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Improvement of Griseofulvin's Solubility and Dissolving Characteristics Using Nano Crystallization

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Abstract

In the present study an attempt has been made to develop nanocrystals of Griseofulvin in order to enhance solubility and dissolution rate by decreasing particle size of drug.The results of compatibility studies by Infrared spectroscopy showed no interaction between the drug and stabilizers.The Griseofulvin nanocrystals were successfully prepared by emulsion solvent diffusion method using different concentrations of stabilizers.The presence of stabilizers made the nanocrystal formulations more stable with increasing drug release.Particle size analyzer used to explore the particle size of Griseofulvin nanocrystals showed a suitable particle size in the range of 80.3nm to 300.6 nm.The polydispersity index of selected nanocrystal formulations(F3C, F3D, F3E, F4D, and F4E) was less than 0.5, which indicated a narrow size distribution of particles. Zeta potential value of Griseofulvin nanocrystals showed a negative surface charge (-19.7mV to -24.7mV). In vitro release study of all the formulations were showed a increased drug release with increase in concentration of different stabilizers. Dissolution rate of all the formulations were improved when compared to pure drug. On the basis of drug release data F3C, F3D, F3E, F4D, and F4E showed a good release profile with more than 80% in 2 hours. The solubility of selected formulations (F3C, F3D, F3E, F4D, and F4E) in phosphate buffer pH(6.8) increased ten folds when compared to pure drug. SEM studies confirmed the morphology of the nanocrystal formulations. The crystalline state of the nanocrystal formulation was not altered according to the XRPD analysis. it was concluded that nanocrystallization was a good approach to enhance the dissolution property of Griseofulvin by emulsion solvent diffusion method. The solubility and in vitro dissolution studies suggested that the nanocrystal formulations can improve the bioavailability of the Griseofulvin by improving its solubility and dissolution rate when compared

to pure drug. Thus nanocrystal drug delivery system can adopted to increase the solubility and dissolution rate of poorly soluble drug like Griseofulvin to enhance their bioavailability.

Keywords: Formulation, Evaluation, Griseofulvin, Nanoparticles

INTRODUCTION

Over the years, nanoparticles (NPs) made of both organic and inorganic materials have been engineered to circumvent the biological barriers and deliver drugs for a variety of indications.[1], [2] Water-insoluble or hydrophobic drugs, pose a challenge in terms of achieving optimal bioavailability and thereby, adequate efficacy.[3] As reported in 2015, 40% of drugs on the market and 90% of drugs within the discovery pipeline face solubility issues.[4] Other statistics, cite 40% of all potential drug candidates were shelved as a result of intrinsic aqueous solubility issues.[5] Thus, a number of hydrophobic drugs, which could potentially be useful for treatments are in need of clinically acceptable carriers.[6]

For the purpose of this review article, drug nanocrystals may be defined as pure solid particles with a mean diameter <1 μm and a crystalline character. The platform offers an exceptional opportunity to deliver hydrophobic drugs. Its uniqueness originates from the fact that nanocrystals are composed entirely of 100% drug or the payload thereby eliminating the ancillary role of a carrier.[7] In addition, surfactants or stabilizers are commonly used to stabilize the crystalline dispersions in liquid media.

Nanocrystalline drug technology improves the solubility of hydrophobic drugs due to an increased surface area to volume ratio and improved dissolution rates (i.e., dissolution velocity) associated with nanosizing.[8] The drug crystals are singularly well-suited for the rehabilitation of previously unsuccessful Biopharmaceutics Classification System (BCS) Class II and IV drugs (low solubility drugs).[9] The BCS classification system is an experimental model that measures permeability and solubility under prescribed conditions. The system divides the drugs into four classes. While Class I drugs have high solubility and high permeability, Class II molecules have low solubility and high permeability, Class III identifies with high solubility and low permeability, and drugs in Class IV have low solubility and low permeability.[4]

Nanocrystal drug formulations have also been shown to be stable in suspensions and are often referred to as nanocrystal colloidal dispersions (NCD's). The dispersions provide a platform for easy scale-up and manufacturing of highly stable and marketable products. Their synthesis and scale-up considerations have been described at length elsewhere.[10], [11] Commonly used synthesis techniques include the use of microfluidic based platforms or the milling method, which, among others, is both flexible and tunable.[7], [12], [13], [14], [15], [16], [17] Taken together, the nanocrystal drug technology has been studied extensively and is well positioned for further exploration in the field of drug delivery.

Several hydrophobic drugs have been salvaged via the nanocrystal formulation method. The drugs were successfully developed, and approved by the FDA to treat a variety of indications ranging from dental disorders to cancer in the clinic.[14], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28] Depending on the disease, the approved formulations can be administered via different routes including oral, dermal, and parenteral.

This highlights the versatility of a nanocrystal drug platform. Pharmacokinetic, biodistribution, and bioavailability data for organs involved in delivery routes tested using nanocrystal technology have been addressed at length previously.[10], [13], [18], [24], [25], [29], [30], [31], [32], [33], [34] Specifically, the reviews of Lu et al. 2016 and 2017 delve into the biodistribution pattern of nanocrystal drugs in the blood, heart, liver, spleen, lung, kidney, tumor, and thymus (i.e., the organs involved in clearance/circulation and host immune responses).[24], [35]

Griseofulvin is for the treatment of ringworm infections of the skin, hair, and nails, namely: tinea corporis, tinea pedis, tinea cruris, tinea barbae, cradle cap or other conditions caused by Trichophyton or Microsporum fungi.

The present study is carried out to develop nanocrystal of Griseofulvin, in order to enhance solubility and bioavailability, by decreasing the particle size of the drug. Drugs nanocrystals are prepared by emulsion solvent diffusion method. Solubility and dissolution profile of obtained nanocrystals are compared with pure drug.

