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Research Article

Bioavailability Of Griseofulvin Nanosponges

Development and Characterization of Griseofulvin Nanosponges to Enhance Bioavailability

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Abstract: The pharmaceutical industries are more interested in development of novel drug delivery system to overcome the disadvantages of traditional drug delivery system. Now a days, fungal infections are increasing worldwide. Hence the demand of novel dosage form is increased with improved therapeutic effect and minimum side effect. The main aim of this study was to develop griseofulvin loaded ß -cyclodextrin based nanosponges for improving dissolution, oral bioavailability. In the present study, the griseofulvin loaded Nano sponges were prepared by emulsion solvent diffusion method. The six different formulation batches were formulated with varying concentration ratio of drug and polymer. The Preformulation study of the drug was done by UV-visible spectrophotometer. Drug, polymer and excipient compatibility studies were analyzed by FTIR studies. The prepared Nanosponges formulation was evaluated for particle size, zeta potential, polydispersity index, scanning electron microscopy and in-vitro drug release studies. The SEM studies showed the highly porous structure of griseofulvin loaded nanosponges having a sponges-like shape. The entrapment efficiency of optimized batch F4 was found to be 97.69±0.006%. The particle size and zeta potential of optimized batch F4 was found to be 488.59nm and -20.77 respectively. The in-vitro drug release of optimized batch F4 showed the maximum drug release of 75.56% in 8 hrs. The current study successfully developed griseofulvin loaded ß -cyclodextrin based nanosponges to improve dissolution rate, oral bioavailability and masking bitter taste. The polymer used in preparation of nanosponges shows efficient drug release. Griseofulvin loaded nanosponges drug delivery showed prolonged release which is beneficial for chronic fungal infection. Another advantage of formulating novel dosage forms is reduction in dose, dosing frequency and reduced side effects.

Keywords: Griseofulvin, Cyclodextrin, Nanosponges, Oral Bioavailability, Emulsion Solvent Dispersion Method.

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1. INTRODUCTION

The antifungal agent Griseofulvin (GRI) is produced by the mold Penicillium griseofulvum.[1] According to FDA, griseofulvin has long been the medicine of choice for treating tinea capitis in childrens.[2] It has a well-established efficacy and safety profile, as well as inexpensive. GRI is classified as a class II drug by the Biopharmaceutics Classification System (BCS), which means that, the dissolution rate is the main factor limiting absorption. Furthermore, one of the possible side effects of this medication is a bitter taste. Liquid formulations are usually ideal for children as they are easy to administer. The manufacturing of such formulations is may be limited by the drug solubility.[3] There are various formulation techniques that are used for increasing the solubility of griseofulvin, like micronisation by supercritical fluid technology, self-emulsifying drug delivery system, solid dispersion, nanocrystallization, polymeric micelles were novel drug delivery system was adapted.[4-6] Nanosponges are tiny sponges with size of about a virus (250nm-1µm), which consist of cavities that can be filled with a wide variety of drugs. Three-dimensional networks of spherical porous particles are used to produce nanosponges.[7] Because of their inherent cyclodextrin cavities and nanoporous network structure, cyclodextrin-based nanosponges can form inclusion and non-inclusion complexes with medicinal molecules. Because of their great stability, high carrier capacity, and ability to incorporate both hydrophilic and hydrophobic molecules, nanosponges are thought to be one of the most promising nanoscale delivery systems. Also, nanosponges can improve drug solubility, chemical stability and, consequently, the bioavailability of lipophilic drugs. [8] Cyclodextrins are particularly important in the pharmaceutical industries because they can increase drug solubility while masking some organoleptic properties, resulting in better compliance. However, there are some drawbacks to using native cyclodextrin for the preparation of inclusion complexes, such as the ease with which the complex can be separated after dilution and the size requirements of the drug molecules, which prevent complexation of molecules with high molecular weight or aqueous solubility. Furthermore, due to strong intermolecular hydrogen bonding in the crystal state, cyclodextrins have low water solubility. It was recently reported that cyclodextrin-based nanosponges have been effectively created as a gabapentin carrier with possible taste masking potential.[9, 10] Formulation of cyclodextrin –based nanosponges of griseofulvin as pediatric oral liquid dosage form for masking the bitter taste, enhancing bioavailability.

