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# PREPARATION AND CHARACTERIZATION OF GRISEOFULVIN NANOPARTICLES BY IONOTROPIC GELATION METHOD

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## ABSTRACT

The main objective of this work was to prepare and characterize chitosan loaded Griseofulvin nanoparticles. The FTIR and DSC studies were carried out for pure drug, polymer and drug with polymer in different ratio which shows there was no chemical interaction between the drug and polymer. The F4 formulation shows more entrapment efficacy compared to other formulations nearly 92.87% and the drug content was found to be 96.7%. The particle size and the surface morphology results revealed that griseofulvin nanoparticles (GNPs) were smoothed spheroidal with a size ranging from260 nm-632 nm. The invitro dissolution studies were carried out for all the Formulation. Formulation F4 shows 97.67% of drug release at the end of 24 hr. The results suggest that chitosan polymer based nanoparticulate formulations are potential means to achieve release of Griseofulvin for the prolonged period of time for effective therapy. The results showed that this method is reproducible easily.

**KEYWORDS:** Griseofulvin, Nanoparticles, Chitosan, Inotropic gelation method.

## INTRODUCTION

Nanotechnology is termed as design, production, characterization and applications of structures, systems and devices by controlling shape and size at nanometer scale. Nanoparticles (NPs) are defined as solid particles or particulate dispersion drug carriers that may or may not be biodegradable. The drug is entrapped, dissolved encapsulated or attached to a nanoparticle matrix. The term nanoparticle can be used for both nanospheres and nanocapsules.[1]

The nanoparticles (NPs) are prepared by using biodegradable and biocompatible polymers in size range between 10-1000 nm where the drug is entrapped, dissolved, encapsulated or attached to a nanoparticle matrix. The field of polymer nanoparticles (NPs) is gaining its prominence and expanding quickly and playing vital role in a wide spectrum of areas ranging from photonics, electronics, conducting materials, medicine, sensors biotechnology, environmental technology and pollution control. NPs are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to targeted site. Polymer- based nanoparticles effectively carry drugs, DNA and proteins to target cells and organs. Their nanometer-size faciliitate effective permeation through cell membranes and stability in the blood stream.[2-6]

## MATERIALS AND METHODOLOGY

### 1.1 Development of Calibration Curve of Griseofulvin

**Preparation of standard stock solution**

Griseofulvin. Pure 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in DCM It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 5µg/ml solution was prepared from the stock solution was prepared using diluent, which was used as working standard.[7-8]

**Preparation of Stock Solution**

Griseofulvin (10 mg) was dissolved in 100 mL DCM From these stock solution aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL were withdrawn in 10 mL volumetric flask and diluted up to the volume with pH 7.4 phosphate buffer solutions to give concentrations of 5, 10, 15, 20, 25, and 30 mg/mL. Absorption of each solution was measured at 291 nm using Shimadzu HPLC and pH 7.4 phosphate buffers as a reference standard.

## 1.2. PREPARATION OF NANOPARTICLES CONTAINING GRISEOFULVIN

A Chitosan nanoparticle was prepared by ionotropic gelation process. Chitosan solution (0.1% w/v) as prepared by dissolving 100 mg of chitosan in 100ml of 1% v/v acetic acid. TPP (tripolyphosphate) solution of 0.1% was prepared by dissolving 100mg of TPP in 100ml of deionized water. Griseofluvin was added to the TPP solution. The chitosan solution was then stirred at1500rpm for 30min in an ultrasonicator (vibronics) and add TPP solution containing Griseofulvin drop by drop (10ml) and kept stirring for 1hr on magnetic stirring. Nanoparticles were obtained upon the addition of a TPP aqueous solution to a chitosan solution.[9] The NP suspension is then centrifuged at 15,000 rpm for 10 min using high-speed centrifuge (Sigma). Discard the sediment and the preserve the supernatant. The formation of nanoparticles results in interaction between the negative groups of TPP and the positively charged amino groups of chitosan.

## 1.3. EVALUATION STUDIES

### 1.3.1. Organoleptic properties

Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

### 1.3.2 Solubility studies

The solubility of drug and polymer was carried out in various solvents such as distilled water, buffer solutions and organic solvents. The resulting solutions were filtered and analyzed for dissolved drug by measuring absorbance at 291 nm.

### 1.3.3. Compatability studies

To know about the interaction between the drug and carriers used in the formulation, the IR analysis was carried out. The IR spectra of pure griseofulvin, Pure Polymer, and Prepared Griseofulvin nanoparticles was studied by FTIR It is scanned over the Frequency range of 4000-500 cm-1.

The fourier transform infrared analysis was conducted for the structure characterization. FTIR spectra ofgriseofulvin, pure polymer and formulated nanoparticles were recorded.

The samples (drug, polymer and mixture of drug and polymers) were mixed with 200- 400 mg of potassium bromide (KBr). The samples were compressed as discs by applying pressure of 5 tons for 5 minutes in a hydraulic press. The prepared pellet was placed in the light path and

the spectrum was recorded from 650 to 4000 $cm^{-1}$.