MATERIALS
MATERIALS USED:

MATERIALS NAME	**SUPPLIERS**
Griseofulvin	n Dr.Reddy's Laboratories,Hydrabad.
Hydroxy Propyl Methyl Cellulose (HPMC) K15M	rom Steril-gene Life science (P)Ltd, Pondicherry.
yrrolidone (PVPK30)	m Shasun Pharmaceuticals,Pondicherry.
β Cyclodextrin(BCD)	Nice chemicals, Cochin.
Sodium Lauryl Sulphate (SLS)	nd chemicals (P) Ltd, NewDelhi.
ne Glycol (PEG)6000	ratory Chemicals (P) Ltd,Mumbai.

EXPERIMENTAL PROTOCOL

1. STANDARD CURVES FOR GRISEOFULVIN:

a) Preparation of calibration medium (Indian Pharmacopoeia 2010)**:**

Phosphate Buffer pH 6.8:

A known volume (50ml) of 0.2M potassium dihydrogen phosphate is placed in a 200ml volumetric flask. 22.4ml of 0.2M sodium hydroxide is added and makeup to the volume with distilled water.

- ***0.2 M potassium dihydrogen phosphate:***

A known quantity (27.218 g) of potassium dihydrogen phosphate is dissolved and diluted to 1000ml with water.

- ***0.2 M sodium hydroxide:***

A known quantity (8 g) of sodium hydroxide is dissolved and makeup to 1000ml with water.

b) Estimation of absorption maximum (λmax):

A known weight (10 mg) of drug (Griseofulvin) is dissolved in sufficient amount of methanol in 100ml volumetric flask and make upto 100ml with phosphate buffer pH (6.8) to prepare a primary stock solution (100µg/ml). The stock solution is further diluted using a phosphate buffer pH (6.8) solution to 10µg/ ml concentration. The resultant solution is scanned in the range of (200- 400nm) by UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) to get absorption maximum (λ max).

c) Preparation of standard curves:

From the above prepared stock solution, (5 to 25µg/ml) concentration solutions are prepared using the phosphate buffer pH (6.8) solution. The absorbance of these solutions are measured at λ max by UV-spectrophotometer (UV-1700 Shimadzu corporation, Japan). A standard curve is plotted using concentration on X- axis and the absorbance obtained on Y-axis. (Harish Chander., *et al*, 2011)

2. PREFORMULATION (COMPATABILITY) STUDIES:

preformulation studies are carried out by Infrared spectrophotometer in orderto evaluate the drug and stabilizer interaction.

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectrum of the drug, stabilizers, and its physical mixtures are obtained by using IR spectrophotometer (Spectrum RX-1 Perkin Elmer, German). The pellets are prepared on KBr-press under hydraulic pressure of 150kg / cm^2; the spectrais scanned over the wave number range of 4000 to 400 cm^{-1} at the ambient temperature (Sinco.C *et al*., 2011).

3. FORMULATION OF GRISEOFULVIN NANOCRYSTALS:

The nanocrystals are prepared by emulsion solvent diffusion method. The preparation process involves following steps (Phanchaxari M Dandagi *et al*., 2011).

a) Formulation of Griseofulvin nanosuspensions;

b) Lyophilization of nanosuspensions to obtain the nanocrystals.

a) Formulation of Griseofulvin nanosuspensions:

i) Preparation of drug solution:

Accurately weighed sample (100 mg) of drug is added to methanol (10 ml) to prepare the drug solution.

ii) Addition of drug solution to aqueous solution containing stabilizers:

The above prepared drug solution is to be added into required quantity (10ml) of water containing stabilizers **(HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000)** with continuous stirring onhomogenizer at 1000 rpm for 2 hours

iii) Removal of solvent:

The organic solvent is removed by continuous stirring for 3-4 hours at 500 rpm (Phanchaxari M Dandagi *et al*., 2011).

b) Lyophilization of nanosuspensions to obtain the nanocrystals:

Griseofulvin nanosuspensions are lyophilized by using freeze dryer (Lyodel- Delvac Pumps Pvt. Ltd, USA) to enhance the chemical stability of nanocrystals. The freshly prepared nanosuspensions are lyophilized with cryoprotective agent (mannitol). Briefly, Griseofulvin nanosuspensions are rapidly cooled down to -50^0C for 2 hours followed by primary drying at 1.03 mbar and secondary drying at 0.001 mbar (Rainer Muller. H *et al*., 2008).

4. CHARACTERIZATION OF GRISEOFULVIN NANOCRYSTALS:

All the formulations are evaluated for its particle size, zeta potential, drug content, and *in vitro* drug release studies

a) Determination of drug content:

Sample containing 10 mg equivalent of Griseofulvin nanocrystals are weighed and dissolved in methanol, and the volume is made upto 100ml with phosphate buffer pH(6.8). From the above solution 10 ml is pipetted out and made upto 100 ml with phosphate buffer pH(6.8). The absorbance of resulting solution is determined at λmax (277 nm) using UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) and the drug content is estimated (Phanchaxari M Dandagi *et al*., 2010).

b) *In vitro* dissolution studies:

USP dissolution apparatus Type II (paddle method) at rotation speed of 100 rpm is used for *in vitro* testing of drug dissolution of all nanocrystal formulations. For each batch, sample of 10 mg equivalent Griseofulvin nanocrystals containing in capsules are taken and subjected to dissolution studies with 900 ml of phosphate buffer pH(6.8) as dissolution medium. Bath temperature is maintained at 37±0.5°C throughout study.

