseofulvin[5]

The pH solubility profile was studied by the shake flask method reported by Higuchi and Conners. The saturation solubility was determined by adding griseofulvin to 1 mL of various buffer solution until no more griseofulvin could be dissolved. The solution was allowed to equilibrate for 24 h by standing in dark and filtered. The concentration of griseofulvin in the solution was determined by UV-Visible spectrophotometer at 295 nm using the linearity equation.

2.2.3. Phase solubility study

Excess amount of griseofulvin (30 mg) was placed in separate amber colored bottles containing 20 ml of aqueous solutions of β-CD (2-16 mM) and samples were stirred continuously until equilibrium was achieved (5 days). Suspensions were filtered using 0.45 µm membrane filter and then analyzed spectro-photometrically at a 295 nm using aqueous solution of respective β-CD as blank. The stability constants was calculated using the Higuchi - Connor equation

$$K(1:1) = \text{slope}/so(1\text{-slope})$$

S0 = Intrinsic solubility of griseofulvin in aqueous complexation media (distilled water) "slope" was calculated from phase solubility diagram.

2.2.4. Preparation of inclusion complexes of griseofulvin with β-CD

Griseofulvin β-cyclodextrin (GCD) inclusion complexes were prepared by two different methods using different molar concentrations of β-cyclodextrin. The molar concentrations used and method adopted are summarized in table 1.

Table 1: Formula for preparation of GCD inclusion complexes

Method Used	Drug- β-CD ratio	Formulation Code
Physical Method	1:1	F1
	1:2	F2
	2:1	F3
Kneading Method	1:1	F4
	1:2	F5
	2:1	F6

2.2.4.1. Physical Method

Griseofulvin and β-Cyclodextrin in different molar ratios (1:1, 1:2 and 2:1) were mixed in a mortar for about one hour with constant trituration, passed through sieve No. 100 and stored in a desiccator over fused calcium chloride.

2.2.4.2. Kneading Method

Griseofulvin and β-cyclodextrin in different molar ratios (1:1, 1:2 and 2:1) were taken. For preparing the

complexes, β-CD was added to the mortar and a small quantity of 50% ethanol was added to it while triturating to get slurry like consistency. Then griseofulvin was slowly incorporated into the slurry and the trituration was further continued for one hour. The slurry was then air dried at 25°C for 24 h, pulverized and passed through sieve no. 100 and stored in desiccator over fused calcium chloride.

2.2.5. Evaluation of GCD inclusion complexes [6]

2.2.5.1. Determination of aqueous solubility

Excess amount of GCD inclusion complexes were kept in amber colored bottles containing 10 mL of distilled water and stirred on thermostatic flask shaker at 25°C for 5 days. Suspensions were filtered through 0.45 µm membrane filter, diluted adequately and analyzed using UV-Visible spectrophotometer at 295 nm.

2.2.5.2. Determination of pH stability

Griseofulvin and complexes with equivalent amounts were kept in amber colored bottles containing 10 mL of buffer solutions. Aliquots were taken at different time intervals, diluted adequately and analyzed spectrophotometrically at 295 nm.

2.2.5.3. Estimation of griseofulvin in complexes

GCD inclusion complexes equivalent to 25 mg were weighed and transferred to 25 mL volumetric flask and volume were made upto the mark with methanol. From this, 1 mL solution was taken in 10 mL volumetric flask and the volume was adjusted upto the mark with methanol. The absorbance of the solution was measured at 295 nm using methanol as the blank. The content of griseofulvin was calculated using calibration curve equation.

2.2.5.4. Stability study

The stability of the GCD inclusion complexes was determined at room temperature as well as at 45°C to accelerate the degradation of griseofulvin. The complexes were dissolved in water and stored at testing conditions. Samples were withdrawn at weekly intervals for duration of 6 weeks and the drug content was estimation by diluting with methanol and measuring the absorbance at 295 nm using UV-Visible spectrophotometer.