### 1.3.4. Differential scanning colorimetry

DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies. A complement to X-ray diffraction, this method is regularly used to determine the extent to which multiple phases exist in the interior or to which the various constituents, including the drug, interact.[6]

Phase transition behavior of Griseofulvin NPs were analyzed by the differential scanning calorimeter. As a control, the pure Griseofulvin, Pure Polymer and Prepared Nanoparticles were analyzed by DSC Approximately 5 mg of samples were loaded to aluminium pan, crimped, sealed and analysed at a scanning rate of 10°C/min from 25°C to 200°C under nitrogen atmosphere (flow rate was100 mL/min).

### 1.3.5. X-Ray Studies

The XRD studies for analyzing structural nature of Griseofulvin , and nanoparticle formulation of Griseofulvin. The samples were placed in sample cell and spread evenly. The sample cell was placed in X-ray Diffractometer (BRUKER ECO D8). The samples were scanned over the frequency range of 10-80.

### 1.4 Characterization of nanoparticles

The optimized nanoparticles containing Griseofulvin were characterized by studying various physico-chemical properties.

### 1.4.1Particle size[10]

Nanoparticle size was determined using Photon Correlation Spectroscopy (PCS). All samples were diluted with ultra-purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25$^{o}$C. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate.

### 1.4.2. Zeta potential and Surface morphology

Nanoparticles were characterized with Zeta potential using a Zeta Sizer. The measurements were performed by diluting the nanoparticles with distilled water and the samples were placed in the electrophoretic cell using an aqueous dip cell in an automatic mode.

The surface morphology of the particles was studied using Scanning Electron Spectroscopy set at from 290 nm-632 nm, 200 kV by placing an air dried nanoparticle suspension on copper electron microscopy grids.

### 1.4.3 Drug content

The total drug amount in nanosuspension was determined spectrophotomertically.

A residue was dissolved in water and filtered with a 0.45µm filter, and griseofulvin content was assayed spectrophotomertically at 291nm.

$$Total\ Drug\ Conent = \frac{Volume\ total}{Volume\ aliquot} \times drug\ amount\ in\ aliquot$$

### 1.4.4 Drug entrapment efficiency

The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer. Entrapment efficiency was calculated as follows.[11]

$$DEE\% = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

### *1.5. In vitro* release studies

*In-vitro* diffusion studies (drug release studies) were performed by using diffusion apparatus. A semipermeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased towards donar compartment.[12] The glass beaker was filled with 100ml of phosphate buffer sample of 1ml was withdrawn at regular intervals from glass beaker for analysis.1ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100ml.The absorbance of sampling was measured at 291nm by using UV spectrophotometer.

### 1.6. Stability Studies

To evaluate the stability of drug, Griseofulvin, and the effect of polymer after storing at different Temperature and Relative Humidity for 30 days stability studies were carried out .About 100mg of equivalent of Griseofulvin formulations were taken in well closed containers from ideal batches and stored separately at 400C+ 20C/75% RH + 6% (Accelerated testing) and 300C+ 20C/60% RH + 5% (Alternate testing). From these, sample equivalent to 20 mg of Griseofulvin was removed at the interval if 10, 20, 30 days and analyzed the drug content by spectrophotometrically at 291 nm.[13]

## RESULTS AND DISCUSSION

### 2.1. Development of Calibration Curve of Griseofulvin

**Table No.1: Results of Calibration curve at 291 nm for Griseofulvin.**

| S.No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 5 | 0.080 |
| 2 | 10 | 0.176 |
| 3 | 15 | 0.268 |
| 4 | 20 | 0.349 |
| 5 | 25 | 0.455 |
| 6 | 30 | 0.540 |
| 7 | 35 | 0.695 |