A sample (5 ml) of the solution is withdrawn from the dissolution apparatus at predetermined time intravels of 5, 10, 20, 30, 40, 60, 90, and 120mins. The samples are replaced with fresh dissolution medium. Absorbance values of sample solutions are measured at λmax (277 nm) in UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan). The cumulative percentage drug release is calculated (Doaa Ahmed El-Setouhy *et al*., 2011)**.**

c) Determination of Particle size and Zeta potential:

The mean particle size (z-average), and zeta potential of Griseofulvin nanocrystal formulations are determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd., UK). The freeze dried powders are redispersed with water to obtain a proper scattering intensity before measurement(Dianrui Zhang *et al*., 2012).

d) Solubility studies:

Solubility of Griseofulvin nanocrystal formulations are studied in different solvents such as distilled water and phosphate buffer pH(6.8). An excess amount of nanocrystal formulation is added in 10 ml of the pertinent solvents. The mixtures are stirred in a mechanical shaker for 24 hours. Visual inspection is carefully made to ensure there are excess Griseofulvin solids in the mixture, indicating saturation have beenreached. The mixtures are then filtered and filtrates are diluted suitably to determine the solubility of Griseofulvin in each solvent (Abdul Hasan Sathali.A., and Gopinath.M., 2013).

5. SELECTION AND EVALUATION OF BEST FORMULATION:

The best formulation is selected depending on the results obtained from particle size, *in vitro* drug release studies and solubility studies.

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectrum analysis are carried out for the selected nanocrystal formulation to find out the interactions between the drug and excipients by using IR spectrophotometer (Spectrum RX-1 Perkin Elmer, German). The pellets are prepared on KBr-press under hydraulic pressure of 150kg / cm^2; the spectra is scanned over the wave number range of 4000 to 400 cm^{-1} at the ambient temperature (Sinco.C *et al*., 2011).

b) Morphological studies of nanocrystals by using Scanning Electron Microscopy(SEM):

Morphological evaluation of the selected Griseofulvin nanocrystal formulation is carried out in scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan). Allsamples are examined on a

brass stub using carbon double-sided tape. Powder samples are glued and mounted on metal sample plates. The samples are gold coated (thickness ≈15–20 nm) with a sputter coater (Fison Instruments, UK) using an electrical potential of 2.0 kV at 25 mA for 10 min. An excitation voltage of 20 kV was used in the experiments (Yuan Gao *et al*., 2011).

c) X-ray Powder Diffraction (XRPD) analysis:

The crystalline state of the samples, including the drug and freeze-dried powders are studied in X-ray diffractometer (XRD-462, Digaku, Japan). XRPD is carried out in symmetrical reflection mode using Copper line as the source of radiation and the wavelength is set at 1.5405A˚. Standard runs using a 40 kV and 30 mA in this process. Samples are performed with a scanning rate of 0.1000˚/min andthe scanning range of the 2θ from the initial angle 4˚ to the final angle 90˚ (Dianrui Zhang *et al*., 2011).

RESULTS AND DISCUSSION

1. STANDARD CURVES FOR GRISEOFULVIN

a) Preparation of calibration medium

The calibration medium pH (6.8) were prepared by using phosphate buffer asper the I.P procedure.

b) Estimation of absorption maximum (λmax)

The λ max of Griseofulvin was estimated by scanning the 10µg/ml concentration of the drug solution in buffer solution of pH (6.8). It showed the λ max of 277nm (Narendra Chary., *et al*., 2012) in buffer solution of pH (6.8).

Preparation of standard curves

The standard curves of Griseofulvin prepared by using phosphate buffer pH(6.8). The linear correlation coefficient was found to be 0.9995 for pH (6.8). Griseofulvin obeys the Beer's law within the concentration range of 5 to 25 µg/ml.

2. PREFORMULATION (COMPATABILITY) STUDIES:

a) Infrared (IR) spectroscopic studies

Infrared (IR) spectroscopic studies were carried out to confirm the compatibility between drug and the stabilizers used for the preparation of nanocrystals. The IR studies were performed for pure drug, stabilizers and physical mixture of drug with stabilizers. The spectra studied at 4000cm^{-1} to 400 cm^{-1}. The principal peaks for pure drug were observed at wave numbers 1373.36 cm^{-1}, 1228.70 cm^{-1}, 1186.26 cm^{-1}, 1063.78 cm^{-1},
815.92 cm^{-1}, 753.23 cm^{-1}, 701.15 cm^{-1}. It was found from the spectra that there wasno major shifting as well as any loss of functional peaks in the spectra of drug, stabilizers and physical mixture of drug with stabilizers. This clearly indicated that there was **no interaction** between the drug and the polymer and the drug was present in its unchanged form.

3. FORMULATION OF GRISEOFULVIN NANOCRYSTALS

The Griseofulvin nanocrystals were prepared by emulsion solvent diffusion method (Phanchaxari M Dandagi *et al*., 2011). The principle of this method was based on the dissolution of the active drug substance in an organic solvent which was then added into a nonsolvent (miscible with the organic solvent). In the presence of, thereafter, the nanocrystals were precipitated. Basic advantage of the precipitation technique was that it was simple and had a low cost. Also, scale up was simple in this method (Phanchaxari M Dandagi *et al*., 2011).

Griseofulvin nanocrystals were prepared by following process:

a) Formulation of Griseofulvin nanosuspensions;

b) Lyophilization of nanosuspensions to obtain the nanocrystals.

Various formulations of Griseofulvin nanocrystals (F1 - F25) were prepared by using different stabilizers like HPMC K15M, PVP K30, β Cyclodextrin, SLS, and PEG 6000 at different concentrations (0.1%, 0.2%, 0.3%, 0.4%, 0.5%).

CHARACTERIZATION OF GRISEOFULVIN NANOCRYSTALS

All the formulations were evaluated for its drug content, particle size, polydispersity index, and zeta potential, *in vitro* drug release studies and solubility studies.

a) Determination of drug content

The drug content of all nanocrystal formulations (F1 to F25) was in the range of 88.00% to 96.00%. The results suggest that the process employed to prepare the nanocrystals shown uniform distribution of drug.

b) *In vitro* dissolution studies

The *invitro* dissolution studies of all formulations were compared with pure drug.. when compared the *In vitro* release profile of all the formulations are significantly greater than that pure drug Griseofulvin.