3. RESULTS AND DISCUSSION

Solubility of Griseofulvin was soluble in methanol and chloroform, slightly soluble in ethanol, insoluble in water. The melting point of Griseofulvin was 224-226°C and λ_{max} of Griseofulvin was found to be 295 nm by using U.V. spectrophotometer (Labindia-3000+) in linearity range 10-60 µg/ml (Fig.1). The FTIR spectrum of griseofulvin exhibited significant peaks of C-N stretch, C=O stretch, C-O-C stretch, N-H and O-H stretch and the peaks were compared to the standard spectra available at NIST. No deletion of the characteristic peaks of griseofulvin was found in the FTIR spectrum of the physical mixtures of drug and polymer. All the peaks were present in the physical mixture of griseofulvin and β-CD indicating a compatibility between the both the components (Figs.2-4). The saturation solubility of griseofulvin in various buffer solutions was determined spectrophotometrically and the results are presented in table 2. The results show that the solubility of griseofulvin was higher in the acidic solution as compared to neutral or basic solution. This may be attributed to the rapid degradation of griseofulvin in neutral and alkaline medium in comparison to the acidic solution. The stability constant for griseofulvin was calculated from the phase solubility diagram using the Higuchi-Connor equation. The result of phase solubility is presented in table 3.

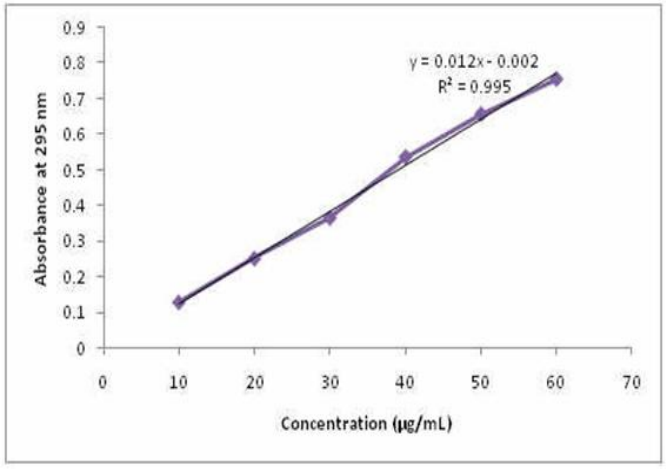

Fig. 1: Calibration curve of griseofulvin in methanol

Table 2: Saturation solubility of griseofulvin in various pH buffers

pH	Absorbance	Concentration of griseofulvin in solution (µg/mL)
1.2	1.246	42.79
2	0.963	33.03
4	0.628	21.48
5.8	0.522	17.82
7	0.325	11.03
7.2	0.322	10.93
8	0.312	10.58

From the diagram the following parameters were obtained for calculating the stability constant. Slope = 1.378, Intercept= 6.722, R^2= 0.701. The stability constant of griseofulvin [$K_{(1:1)}$] was calculated to be 774.89 M^{-1}. Phase solubility diagram of griseofulvin with β-CD illustrates the solubility enhancement capability of cyclodextrin. The aqueous solubility of the GCD inclusion complexes was determined using the Higuchi-Connor method (table 4, fig. 5). The results indicated that the use of kneading method for preparation of complexes was more effective than the physical mixture method. The solubility increased manifold in the inclusion complexes and was doubled by kneading method in comparison to the physical method. The result also revealed that the on increasing the molar ratio of β-CD increased the solubility whereas higher molar ratio of griseofulvin decreased the solubility. The stability of griseofulvin and the GCD inclusion complexes was determined at varioustime intervals in buffer solutions of varying pH (table 5, 6).

Table 3: Phase solubility data of griseofulvin

Concentration of β-CD (mM)	Abs	Conc of griseofulvin in solution (µg/mL)
0	0	0
2	0.239	8.06
4	0.533	18.20
8	0.712	24.37
12	0.699	23.93
16	0.691	23.65

Table 4: Aqueous solubility data of GCD inclusion complexes

Complex code	Aqueous solublity (µg/mL)
Griseofulvin	2.79
F1	127.29
F2	143.68
F3	109.75
F4	185.10
F5	193.79
F6	152.62