2. MATERIAL AND METHODS

2.1 Materials

Griseofulvin (GRI) was purchased from yarrow chem (Mumbai). β -Cyclodextrin (β -CD), polyvinyl alcohol, methanol and dichloromethane were purchased from modern science (Nashik). All of the other chemicals and solvents were commercially available analytical grade materials.

2.2. Methods

2.2.1. Preformulation studies of griseofulvin and polymers

Preformulation is the initial step in the logical development of any new drug's pharmaceutical dosage form. Preformulation studies focus on the new compound's physicochemical properties that may affect the drug performance and the development of an effective dosage form. These preformulation studies shows that there are no significant obstacles to the development of compounds.[12]

2.2.2. Physical characteristics

The drug was identified for physical characteristics like color, odor and texture by visual method.[13]

2.2.3. Solubility studies

The solubility of drug griseofulvin was determined in distilled water, phosphate buffer 7.4, and organic solvents like N, N-Dimethylformamide, ethanol, chloroform, acetone, and dichloromethane. The solubility of griseofulvin in different solvents was determined by the shake flask method; the examined compound was dissolved in excess 10 ml of respective solvents. The solutions were stirred for 24 hrs. with the help of a magnetic stirrer at 37°C and allowed to equilibrate. After 24 hrs, the samples were withdrawn and filtered through a membrane filter and analyzed in a UV-visible spectrophotometer (shimadzu UV-2600). [14]

2.2.4. Melting point

Melting point of griseofulvin is determined by the capillary tube method. Small amount of drug was filled in an capillary tube where, one end of capillary was sealed and kept in the melting point apparatus and the temperature at which drug starts to melt is noted as melting point.[11]

2.2.5. Ultraviolet- visible spectroscopy

2.2.5.1. Determination of λmax in methanol

The UV spectrum of griseofulvin was obtained using UV Shimadzu 2600. Accurately weighed 10 mg of the griseofulvin was dissolved in sufficient amount of methanol and volume make up to 10 ml and this was diluted to get a 100 µg/ml concentration. The 1 ml of aliquot was withdrawn and volume was make up to 10 ml by using methanol to obtain 10µg/ml concentration. The resultant solutions was scanned in the range of 200 to 400 nm. The maximum wavelength was measured by recording the spectrum.[15]

2.2.5.2. Construction of Beer'sLambert's plot in methanol

The stock solution of 100µg/ml was prepared in methanol, and this stock solution was used to prepare different dilutions in the range 2-10 µg/ml. The absorbance of resulting solutions were measured at 291 nm by UV visible spectrophotometer.

2.2.5.3. Determination of λmax in pH 7.4 phosphate buffer

2.2.5.3.1. Preparation of 7.4 pH phosphate buffer

0.2M of potassium dihydrogen phosphate (KH_2PO_4) solution was prepared by dissolving 2.722 gm KH_2PO_4 in 100 ml distilled water in separate 100 ml of volumetric flask and solution of 0.2 M sodium hydroxide (NaOH) was prepared by dissolving 0.8 gm NaOH in 100 ml distilled water. 50 ml of

0.2M KH_2PO_4 solution was taken in another 500ml beaker and a specified volume of 0.2N NaOH solution (22.4 ml) was added in it and the volume was adjusted with distilled water to 200 ml.

2.2.5.3.2. Determination of λmax in phosphate buffer pH 7.4.

The UV spectrum of griseofulvin was obtained using UV Shimadzu 2600. Accurately weighed 10 mg of the griseofulvin was dissolved in sufficient amount of phosphate buffer pH 7.4 and volume made up to 10 ml. The stock was diluted to get a 100 µg/ml concentration. The 1 ml of aliquot was withdrawn and volume was made up to 10 ml by using phosphate buffer pH7.4 to obtain 10µg/ml (ppm) concentration. The resultant solutions was scanned in the range of 200 to 400 nm. The maximum wavelength was measured by recording the spectrum.[15]

2.2.5.3.3. Construction of beer's Lambert's plot in phosphate buffer pH 7.4.

The stock solution of 100µg/ml was prepared in a phosphate buffer, this stock solution was used to prepare different dilutions in the range 2-10 µg/ml. The absorbance of resulting solutions were measured at 295.20 nm by UV visible spectrophotometer.