Formulations (F1A – F1E) prepared using different concentration of stabilizer (HPMC K15M 0.1% to 0.5%) shown the percentage drug release of 53.39%, 61.10%,
67.97%, 71.12%, and 74.81% at 120 mins respectively.

Formulations (F2A – F2E) prepared using different concentration of stabilizer (PVP K30 0.1% to 0.5%) shown the percentage drug release of 73.29%, 74.04%,
75.68%, 75.67%, and 78.78% at 120 mins respectively.

Formulations (F3A – F3E) prepared using different concentration of stabilizer (β-cyclodextrin 0.1% to 0.5%) shown the percentage drug release of 75.69%, 77.98%,

81.12%, 81.12%, and 83.43% at 120 mins respectively.

Formulations (F4A – F4E) prepared using different concentration of stabilizer (SLS 0.1% to 0.5%) shown the percentage drug release of 60.46%, 72.49%, 78.69%,
81.02%, and 87.92% at 120 mins respectively.

Formulations (F5A – F5E) prepared using different concentration of stabilizer (PEG 6000 0.1% to 0.5%) shown the percentage drug release of 57.18%, 61.06%,
64.17%, 67.23%, and 71.76% at 120 mins respectively.

The percentage drug release of all the formulations were found to be in the following order

0.1% < 0.2% < 0.3% < 0.4% < 0.5%

The release rate of the drug from the nanocrystals were increased, on increasing the stabilizers concentration.

The increased percentage drug release of stabilizer (HPMC K15M) having formulation (F1A to F1E) indicates that, stabilizer HPMC K15M was used for the suspension's stabilization as this water soluble polymer offers adequate surface active properties and it indicates that they have increased the drug release (Amighi.K *et al*., 2005).

The increased percentage drug release of stabilizer (PVP K30) having formulation (F2A to F2E) indicates that, stabilizer PVP K30 was used as water soluble compound, in order to improve the drug dissolution rate (NoushinBolourchian *et al*., 2013).

The increased percentage drug release of stabilizer (β-cyclodextrin) having formulation (F3A to F3E) indicates that, stabilizer β-cyclodextrin to form a network through intermolecular interaction, that colud protect and it can self-associate in aqueous solution to form nano-scale aggregates that have a minimum hydrodynamic radius in order to improve the drug dissolution rate (Phanchaxari M Dandagi *et al*., 2011).

The increased percentage drug release of stabilizer (SLS) having formulation (F4A to F4E) indicates that, SLS was used as dispersion stabilizer, it prevents agglomeration of precipitated nanocrystal in the formulation by increasing the activation energy and reduce the surface tension existing between the drug particle and the solvent by providing wettability to the particls, in order to improve the drug dissolution rate (Phanchaxari M Dandagi *et al*., 2011).

The increased percentage drug release of stabilizer (PEG 6000) having formulation (F5A to F5E) indicates that, stabilizer PEG 6000 had long hydrophilic chain and it captured the water molecule through hydrogen bonding, which were formed between the hydroxyl group and ether bond of PEG and water molecule, in order to improve the drug dissolution rate (Peng Liu *et al*., 2011).

c) Determination of Particle size and Zeta potential:

i) Particle size:

Particle size, size distribution and zeta potential were important characterizations of the nanocrystals

because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances (Dianrui Zhang *et al*., 2012).

In the present study the particle size of Griseofulvin nanocrystals were ranged between 80.3nm to 300.6 nm.

ii) Zeta potential

The zeta potential of the nanocrystals were allowed predictions about the storage stability of colloidal dispersions. In general, the zeta potential value at least
±30 mV can ensure the physically stable nanocrystals when using ionic surfactants forelectric repulsion; however, a zeta potential about ±20 mV can also signify long-term stability of the system when nonionic surfactants were applied for steric hindrance. In addition, the type of a key variable that had prominent effect on the mean zeta potential value (Dianrui Zhang *et al*., 2012).

The Griseofulvin nanocrystals were characterized to evaluate the effect of stabilizers at different ratios and different on surface charge of nanocrystals.

Zeta potential values of the formulations code (F3C – F3E and F4D – F4E) prepared with different showed negative zeta potential (-19.7mV to -24.7mV) which indicated a stable preparation.

d) Solubility studies

Solubility studies of pure drug (GRISEOFULVIN) and selected formulation (F3C to F3E and F4D to F4E). Nanocrystal formulations (F3C to F3E and F4D to F4E) shown highest solubility in distilled water as compared with pure drug.

The solubility of formulations (F3C, F3D, F3E, F4D, F4E) and pure drug in phosphate buffer pH (6.8) were 1.177mg/10ml, 2.791mg/10ml, 5.680mg/10ml, 2.294mg/10ml, 5.182mg/10ml and 0.547mg/10ml respectively. Thus the solubility of GRISEOFULVIN in nanocrystal formulations (F3E and F4E) was increased approximately by ten folds when compared to pure drug.

Hence, the noticeable increased saturation solubility of Griseofulvin in the formulation of nanocrystals was mainly attributed to the decreased particle size and increased surface area. The results can be explained by the Ostwald–Freundlich equation which demonstrates that the saturation solubility of the drug increases with reduction of particle size (Dianrui Zhang *et al*., 2012).

4. SELECTION AND EVALUATION OF BEST FORMULATION:

From the above results characterization, the following two formulations were selected as the best formulation showing,

For F3E

Particle size : 80.3 nm.

In vitro drug release : 85.74% in 2 hours Solubility studies :
3.565mg/10ml distilled water
5.68mg/10ml phosphate buffer pH(6.8)

For F4E

: 100.8 nm.