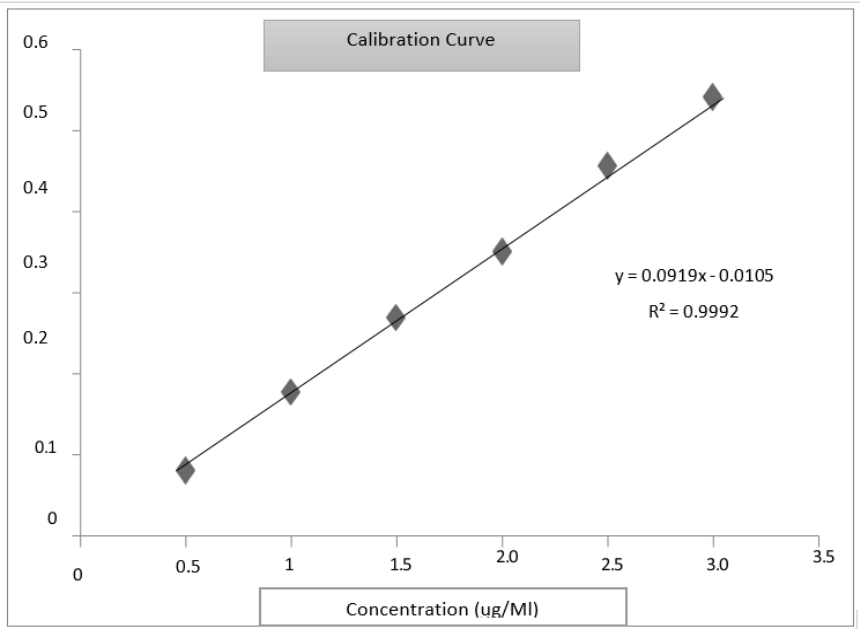


**Figure 1: Calibration curve of griseofulvin.**

**Table 2: Formulation of Griseofulvin nanoparticles.**

| S.NO | Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| 1 | Griseofulvin | 40mg | 40mg | 40mg | 40mg | 40mg | 40mg |
| 2 | Chitosan | 0.2% | 0.3% | 0.35% | 0.5% | 0.65% | 0.75% |
| 3 | Tripoly phosphate | 40ml | 40ml | 40ml | 40ml | 40ml | 40ml |
| 4 | 0.1% Acetic acid solution | 100ml | 100ml | 100ml | 100ml | 100ml | 100ml |
| 5 | Dichloro methane | Q.S | Q.S | Q.S | Q.S | Q.S | Q.S |

### 2.3.1. Organoleptic properties

Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

**Table 3: Organoleptic properties.**

| Properties | Results |
|---|---|
| Description | Crystalline |
| Taste | Tasteless |
| Odour | Odourless |
| Colour | White to pale creamed colored powder |

### 2. 3.2 Solubility studies

Solubility of griseofulvin was performed in various solvents like water, 0.1 N HCL, methanol, ethanol, Dichloro methane and phosphate buffer (pH 7.4). From the above solvent griseofulvin was freely soluble in Dichloro methane and ethanol, whereas remaining solvents shows insoluble particle sediment in the bottom of test tube.

### 2.3.3. Compatability studies

The FTIR studies showed that the significant peaks of griseofulvin where C-N stretching at 1444.02 cm-1, C=O $cm^{-1}$ vibration at 1682.16 $cm^{-1}$, C-O-C at 1085.35 $cm^{-1}$, N-H $cm^{-1}$ at 3301.77 $cm^{-1}$, C=C group vibration at 1630.68 $cm^{-1}$ and O-H vibration at 2799.45 $cm^{-1}$ (Figure 2). Based on that FTIR spectrum of griseofulvin functional groups peak was coincided with standard griseofulvin pure drug.