2.2.5.3.4. Drug- Excipient Compatibility Studies

A compatibility analysis was conducted in order to ensure that the drug and excipients utilized in the formulation did not interact. The vial was filled with the drug and physical mixtures of the drug: polymer (1:1) and sealed. The sealed vials were stored in a desiccator for 45 days at a particular temperature. After 45 days, the mixture was analyzed using FTIR. FTIR was used to determine preliminary compatibility (Shimadzu: IR Affinity-1S). The sample was analyzed in the region of 4000-400cm^{-1}. [16]

2.2.5.3.5. FTIR Analysis

To study the interaction (if any) of griseofulvin with the excipients added in formulation and the stability of the drug during the loading process of nanosponges , FT-IR spectroscopic studies were carried out for griseofulvin, β -cyclodextrin, polyvinyl alcohol and optimized batch F4. Samples were mixed with KBr to prepare pellets by applying 5 ton pressure. By powder diffuse reflectance in FTIR; spectra were obtained in a scanning period of 3min.

2.3. Method of Preparation of Nanosponges

2.3.1. *Emulsion solvent dispersion method*

Table.1*: Formulation Batches for Nanosponges Preparation*

Sr. no	Formulation code	Organic phase		Aqueous phase		
		Drug (mg) Griseofulvin	Polymer (mg) β -cyclodextrin	Solvent (ml) dichloromethane	Polyvinyl alcohol (mg)	Distilled water (ml)
1	F1	125	125	20	300	100
2	F2	125	250	20	300	100
3	F3	125	375	20	300	100
4	F4	125	500	20	300	100
5	F5	125	625	20	300	100
6	F6	125	750	20	300	100

Table.1 illustrate the formulation batches of griseofulvin loaded Nanosponges. Six different batches of Nanosponges formulations are prepared by emulsion solvent dispersion method. The organic internal phase was first prepared by dissolving β -cyclodextrin in 20 ml dichloromethane and griseofulvin was added and stirred by using a magnetic stirrer to dissolve . In 100 ml of distilled water, the polyvinyl alcohol was dissolved. This was considered as aqueous external phase. Then organic internal phase was added drop by drop using 100 ml syringe to aqueous external phase and agitated for 2 hr. by using magnetic stirrer at 1000 rpm. Finally, the resultant dispersion was filtered, desiccated for 24 hr. at 40°C and then resultant powder obtained was used for characterization. [11]

2.4. Evaluation of nanosponges

2.4.1. *Determination of percentage yield*

The percentage yield of prepared nanosponges have been calculated by weighing the final weight of prepared nanosponges and initial weight of drug with the excipients used in formulation of nanosponges i.e. weight of drug, polymer and other excipients used in formulation.[17] Percentage yield of prepared nanosponges was calculated by using following formula.

$$\text{Percentage Yield} = \frac{\text{Actual weight of nanosponges prepared}}{\text{Weight of drug + weight of polymer + weight of excipients}} \times 100$$

2.4.2. Drug entrapment efficiency

Weighed 10 mg samples of drug-loaded nanosponges was dissolved in 10 ml of methanol under sonication for 1 hr. The sample are then centrifuged at a high speed of 9000 rpm for 30 min and the supernatant liquid was analyzed for non-bound drug in UV spectrophotometer at 291 nm.[18] Then percentage encapsulation efficiency was calculated by using following formula :

$$\% \text{ entrapment efficiency} = \frac{\text{Total drug added} - \text{free unentrapped drug}}{\text{Total drug added}} \times 100$$

Total drug added – free unentrapped drug
% entrapment efficiency = ×100
Total drug added

2.4.3. *Particle size, polydispersity index and zeta potential determination*

The mean particle size, polydispersity index (PDI), and zeta potential of all the batches were determined using Malvern@ zetasizer. Every sample to be analyzed was diluted suitably with distilled water prior to the measurements. The temperature was maintained at 25±0.5°C throughout the experiment. All the samples were measured in triplicates and results were represented as mean value standard deviation.[19]

2.4.4. *Scanning electron microscopy (SEM) examination*

The surface morphology of griseofulvin loaded nanosponges of optimized batch (F4) was examined by scanning electron microscope using high vacuum mode. The samples were coated with gold sputter coater and digital image of the sample were obtained at an accelerating voltage of 20 kV. [19]