In vitro drug release

Particle size

: 87.91% in 2 hours Solubility studies :

3.068mg/10ml distilled water

5.182mg/10ml phosphate buffer pH(6.8)

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectroscopic studies were carried out for pure drug, stabilizers and selected nanocrystal formulations. It was found from the spectra that there was no major shifting as well as any loss of functional peaks in the spectra of drug, stabilizers and selected formulations **(F3E and F4E)**. This clearly indicated that there was **no interaction** between the drug and the polymer and the drug was present in its unchanged form.

b) Morphological studies of nanocrystals by using Scanning Electron Microscopy(SEM):

The morphology of selected nanocrystal formulations (F3E and F$E) was examined by Scanning Electron Microscopy (SEM). It was observed that the particle size of Griseofulvin nanocrystal formulations (F3E and F4E) was relatively small, uniform,crystal in shape and the mean size of particles were lower than 1 µm. This data was agreement with obtained data from Malvern zeta sizer analysis **(Rainer Muller .H *et al*., 2008)**.

c) X-ray Powder Diffraction (XRPD) analysis:

The XRPD patterns of pure drug (Griseofulvin) and formulations (F3E, F4E) were presented.

The XRPD patterns of pure drug showed numerous sharp peaks (at 2θ 15.00°, 16.80°, 20.90°, 23.00°, 25.40° and 34.31°) which are the characteristic of a crystallinecompound.

This result confirmed that the characteristic peaks were still preserved indicating the crystalline state was not changed.

As we know, the amorphous form can generally enhance the dissolution rate and bioavailability of drugs due to its high-energy. According to that principle and with the XRPD analysis considered, the enhancement of dissolution rate of Griseofulvinmay be due to the reduction of particle size or the influence of stabilizers rather than the appearance of amorphous form. Moreover, compared with the amorphous form, the maintenance of crystalline state was beneficial to a long-term stability **(Dianrui Zhang *et al*., 2012)**.

TABLE 1: CALIBRATION OF GRISEOFULVIN USING PHOPSPHATE BUFFER pH (6.8)

S. NO	CONCENTRATION(µg/ml)	ABSORBANCE ± SD*
1	05	0.023 ± 0.0035
2	10	0.042 ± 0.0025
3	15	0.065 ± 0.0060
4	20	0.083 ± 0.0083
5	25	0.102 ± 0.0090

n=3* **γ = 0.99958**

TABLE 2: IR PEAKS OF DRUG, STABILIZERS, PHYSICAL MIXTURE OF DRUG WITH STABILIZERS AND FORMULATIONS

S. NO	DESCRIPTION	ERISTIC PEAKS (cm^{-1})OBTAINED
1	**GRISEOFULVIN**	1373.36, 1228.70, 1063.78, 815.92, 753.23, 701.15
2	**HPMC K15M**	3642.69, 2934.79, 1650.16, 1459.20, 945.15
3	**PVP K30**	3748.78, 3398.69, 2956.01, 1667.52,1502.60
4	**β-CYCLODEXTRIN**	1490.06, 1457.27, 1363.72, 948.04, 651.00, 431.10

5	**SLS**	1654.01, 1470.77, 1248.95, 834.24, 634.60, 591.20
6	**PEG 6000**	2165.17, 1650.16, 1469.81, 1240.27, 961.55
7	**GRISEOFULVIN + HPMC K15M**	2935.76, 1743.71, 1187.23, 755.16, 355.88
8	**OFULVIN + PVP K30**	2867.28, 1654.01, 1442.80, 1187.23, 701.15
9	**GRISEOFULVIN+ β-CD**	2932.86, 1653.05, 1157.33, 1029.06, 702.11
10	**GRISEOFULVIN+ SLS**	2920.32, 1743.71, 1654.01, 1465.95, 1081.44
11	**OFULVIN + PEG 6000**	3281.02, 1349.25, 1280.78,845.81, 751.30
12	**ULATION(F3E)**	2932.86, 1653.05, 1157.33, 1029.06, 702.11
13	**ULATION(F4E)**	2920.32, 1743.71, 1654.01, 1465.95, 1081.44

TABLE 3: COMPOSITION OF GRISEOFULVIN NANOCRYSTALS

S.NO	FORMULATION CODE	SOLVENT/ORGANIC PHASE	DRUG CONCENTRATION(mg/10ml)	STABILIZERS CONCENTRATION(%)
1	F1A	METHANOL	100mg in 10ml	HPMC K15M(0.1%)
2	F1B	METHANOL	100mg in 10ml	HPMC K15M(0.2%)
3	F1C	METHANOL	100mg in 10ml	HPMC K15M(0.3%)
4	F1D	METHANOL	100mg in 10ml	HPMC K15M(0.4%)
5	F1E	METHANOL	100mg in 10ml	HPMC K15M(0.5%)
6	F2A	METHANOL	100mg in 10ml	PVP K30(0.1%)
7	F2B	METHANOL	100mg in 10ml	PVP K30(0.2%)
8	F2C	METHANOL	100mg in 10ml	PVP K30(0.3%)
9	F2D	METHANOL	100mg in 10ml	PVP K30(0.4%)
10	F2E	METHANOL	100mg in 10ml	PVP K30(0.5%)
11	F3A	METHANOL	100mg in 10ml	BCD(0.1%)
12	F3B	METHANOL	100mg in 10ml	BCD(0.2%)
13	F3C	METHANOL	100mg in 10ml	BCD(0.3%)
14	F3D	METHANOL	100mg in 10ml	BCD(0.4%)
15	F3E	METHANOL	100mg in 10ml	BCD(0.5%)
16	F4A	METHANOL	100mg in 10ml	SLS(0.1%)
17	F4B	METHANOL	100mg in 10ml	SLS(0.2%)
18	F4C	METHANOL	100mg in 10ml	SLS(0.3%)
19	F4D	METHANOL	100mg in 10ml	SLS(0.4%)
20	F4E	METHANOL	100mg in 10ml	SLS(0.5%)
21	F5A	METHANOL	100mg in 10ml	PEG6000(0.1%)
22	F5B	METHANOL	100mg in 10ml	PEG6000(0.2%)
23	F5C	METHANOL	100mg in 10ml	PEG6000(0.3%)
24	F5D	METHANOL	100mg in 10ml	PEG6000(0.4%)
25	F5E	METHANOL	100mg in 10ml	PEG6000(0.5%)