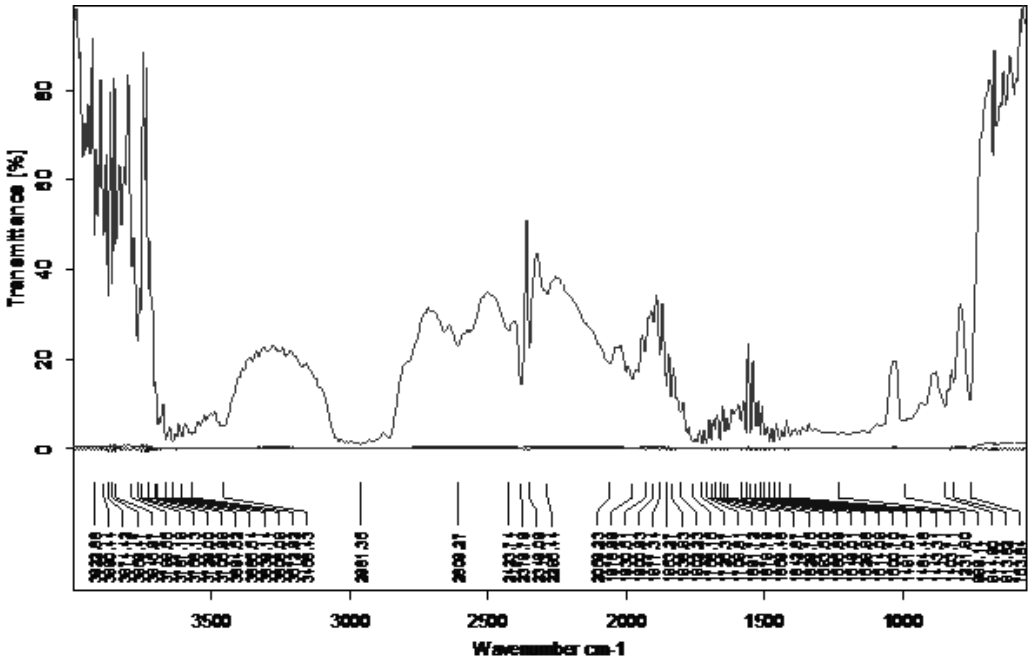


**Fig. 2: FTIR Spectra of Griseofulvin.**

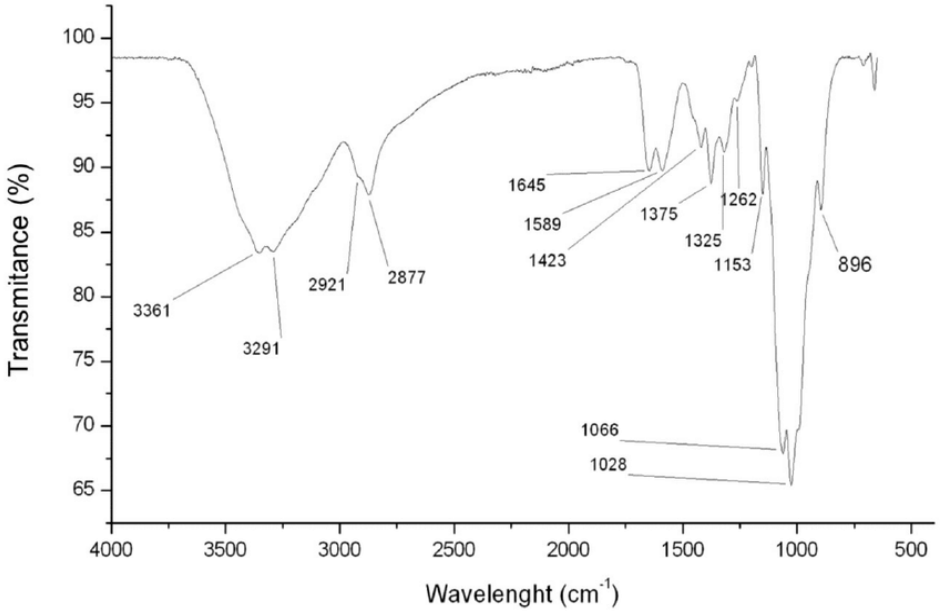


**Fig 3: FTIR Spectra of Chitosan Polymer.**

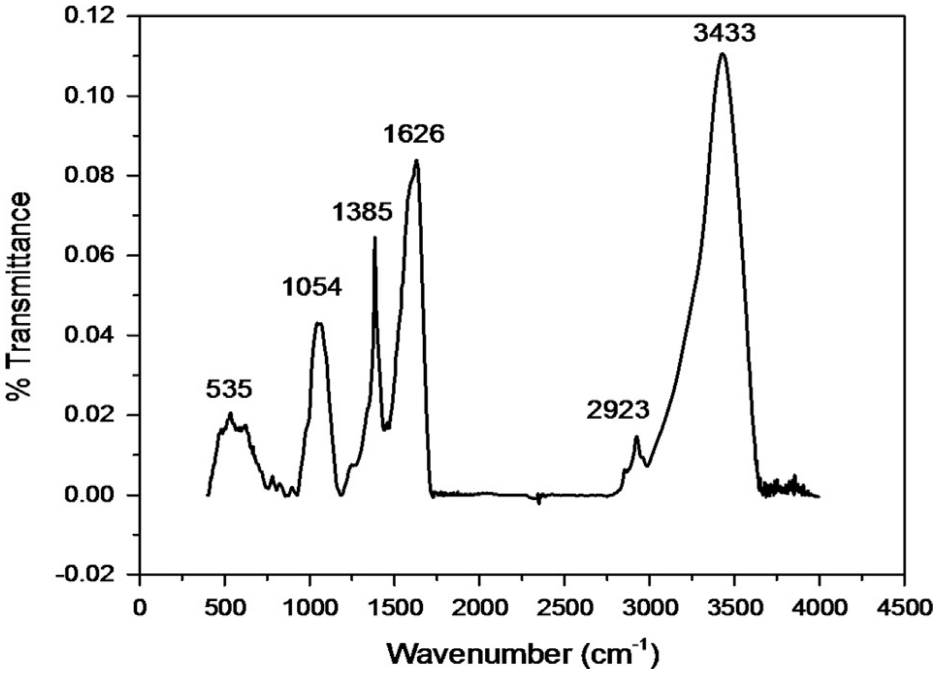


**Fig. 4: FTIR spectrum recorded by making KBr pellet with synthesized griseofulvin nanoparticles.**

### 2.3.4. Differential scanning colorimetry

The DSC analysis was carried out for standard griseofulvin, chitosan and griseofulvin-chitosan mixture. The DSC studies performed in order to determine the compatibility studies. The peak that is obtained for griseofulvin in nanoparticles was compared with standard griseofulvin and its ensure there was no interaction between drug and drug with polymer.