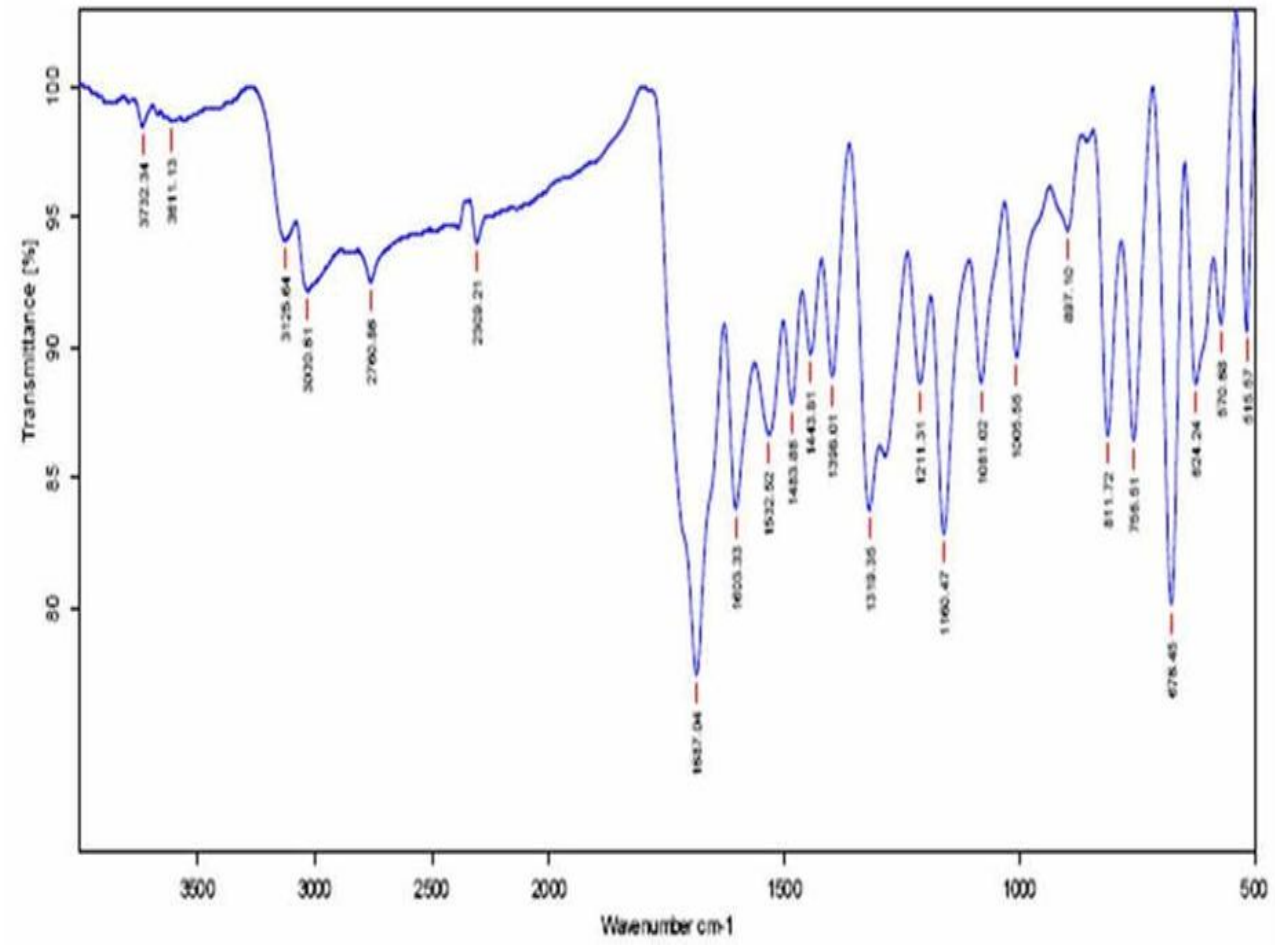

Fig. 2: FTIR spectra of griseofulvin

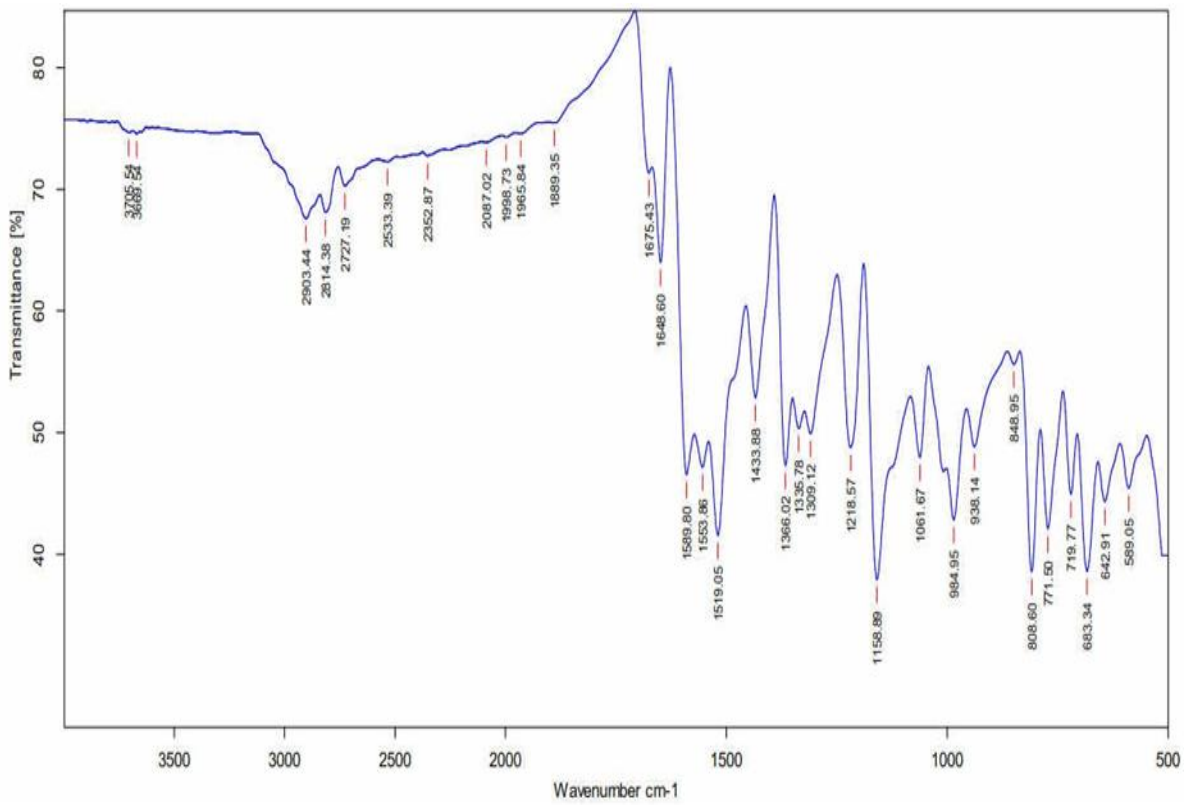

Fig. 3: FTIR spectra of β-CD

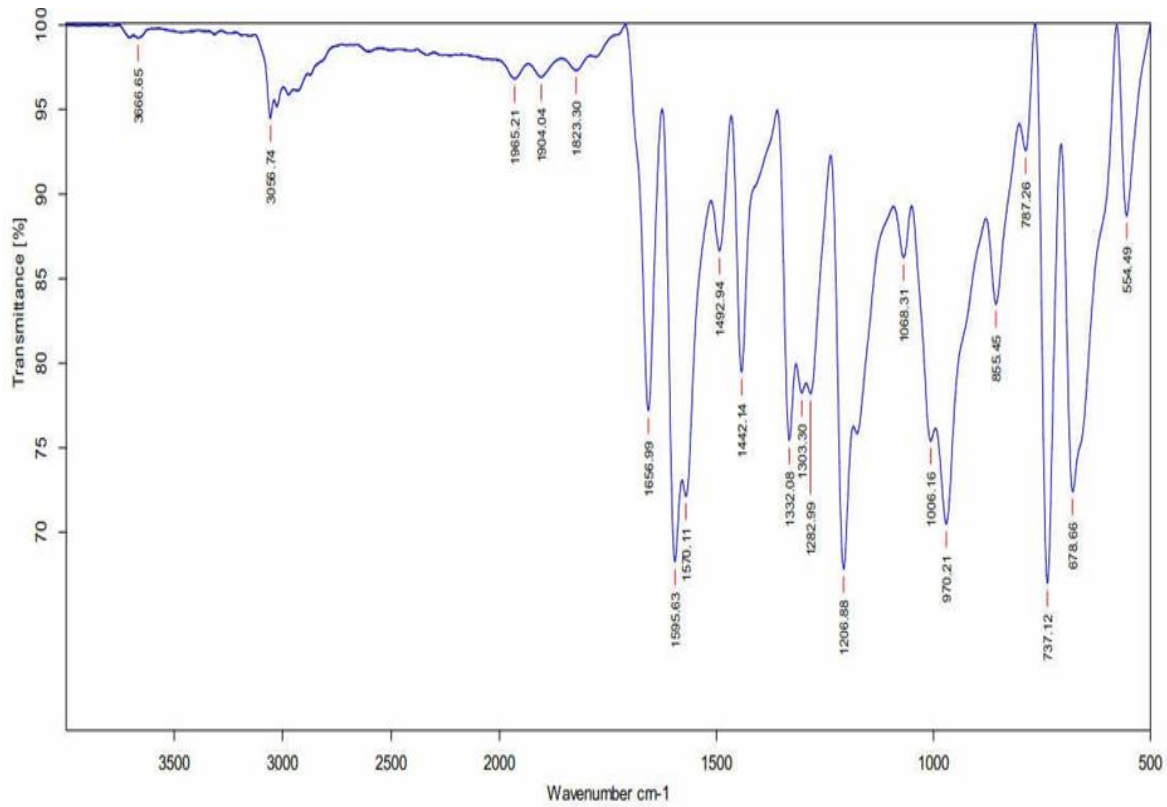

Fig. 4: FTIR spectra of physical mixture of griseofulvin and β-CD

Table 5: pH stability of griseofulvin in solution

Time (h)	% griseofulvin in solution						