TABLE 4: DRUG CONTENT OF GRISEOFULVIN NANOCRYSTALS

S.No	STABILIZERS CONCENTRATION	FORMULATION CODE	AVG±SD
1	HPMC K15M(0.1%)	F1A	93.63±1.6226
2	HPMC K15M(0.2%)	FIB	89.63±1.9561
3	HPMC K15M(0.3%)	F1C	89.70±1.3856
4	HPMC K15M(0.4%)	F1D	88.00±0.0000
5	HPMC K15M(0.5%)	F1E	89.50±2.0621
6	PVP K30(0.1%)	F2A	92.86±1.8013
7	PVP K30(0.2%)	F2B	92.00±1.6055
8	PVP K30(0.3%)	F2C	90.33±1.0237
9	PVP K30(0.4%)	F2D	93.66±1.1547
10	PVP K30(0.5%)	F2E	93.00±1.4641
11	BCD(0.1%)	F3A	95.86±0.5011
12	BCD(0.2%)	F3B	95.33±1.5166
13	BCD(0.3%)	F3C	91.66±1.1547
14	BCD(0.4%)	F3D	95.33±1.5166
15	BCD(0.5%)	F3E	93.00±1.0000
16	SLS(0.1%)	F4A	90.66±1.5166
17	SLS(0.2%)	F4B	91.66±1.1547
18	SLS(0.3%)	F4C	94.33±1.1547
19	SLS(0.4%)	F4D	92.33±1.1547
20	SLS(0.5%)	F4E	96.00±1.7320
21	PEG6000(0.1%)	F5A	93.66±1.1547
22	PEG6000(0.2%)	F5B	95.33±0.5166
23	PEG6000(0.3%)	F5C	96.00±1.7320
24	PEG6000(0.4%)	F5D	95.33±1.5166
25	PEG6000(0.5%)	F5E	96.00±1.7320

n=3*

TABLE 5a: *IN VITRO* RELEASE PROFILE OF GRISEOFULVIN NANOCRYSTALS CONTAINING HPMC K15M AS STABILIZER

IME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F1A±SD	F1B±SD	F1C±SD	F1D±SD	F1E± SD	
5	12.85±1.23	21.05±1.29	24.04±1.23	30.00±1.29	27.02± 1.29	9.87±1.29
10	18.89±1.42	27.14±1.29	30.88±1.24	36.88±1.29	33.88± 1.29	15.89±1.29
20	25.70±1.44	32.51±1.30	37.02±1.60	43.05±1.29	40.03± 2.24	22.69±1.30
30	31.16±1.49	38.07±1.32	42.65±1.64	48.77±1.31	47.21± 1.32	28.10±1.32
40	37.03±1.36	43.87±1.30	49.90±1.45	54.47±1.30	53.68± 2.24	34.00±1.30
60	43.20±2.64	50.82±1.31	56.14±1.53	59.99±1.29	60.68± 1.25	40.90±1.31
90	47.91±0.66	55.58±1.32	61.66±1.49	66.28±1.31	67.73± 2.26	45.60±1.32
120	53.39±1.51	61.10±2.26	67.97±2.33	71.12±1.32	74.81± 2.28	50.32±1.33

n=3*

TABLE 5b: *IN VITRO* RELEASE PROFILE OF GRISEOFULVIN NANOCRYSTALS CONTAINING PVP K30 AS STABILIZER

IME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F2A±SD	F2B±SD	F2C±SD	F2D±SD	F2E±SD	
5	28.51±2.23	25.53±1.29	32.24±1.29	34.48±1.29	36.71±1.29	9.87±1.29
10	33.14±2.24	32.38±1.29	38.38±1.29	39.14±1.29	42.88±1.29	15.89±1.29
20	40.04±2.26	39.27±1.30	45.31±1.30	43.83±1.30	48.34±1.30	22.69±1.30
30	45.71±2.29	45.69±2.27	51.06±1.32	49.56±1.32	54.14±1.32	28.10±1.32
40	52.19±1.32	53.65±2.25	57.49±1.30	56.75±1.30	59.79±1.30	34.00±1.30
60	59.19±1.33	60.66±2.26	63.02±2.24	62.28±1.28	66.08±1.30	40.90±1.31
90	66.22±1.33	66.96±2.61	69.33±1.32	68.59±1.31	73.16±1.31	45.60±1.32
120	73.29±1.34	74.04±2.62	75.68±1.31	75.67±1.32	78.78±1.32	50.32±1.33

n=3*

TABLE 5c: *IN VITRO* RELEASE PROFILE OF GRISEOFULVIN NANOCRYSTALS CONTAINING β-CYCLODEXTRIN A STABILIZER

IME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F3A±SD	F3B±SD	F3C±SD	F3D±SD	F3E±SD	
5	32.24±1.29	34.48±1.29	38.20±1.29	40.44±1.29	42.68±1.29	9.87±1.29
10	38.38±1.29	41.38±1.29	45.13±1.29	47.38±1.29	49.63±1.29	15.89±1.29
20	45.31±1.30	47.57±1.29	52.09±1.30	54.35±1.30	56.61±1.30	22.69±1.30
30	51.06±1.32 n=3*	53.36±1.31	57.94±1.32	60.24±1.32	62.53±1.32	28.10±1.32
40	58.23±2.24	59.02±1.30	64.31±1.30	66.58±1.30	68.85±1.30	34.00±1.30
60	63.77±1.32	63.82±1.31	70.62±1.31	72.91±1.31	75.20±1.31	40.90±1.31
90	70.09±2.26	70.88±1.32	75.49±1.32	77.78±1.32	80.08±1.32	45.60±1.32
120	75.69±1.33	77.98±1.32	81.12±1.31	83.43±1.31	85.74±1.31	50.32±1.33