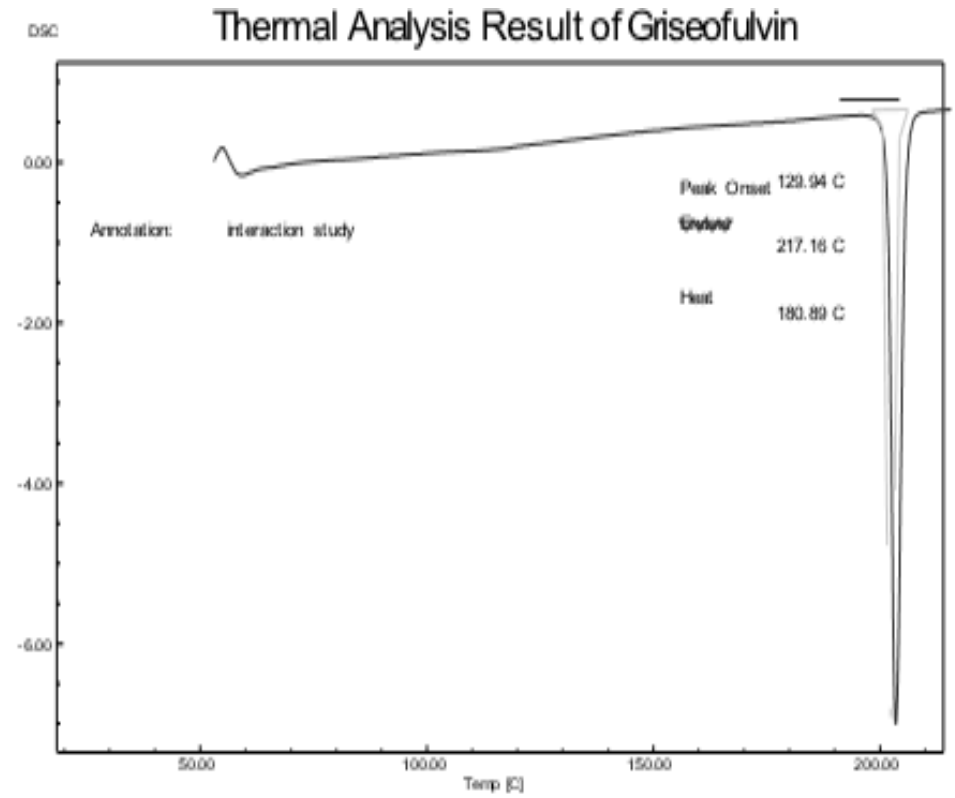


**Fig. 5: DSC Curve of Griseofulvin.**

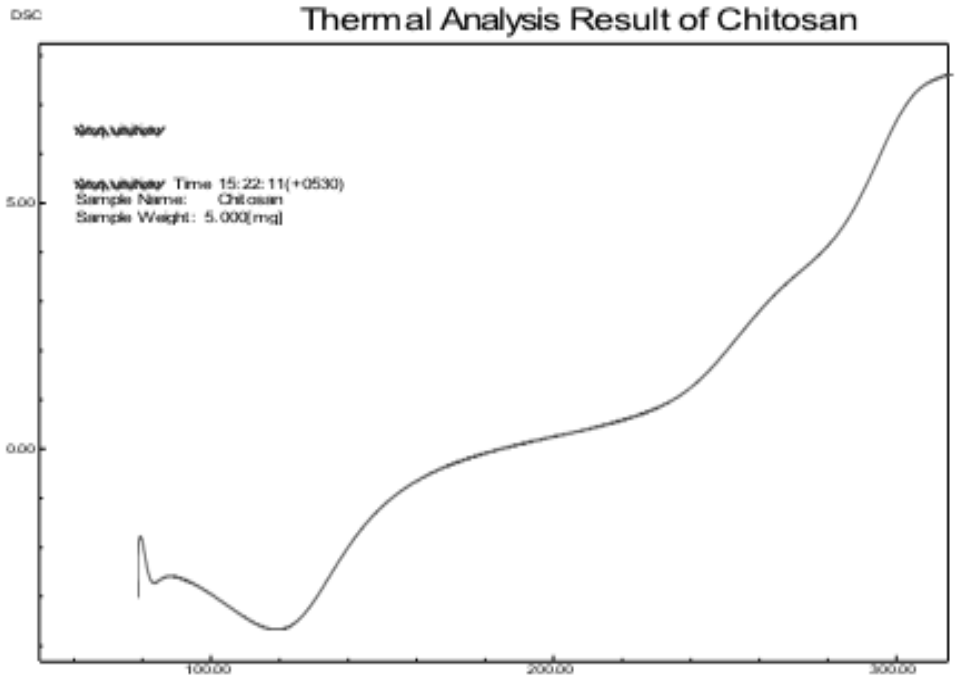


**Fig. 6: DSC Curve of Chitosan.**

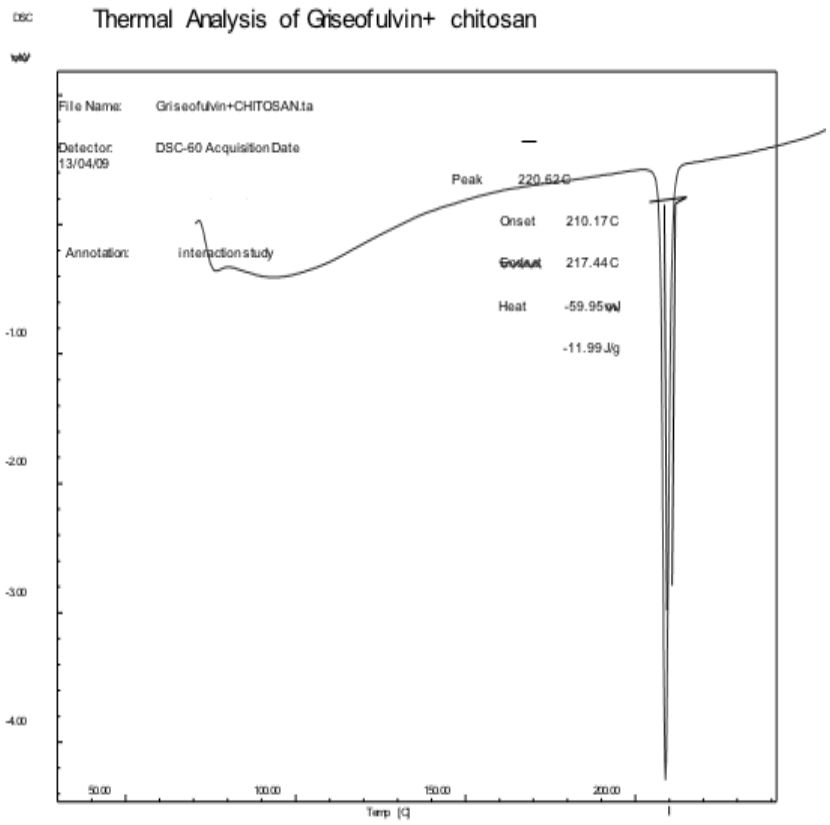


**Fig. 7: DSC Curve of Griseofulvin+ Chitosan.**

### 2.3.5. XRD Studies

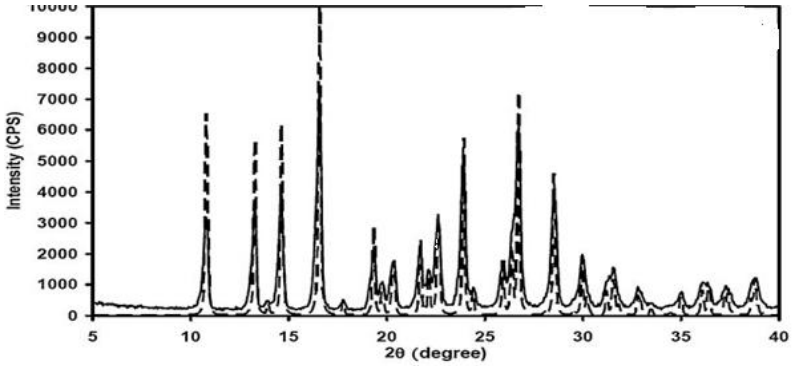


**Fig. 8: XRD of pure drug of Griseofulvin.**

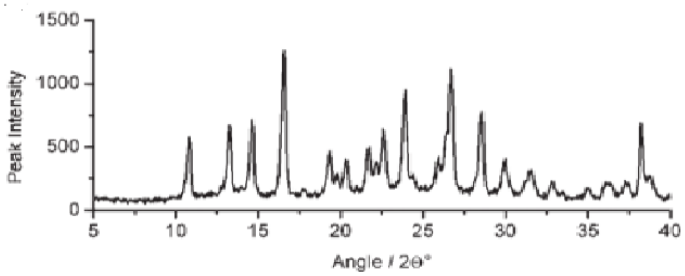


**Fig. 9: XRD of Griseofulvin Nanoparticles.**

### 2.4.1 Measurement of particle size of Nanoparticles

The particle sizes of prepared nanoparticles were measured from the microphotograph of 100 particles. The particle size ranged from 260 to 632 nm for various batches. The results of Particle Size were presented in Table 4 and fig 10.

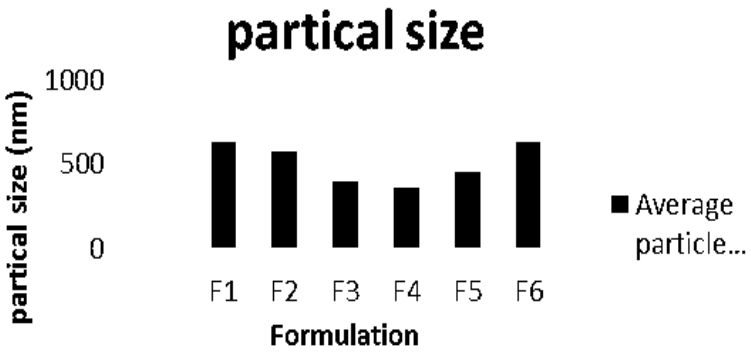


**Fig 10: Particle size of Griseofulvin NP formulations.**

Particle size is often used to characterize the nanoparticles facilitation via skin andunderstanding of aggregation. In the case of large surface area, the attractive force between the particles and chance for possible aggregation in smaller sized particles. To overcome such aggregation, addition of a surfactant in the preparation was necessary. The formulations F1-F5 shows the particle size range between 260.6 ± 3.4 nm to 632.6 ± 1.2 nm. It is indicated that the particle size depends on the concentration of the polymer.