2.4.5. *In-vitro drug release studies*

The release studies were performed for griseofulvin loaded nanosponges using USP dissolution tester apparatus II (paddle method). The paddle was rotated at speed of 50 rpm and temperature was maintained at 37°C±0.5°C. Amount of griseofulvin loaded nanosponges equivalent to 10 mg of drug was filled in the dialysis bag. Dialysis bags were tied with a paddle and immersed in a 900 ml dissolution medium. Initial studies were conducted in 0.1N HCL for 2 hrs. The dialysis bags were then transferred into a phosphate buffer of pH 7.4. 5ml of sample were withdrawn after each 1 hr. Samples were analyzed by using UV-spectrophotometer.[20]

4. RESULTS AND DISCUSSION

3.1. *Preformulation Studies*

3.1.1. Organoleptic properties

The sample of griseofulvin received was studied for its organoleptic properties such as color, odor, and appearance, as it is one of the first criteria for identification of compound and it showed properties which comply with standards. The color of griseofulvin was creamy white, odorless and the appearance of the sample was crystalline powder.[21]

3.1.2. *Solubility studies*

Table. 2: *Solubility profile of griseofulvin*

Solvent	Solubility
Dimethyl formamide	Freely soluble
Acetone	Soluble
Chloroform	Soluble
Ethanol	Slightly soluble
Water	Practically insoluble
Phosphate buffer 7.4	Soluble

Table.2 illustrate the solubility of drug griseofulvin in different types of solvents. According to the standard specified; it was found that Griseofulvin is very slightly soluble in water (0.2 g/L at 25 °C). Solubility study of drug sample was studied in different types of solvent and data shows that drug was very slightly soluble in ethanol and methanol; soluble in chloroform and acetone, and freely soluble in dimethyl formamide.[22]

3.1.3. Melting point

The melting point of a substance is defined as the temperature at which the substance begins to melt and is completely melted except as defined otherwise for certain substances. The melting point of pure griseofulvin was found to be in the range 220 to 223°C. The reported melting point of griseofulvin was 220.24 °C. Confirmation of melting point shows that sample of griseofulvin obtained was of pure quality.[22]

3.1.4. Ultraviolet – Visible spectroscopy study

Determination of λmax Of Griseofulvin In Methanol

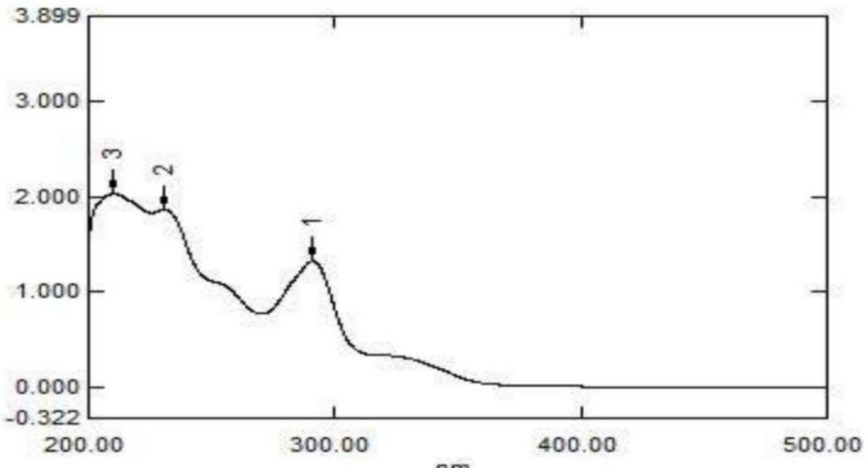

Fig. 1 ***UV –visible spectrum of griseofulvin in methanol.***

Figure. 1 illustrate the UV–visible spectrum of griseofulvin in methanol. The UV spectrum of pure griseofulvin solution in methanol exhibits wavelength of absorbance maximum at 291 nm which is exactly the same as standard λ max. However, the probable concentrations likely to be encountered while carrying out in vitro drug release studies and considering the predicted theoretical λ max was decided as 291nm.[23]

Construction of beers lamberts plot

Table.3: ***Absorbance of Griseofulvin in methanol.***

Sr. no	Concentration(ug/ml)	Absorbance (nm)
1	2	0.09
2	4	0.199
3	6	0.296
4	8	0.388
5	10	0.546