TABLE 5d: *IN VITRO* RELEASE PROFILE OF GRISEOFULVIN NANOCRYSTALS CONTAINING SLS AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F4A±SD	F4B±SD	F4C±SD	F4D±SD	F4E±SD	
5	28.51±2.23	24.04±2.23	32.24±1.29	35.22±2.23	39.69±2.23	9.87±1.29
10	32.40±1.30	30.88±2.24	39.13±1.29	42.13±2.24	46.63±2.24	15.89±1.29
20	36.30±1.29	37.76±2.26	46.06±1.30	49.07±2.26	53.60±2.26	22.69±1.30
30	40.42±2.61 n=3*	43.40±2.29	51.82±1.32	54.88±2.29	59.47±2.29	28.10±1.32
40	46.20±2.43	51.39±2.27	58.24±0.02	60.53±2.27	66.57±1.32	34.00±1.30
60	50.93±2.45	58.39±2.28	64.53±1.28	66.83±2.42	73.64±1.33	40.90±1.31
90	55.68±1.47	65.42±2.29	71.59±1.28	73.90±1.64	80.76±1.33	45.60±1.32
120	60.46±2.49	72.49±2.31	78.69±1.29	81.02±1.65	87.91±1.34	50.32±1.33

TABLE 5e: *IN VITRO* RELEASE PROFILE OF GRISEOFULVIN NANOCRYSTALS CONTAINING PEG 6000 AS STABILIZER

IME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F5A±SD	F5B±SD	F5C±SD	F5D±SD	F5E±SD	
5	15.09±2.23	19.56±2.23	22.54±1.29	24.78±1.29	27.02±1.29	9.87±1.29
10	21.88±2.24	26.38±2.24	28.63±2.24	31.63±1.29	33.13±2.24	15.89±1.29
20	26.48±2.26	31.75±1.31	35.50±2.25	37.77±2.24	37.79±2.25	22.69±1.30
30	31.95±2.29	37.30±1.33	41.11±2.28	43.41±2.28	43.43±2.28	28.10±1.32
40	37.81±2.27	43.11±1.32	46.89±2.26	49.16±2.26	50.68±2.44	34.00±1.30
60	44.73±2.28	48.56±1.61	53.85±2.28	55.40±1.61	57.67±1.46	40.90±1.31
90	50.94±1.47	54.05±2.28	59.37±1.34	61.67±1.46	64.69±1.48	45.60±1.32
120	57.18±1.65	61.06±2.29	64.17±1.34	67.23±1.65	71.76±1.50	50.32±1.33

n=3*

TABLE 6a: EFFECT OF PARTICLE SIZE ON *IN VITRO* RELEASE STUDIESOF GRISEOFULVIN NANOCRYSTALS CONTAINING β-CYCLODEXTRIN AND SLS AS STABILIZER

MULATION CODE	STABILIZERS CONCENTRATION	MEAN DIAMETER(nm)	% DRUG RELEASE
F3C	BCD(0.3%)	300.6	81.12
F3D	BCD(0.4%)	110.6	83.43
F3E	BCD(0.5%)	80.3	85.74
F4D	SLS(0.4%)	109.3	81.02
F4E	SLS(0.5%)	100.8	87.91

TABLE 7: EFFECT OF PARTICLE SIZE OF GRISEOFULVIN NANOCRYSTALS CONTAINING β-CYCLODEXTRIN AND SLS AS STABILIZER

MULATION CODE	STABILIZERS CONCENTRATION	MEAN DIAMETER(nm)	PDI
F3C	BCD(0.3%)	300.6	0.324
F3D	BCD(0.4%)	110.6	0.416
F3E	BCD(0.5%)	80.3	0.232
F4D	SLS(0.4%)	109.3	0.457
F4E	SLS(0.5%)	100.8	0.416

TABLE 8: ZETA POTENTIAL VALUES OF GRISEOFULVIN NANOCRYSTALS

MULATIONCODE	STABILIZERS CONCENTRATION	ZETA POTENTIAL(mV)
F3C	BCD(0.3%)	-20.5
F3D	BCD(0.4%)	-22.3
F3E	BCD(0.5%)	-19.7
F4D	SLS(0.4%)	-24.7
F4E	SLS(0.5%)	-21.4

TABLE 9: COMPARISON OF SOLUBILITY OF SELECTEDFORMULATION WITH PURE DRUG

S. NO	)LVENT USED	SOLUBILITY IN EACH SOLVENT(mg/10ml)					
		PURE DRUG	F3C	F3D	F3E	F4D	F4E
1	DISTILLED WATER	0.174±0.02	0.879±0.05	2.238±0.09	3.565±0.09	1.077±0.05	3.068±0.09
2	PHOSPHATE BUFFER Ph(6.8)	0.547±0.05	1.177±0.05	2.791±0.09	5.68±0.14	2.294±0.09	5.182±0.14