### 2.4.2. Zeta potential and Surface morphology

**Table 4: The results of Particle size, polydispersity index and zeta potential of prepared Griseofulvin Nanoparticles.**

| Samples | PS (nm) | PI (nm) | ZP (mV) |
|---|---|---|---|
| F1 | 510±0.3 | 0.37±0.06 | -11.1 |
| F2 | 480.7±0.2 | 0.28±0.08 | -12.9 |
| F3 | 350.4±0.4 | 0.15±0.04 | -18.74 |
| F4 | 260.5±0.6 | 0.14±0.08 | -30.79 |
| F5 | 585.2±0.7 | 0.50±0.07 | -.22.12 |
| F6 | 632.1±1.2 | 0.12±0.04 | -15.75 |

Zeta potential of griseofulvin polymeric nanoparticles is presented in the Table 4. Zeta potential is an essential factor to evaluate the stability of nanosuspension. Zeta potential values mainly reflect the electrical repulsion between the particles The average zeta potential value of griseofulvin polymeric nanoparticle was in the range of -22.04 ± 1.82 mV to -30.64 ± 1.84 mV. Even though a high zeta potential could provide an electric repulsion between the

particles. Surface charge of nanoparticlesinfluences their skin penetration. The only the negative charged particles were able to penetrate the SC to reach the inner epidermis. Whereas griseofulvin polymeric nanoparticles shows -22 to – 30 mV with small particle size, which abundantly influence the penetration of nanoparticles through stratum corneum.