Table.3 Illustrate the Absorbance of Griseofulvin in methanol**.**The Absorbance of drug is determined to check the purity of drug sample. The dilutions of 2-10 ug/ml were prepared from 100 ug/ml solution and analyzed by UV- visible spectrophotometer. Absorbance was increased with increase in concentration of griseofulvin as shown above.[23]

Calibration curve of griseofulvin in methanol

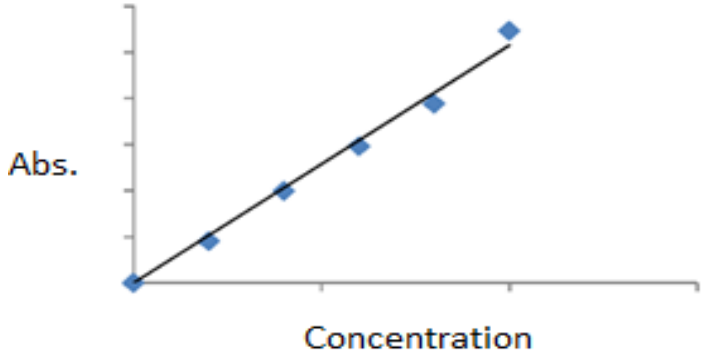

Fig.2 ***Calibration curve of Griseofulvin in methanol.***

Figure.2 Illustrate the calibration curve of griseofulvin in methanol which was found to be linear in concentration range of 2 to 10 µg/ml, having coefficient of regression value R^2= 0.9904. The regression line is good consideration for the true relationship of concentration and absorbance. slope y= 0.0514x

Determination of λ max of griseofulvin in phosphate buffer pH 7.4.

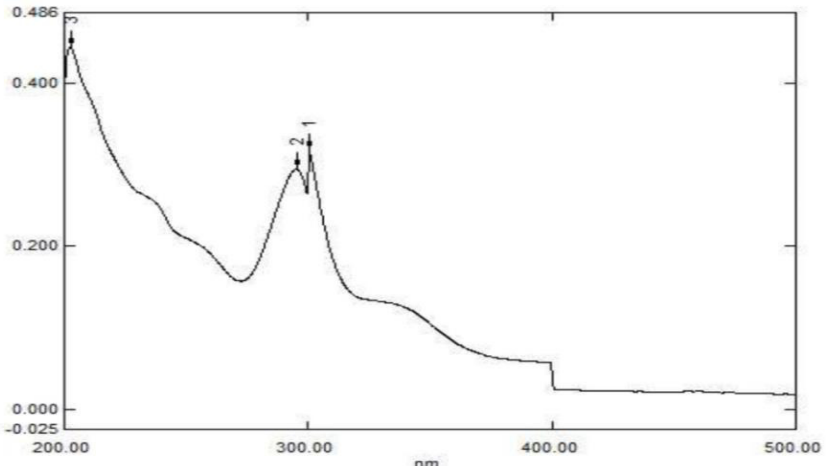

Fig.3 *UV –visible spectrum of griseofulvin in phosphate buffer* pH *7.4*

Figure. 3 illustrate the UV–visible spectrum of griseofulvin in phosphate buffer pH 7.4. The UV spectrum of pure griseofulvin solution in phosphate buffer pH 7.4 exhibitswavelength of absorbance maximum at 295.20 nm and standard λ max is 296 nm which is near to the reported λ max. This shows the purity of drugs. Spectrum of griseofulvin in phosphate buffer pH7.4.[23]

Construction of beers lamberts plot

Table .4: *Absorbance of Griseofulvin in phosphate buffer pH 7.4*

Sr. no	Concentration(ug/ml)	Absorbance (nm)
1	2	0.076
2	4	0.192
3	6	0.236
4	8	0.317
5	10	0.397

Table.4 Illustrate the Absorbance of Griseofulvin in phosphate buffer pH 7.4**.** The Absorbance of drug is determined to check the purity of drug sample. The dilutions of 2-10 ug/ml were prepared from 100 ug/ml solution and analyzed by UV- visible spectrophotometer. Absorbance was increases with increased in concentration of griseofulvin.[23]