n=3*

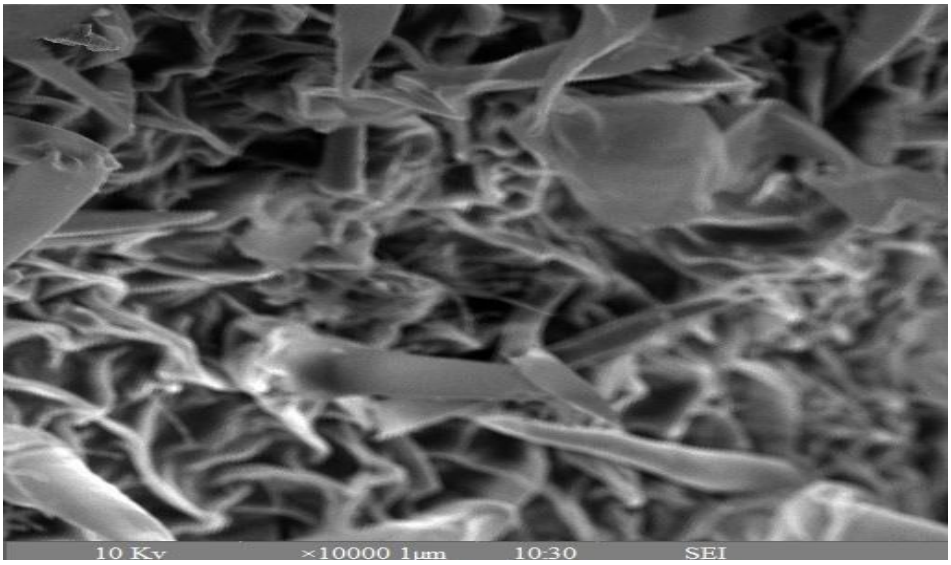

FIGURE 1: SCANNING ELECTRON MICROSCOPY (SEM) IMAGE OFBEST FORMULATION (F3E)

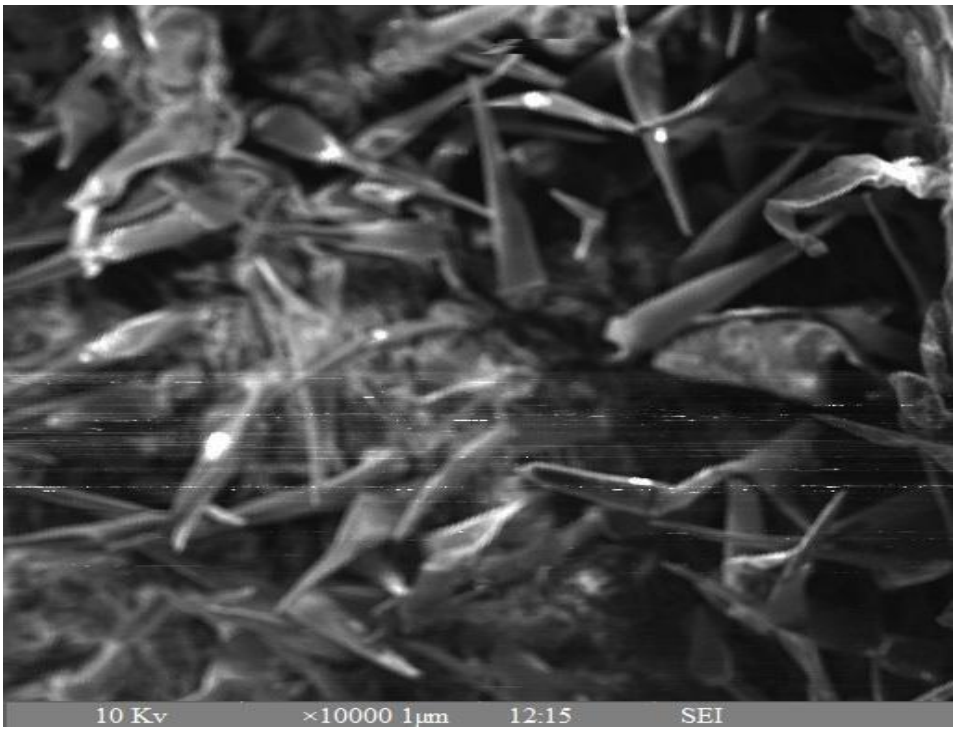

FIGURE 2: SCANNING ELECTRON MICROSCOPY (SEM) IMAGE OFBEST FORMULATION (F4E)

SUMMARY

In the present study an attempt has been made to develop nanocrystals of Griseofulvin in order to enhance solubility and dissolution rate by decreasing particle size of drug.

- The results of compatibility studies by Infrared spectroscopy showed no interaction between the drug and stabilizers.

- The Griseofulvin nanocrystals were successfully prepared by emulsion solvent diffusion method using different concentrations of stabilizers (HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000).
- The presence of stabilizers made the nanocrystal formulations more stablewith increasing drug release.
- Particle size analyzer used to explore the particle size of Griseofulvin nanocrystalsshowed a suitable particle size in the range of 80.3nm to 300.6 nm.
- The polydispersity index of selected nanocrystal formulations(F3C, F3D, F3E, F4D, and F4E) was less than 0.5, which indicated a narrow size distributionof particles.
- Zeta potential value of Griseofulvin nanocrystals showed a negative surface charge (-19.7mV to -24.7mV). *In vitro* release study of all the formulations were showed a increased drug release with increase in concentration of different stabilizers (HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000). Dissolution rate of all the formulations were improved when compared to pure drug.
- On the basis of drug release data F3C, F3D, F3E, F4D, and F4E showed a good release profile with more than 80% in 2 hours.
- The solubility of selected formulations (F3C, F3D, F3E, F4D, and F4E) in phosphate buffer pH(6.8) increased ten folds when compared to pure drug.
- SEM studies confirmed the morphology of the nanocrystal formulations. The crystalline state of the nanocrystal formulation was not altered according tothe XRPD analysis.

CONCLUSION:

Hence, it was concluded that nanocrystallization was a good approach to enhance the dissolution property of Griseofulvin by emulsion solvent diffusion method. The solubility and *in vitro* dissolution studies suggested that the nanocrystal formulations can improve the bioavailability of the Griseofulvin by improving its solubility and dissolution rate when compared to pure drug. Thus nanocrystal drug delivery system can adopted to increase the solubility and dissolution rate of poorly soluble drug like Griseofulvin to enhance their bioavailability.

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