**Surface Morphology**

The griseofulvin nanoparticles had spherical shape with rough surface, as the polymer did not completely dissolved in solvent and secondly due to faster evaporation of solvent. In the case of, F4 formulations, it showed smooth surface of spherical shaped nanoparticles (Figure 11).

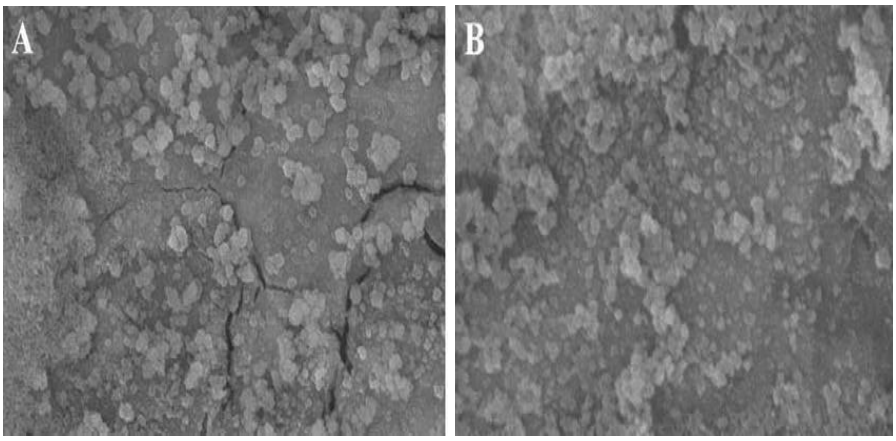


**Fig. 11: Griseofulvin chitosan loaded nanoparticle.**

**2.4.3 Drug content**

The total drug amount in nanosuspension was determined spectrophotomertically. A0.50-mlaliquot residue was dissolved in dichloro methane and filtered with a 0.45µm filter, and Griseofulvin content was assayed spectrophotomertically at 291nm. The results of total amount of drug content of prepared formulations were represented in table 8.

**2.4.4 Drug entrapment efficiency**

The drug entrapment efficiency of nanosuspension was determined spectrophotomertically A 0.50-ml aliquot residue was dissolved in dichloro methane and filtered with a 0.45µm filter, and Griseofulvin content was assayed spectrophotomertically at 291nm. The results of entrapment efficiency of prepared formulations were represented in table 5.

**Table 5: Drug content and entrapment efficiency of Griseofulvin nanoparticle formulations.**

| Formulation | Drug content (%) | Average entrapment efficiency |
|---|---|---|
| F1 | 65.4 | 57.1% |
| F2 | 58.8 | 65.26% |
| F3 | 80.2 | 68.14% |
| F4 | 93.7 | 92.87% |
| F5 | 72.4 | 82.41% |
| F6 | 84.7 | 59.28% |

The entrapment efficiency is the functional characteristic of polymers, drug and surfactant etc. the entrapment efficiency was high in the case of F1-F6 formulations, due to high affinity of drug and the polymer in the same solvent. Entrapment Effeciency of the prepared Nanoparticles were in the Range of 57.4 7 to 92.87 (Table 5). The entrapment efficient depends on the polymer- drug concentration and the method used to prepare nanoparticles.

### *2.5. In vitro* release studies

*In vitro* diffusion studies (drug release studies) were performed by using diffusion apparatus. A semi permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was placed towards donor compartment.

**Table 6: Percentage Drug Release.**

| Formulation code | Percentage drug release | | | | | |
|---|---|---|---|---|---|---|
| | 2 h | 4h | 8h | 10h | 12h | 24h |
| F1 | 19.2 ± 0.6 | 26.5 ± 0.8 | 35.0 ± 1.4 | 47.9 ± 1.8 | 61.6 ± 0.2 | 78.9 ± 0.2 |
| F2 | 20.2± 1.2 | 32.4± 1.4 | 40.6 ± 0.4 | 56.4 ± 0.2 | 69.4± 1.8 | 80.1 ± 1.4 |
| F3 | 24.4 ± 0.6 | 35.6 ± 1.8 | 52.3± 1.4 | 63.9± 1.2 | 75.2± 0.2 | 84.6 ± 0.6 |
| F4 | 26.6 ± 1.2 | 37.4 ± 0.4 | 50.8 ± 1.4 | 65.0 ± 1.2 | 84.1± 0.8 | 97.9 ± 1.2 |
| F5 | 30.4 ± 0.8 | 47.7 ± 1.4 | 78.2 ± 1.0 | 90.6 ± 0.2 | - | - |
| F6 | 40.2± 0.3 | 52.9±0.5 | 75.9±6.0 | 92.3±1.8 | - | - |

The *in vitro* release of griseofulvin from different biodegradable nanoparticles is shown in Table 6. The quantity of drug release in the all formulations (F1-F6) of polymeric nanoparticle was as very low to medium in the range of 19.2 ± 0.6% to 40.4 ± 0.3% in the initial period (2h). From this, it is obvious that the decreased percentage of drug release was due to the formulation of more compact wall around the drug by the biodegradable polymer and it significantly possess the sustained drug release for a prolonged period of time. At the end of 24 h limited percentage of drug was released in the range of 78.2 ± 1.2 to 97.9 ± 1.2% (Table 6, Figure 12).