Calibration curve of griseofulvin in phosphate buffer 7.4

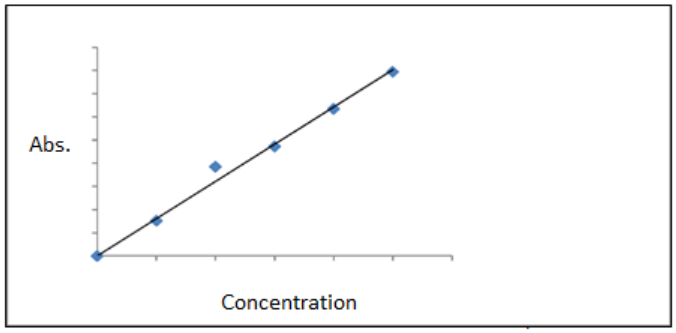

Figure.4 Illustrate the calibration curve of griseofulvin in phosphate buffer pH7.4 which was found to be linear in concentration range of 2 to 10 µg/ml, having coefficient of regression value R^2= 0.09902 and slop y=0.0402x.

Fig.4 *Calibration curve of Griseofulvin in phosphate buffer pH 7.4*

3.1.5. FTIR spectroscopy

FTIR spectra of pure GF were reported at wave numbers 1708, 1660, 1619, 1598, 1504, 1466, 1428, 1353, 1337, 1238, 1182,1061, 960, 888, 821, 801 and 682 cm^{-1}. FTIR spectra of griseofulvin loaded nanosponges reported at wave numbers 1705.07, 1651.07, 1624.06, 1508.33, 1454.33, 1417.68, 1340.53,1215.15, 1153.43, 1031.92, 941.26, 858.32,705.95.

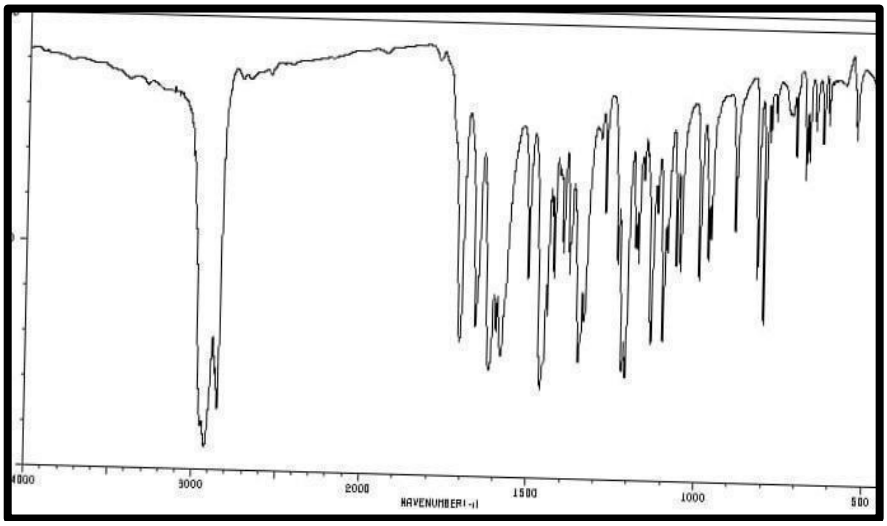

Fig.5 *FTIR spectrum of Griseofulvin*

Figure 5 illustrates the FTIR spectra of pure griseofulvin, in which characteristic peak at wave number 682 cm^{-1} showed presence of benzene derivatives. Peak at 1353 cm^{-1}indicates presence of O-H bending of aromatic ring peak at 1238 cm^{-1} indicates the presence of C-O stretching of alkyl, aryl ether. Sharp peak at 888 cm^{-1} and 821cm^{-1} indicates C-Cl stretch of chloro-benzene ring. The peak at 1708, 1660 cm^{-1} indicates C=O stretch of lactum and three peaks at 1619, 1598 and 1504 cm^{-1} indicates C=C stretch of aromatic ring.[24]