	pH 1.2	pH 2.0	pH 4.0	pH 5.8	pH 7.0	pH 7.2	pH 8.0
0	100	100	100	100	100	100	100
0.5	88.27	85.35	84.63	81.22	65.27	60.24	58.31
1	76.81	75.19	74.91	71.69	61.59	57.96	54.62
2	70.11	68.26	65.84	62.48	54.33	51.42	48.94
4	62.49	59.54	56.63	51.95	47.65	45.11	41.29
6	58.33	53.22	51.18	44.26	41.28	38.66	35.56
8	54.17	50.44	48.92	41.66	35.85	34.39	31.67

Table 6: pH stability of griseofulvin in F5

Time(h)	% griseofulvin in solution						
	pH 1.2	pH 2.0	pH 4.0	pH 5.8	pH 7.0	pH 7.2	pH 8.0
0	100	100	100	100	100	100	100
0.5	96.44	95.53	90.67	85.23	81.22	76.87	73.69
1	91.81	89.85	85.94	81.14	78.19	74.32	68.66
2	87.88	85.17	81.39	76.58	73.96	71.29	61.08
4	85.67	81.29	78.61	74.93	70.45	62.49	59.54
6	82.29	80.05	74.98	68.27	65.67	58.33	53.22
8	80.15	76.88	69.56	65.84	60.24	54.17	50.44