The formulation F1 shows 78.9% of drug release at the end of 24 hr.

The formulation F2 shows 80.42% of drug release at the end of 24 hr.

The formulation F3 shows 84.06% of drug release at the end of 24 hr

The formulation F4 shows 97.9% of drug release at the end of 24 hr compared to other formulation. The rate of release was based on concentration of a polymer, if the concentration of polymer increase the rate of release of a drug was slow. Based on the solubility nature of an polymer the drug will get release slowly in a desire amount at the particular site of action there by fluctuation of drug was avoided and the plasma concentration of an drug was maintained there by the therapeutic action of an drug was more at the site of action there by the side effects were avoided. The formulation F5 and F6 shows 90 % of drug release with in 10 hrs. There by the sustained action of an drug at the targeted site was not obtained.

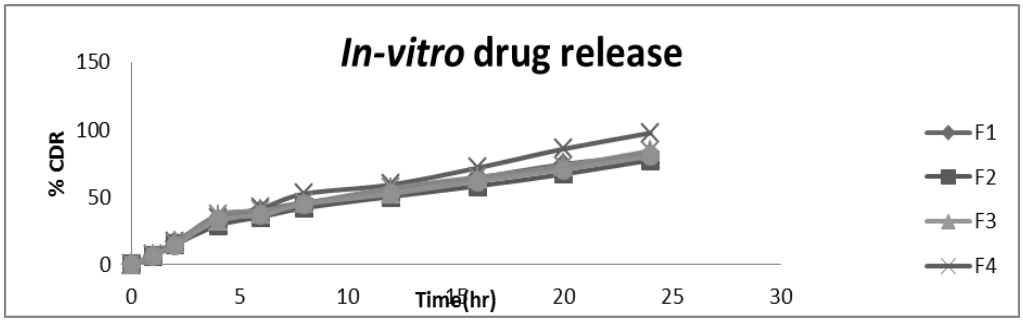


**Fig. 12: Invitro release of Griseofulvin Nanoparticles.**

### 2.6. Stability Studies

Formulations were stored at 40C+ 20C/75% RH + 6% (Accelerated testing) and 30C+ 20C/60% RH + 5% (Alternate testing). After 30 days of storage, the formulations were observed physically and no color changes occurred. The content of Griseofulvin in all best formulations at various intervals of 10, 20, and 30 days was calculated. The result proved that the percentage of griseofulvin was not less than 2-3 % in all the formulations as shown in TableNo: 7.

| Formulation code | Temperature(°C) | % of Griseofulvin | | | |
|---|---|---|---|---|---|
| | | 0 Days | 10 Days | 20 Days | 30 Days |
| F1 | 40°C± 2°C/75% RH± 6% | 98.20 | 98.13 | 98.11 | 98.02 |
| | 30°C± *2°C!60%* RH±5% | 98.20 | 98.19 | 98.14 | 98.11 |
| F2 | 40°C± 2°C/75% RH± 6% | 98.60 | 98.58 | 98.53 | 98.52 |
| | 30°C± *2°C!60%* RH±5% | 98.70 | 98.65 | 98.64 | 98.58 |
| F3 | 40°C± 2°C/75% RH± 6% | 96.40 | 96.38 | 96.34 | 96.22 |

| | | | | | |
|---|---|---|---|---|---|
| | 30°C± 2°C/60% RH± 5% | 96.40 | 96.32 | 96,27 | 96.17 |
| **F4** | 40°C± 2°C/75% RH± 6% | 98.40 | 98.39 | 98.43 | 98.37 |
| | 30°C± 2°C/60% RH± 5% | 98.40 | 98.42 | 98.42 | 98.39 |
| **F5** | 40°C± 2°C/75% RH± 6% | 98.20 | 98.30 | 98.17 | 98.24 |
| | 30°C± *2°C!60%* RH±5% | 98.20 | 98.19 | 98.13 | 98.24 |
| **F6** | 40°C± 2°C/75% RH± 6% | 98.60 | 98.69 | 98.57 | 98.63 |
| | 30°C± 2°C/60% RH± 5% | 98.60 | 98.62 | 98.58 | 98.64 |

## CONCLUSION

The present Research relates to the Preparation of Griseofulvin Chitosan loaded Nanoparticles. The Griseofulvin Nanoparticles were prepared by using Ionotropic Gelation Method, wherein the polymers used in the preparation were chitosan. This study confirms that Inotropic gelation method is suitable for the preparation of Griseofulvin nanoparticles. Out of all the prepared formulations F4 with the Griseofulvin concentration of 40mg showed the highest entrapment efficiency of 92.87% when compared to that of other formulations and the highest extent of release (97.67% at 24thhr). the possibility to achieve a therapeutic dose was much higher by using the Prepared Nanoparticles. The prepared Griseofulvin chitosan loaded nanoparticles would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional Griseofulvin tablets. According to the data obtained, this chitosan-based nanotechnology opens new and interesting perspectives for anti-fungal activity.

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