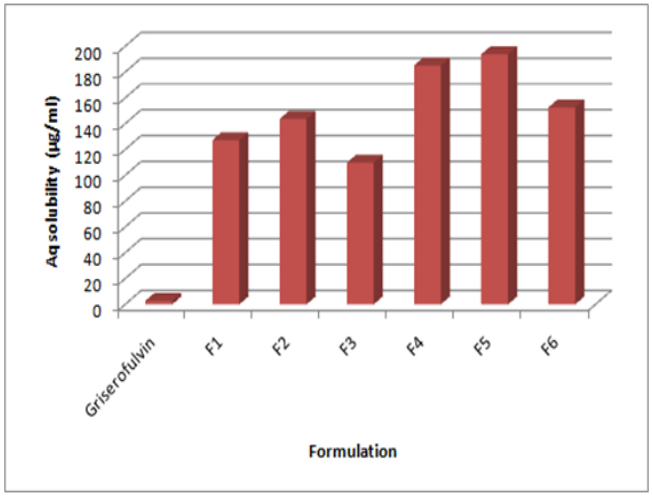

Fig. 5: Comparison of aqueous solubility of GCD inclusion complexes

The results show that griseofulvin was very unstable in the buffer solutions especially in the neutral and alkaline solutions. The degradation of griseofulvin occurred to an extent of 70% in the alkaline solution, 65% in the neutral solution and 60% in acidic solutions. The higher the acidity of the buffer solution, the more stable was griseofulvin. The acid buffer pH 1.2 exhibited 56% degradation of griseofulvin. The inclusion complexes were on the other hand was able to prevent the degradation of griseofulvin in all the pH. The degradation of griseofulvin from the inclusion complexes was found to be prevented and around 20 35% drug degraded in the acidic buffer while 40-50% drug degraded in the neutral and alkaline buffers. It was very much evident from the stability data that the inclusion complexes were helpful in improving the solution stability of griseofulvin. F5 was used as the complex of choice for stability analysis as it exhibited the highest aqueous solubility amongst all the inclusion complexes that were prepared. The drug content in the GCD inclusion complexes was determined spectrophotometrically (table 7) The results reveal that the kneading method was helpful in incorporating higher amount of griseofulvin in the complex, making it a method of choice for formation of the complexes. The drug-carrier molar ratio affected the incorporation of drug into the complex to a very little extent which could almost be neglected. 100% drug content was found in GCD complex using the kneading method and 1:2 drug-carrier ratio. The inclusion complex of griseofulvin (F5) was subjected to short term accelerated stability testing by storing the complexes at room temperature and at 45°C. The samples were analyzed at an interval of one week, three weeks and six weeks for their physical appearance and drug content values. No appreciable changes observed with the above parameters (table 8, 9).

Table 7: Griseofulvin content in inclusion complexes

Complex code	Average drug content*
F1	97.38 ± 0.71
F2	97.32 ± 0.58
F3	100.28 ± 0.31
F4	98.84 ± 0.82
F5	100.52 ± 0.29
F6	98.28 ± 0.82

**Average ± standard deviation; Average of three readings*

Table 8: Stability data of F5 at room temperature

Day	Color	Drug content (%)
1	White	100.04
7	White	99.83
21	White	99.71
42	White	98.85

Table 9: Stability data of F5 at 45°C

Day	Color	Drug content (%)
1	White	100.04
7	White	99.81
21	White	98.78
42	White	98.11

4. CONCLUSION

The objective of the study was to improve solution stability of griseofulvin by formulating as inclusion complexes. The following conclusions could be made from the study:

- Cyclodextrin can be used to prepare inclusion complex of griseofulvin with improved solubility of the drug.
- The phase solubility diagram reveals a 1:2 molar ratio complex
- The solubility and stability of griseofulvin was improved by formulating asinclusion complexes
- The formation of inclusion complex reduced the degradation of griseofulvin in various buffer solutions.
- The inclusion complexes were stable in accelerated conditions of stability testing.

Conflicts of Interst

None declared

Source of Funding

None declared

5. REFERENCES

1. Thakkar AL, Kuehn PB, Perrin JH, Wilham WL. *J Pharma Sci.,* 1972 1; **61(11)**:1841-3.
2. Maheswaran MM, Divakar S. *J Scienti Indust Res.,* 1994; **53(12)**:924-32.
3. Szejtli J. *Drug Invest.,* 1990; **2(4)**:11-21.
4. Goswami RG, Mishra B, Mishra R. *J Pharmacol Biomed.,* 2017; **1(4)**:129-133
5. Higuchi T, Connors KA. *Adv Anal Chem Instrument.,* 1965; **4**:117-212.
6. Ansari MJ, Ahmed MM, Fatima F, Anwer MK, Jamil S, Al-Shdefat R, Ali B. *Int J Biol Pharma Allied Sci.,* 2014; **3(11)**:2668-2675.