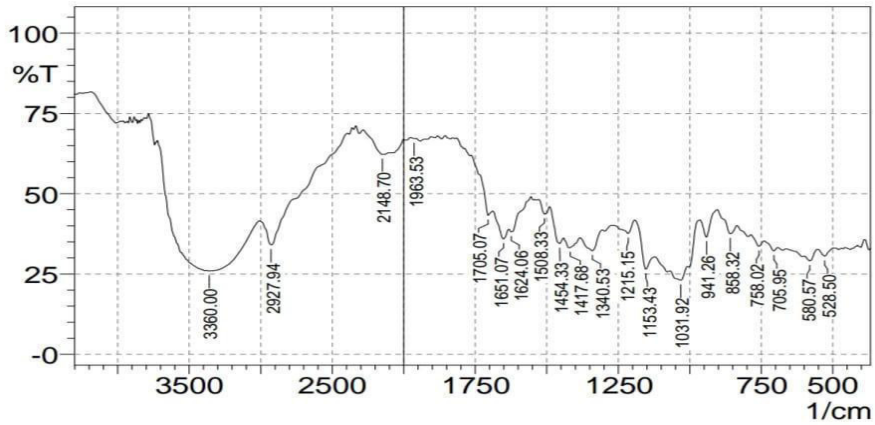

Fig.6 *FTIR spectrum of Griseofulvin Nanosponges*

Figure 6 illustrates the spectra of griseofulvin loaded nanosponges in which the same peaks were observed at wavenumber 1705.07 and 16651.07 cm^{-1}indicate presence of C=O stretch of lactum. The two peaks at 1624.06 and 1508.33 cm^{-1}indicates C=C stretch of aromatic ring. Peak at 1215.15 cm^{-1} indicates the presence of C-O stretching of alkyl, aryl ether. Also sharp peak at 858 cm^{-1} indicates C-Cl stretch of chloro-benzene ring. The absorption band shown by griseofulvin and griseofulvin loaded nanosponges shows all characteristics of groups present in its molecular structure. The presence of an absorption band corresponding to the functional groups present in the structure of griseofulvin confirmed the identification and purity of purchased griseofulvin samples.[24]

3.3. *Evaluation of Nanosponges*

Evaluation of prepared nanosponges of griseofulvin were carried out for percentage yield, entrapment efficiency, particle size, polydispersity index, zeta potential and scanning electron microscopy study.

3.3.1. Percentage yield

Table .5 *Percentage yield of Nanosponges*	
Batch number	**Percentage yield (%)**
F1	30.90± 0.03
F2	27.11±0.006
F3	44±0.02
F4	47.78±0.026
F5	36.72±0.04
F6	35.85±0.005

Table.5 illustrates the percentage yield of nanosponges which were found in the range of 27.11-47.78%. The optimized batch was found to be F4 on the basis of percentage yield. The percentage yield of the optimized batch was found to be 47.78%. The percentage yield of β -cyclodextrin based nanosponges is increased as compared to previous studies is due to change in the method of preparation in which dichloromethane is used as crosslinking agent. It was concluded that β -cyclodextrin concentration and cross-linking time affects the percentage yield of nanosponges. The percentage yield may vary due to the change in polymer concentration.[25]

3.3.2. *Percentage entrapment efficiency*

After preparing nanosponges dispersion, unentrapped drug is separated by centrifugation method described above and the drug remained entrapped in nanosponges is determined by spectrophotometric method.

Table .6 *Entrapment efficiency in Nanosponges.*			
Batch code	**Absorbance at 265nm**	**Unentrapped drug (%)**	**%Entrapment nanosponges**
F1	0.523	10.16	91.86±0.04
F2	0.935	18.08	85.53± 0.02
F3	0.134	2.81	97.74± 0.02
F4	0.139	2.87	97.69±0.006
F5	0.380	7.50	93.99±0.033
F6	0.334	6.61	94.70±0.006

±SD P<0.01 ,n=10

Table.6 illustrates the entrapment efficiency of nanosponges which were found in the range of 85.53±0.02% to 97.74±0.02%. Entrapment efficiency of the optimized batch (F4) was found to be 97.69±0.006. Entrapment efficiency increased as compared to previous studies by changing the method of preparation as well as crosslinking agent and also with increasing polymer concentration up to certain limit. It was also reported that with increasing polymer concentration, the EE also increases as there are more chances of drug entrapment.[26]

3.3.3. Particle size

Table 7: Particle size of all formulation Batches	
Batch code	**Particle size(nm)**
F1	608.15
F2	652.40
F3	909.53
F4	488.59
F5	829.71
F6	863.83

Figure.7 shows the particle size analysis of the griseofulvin loaded nanosponges. Particle size analysis was performed by Malvern@ zetasizer instrument. The griseofulvin nanosponges were successfully prepared by using different concentration of polymers. The particle size was found to be between 488.59nm to 909.53 nm. which is in the range of standard paticle size of nanosponges which is in increasing order due to an increase in the concentration of polymer, but after a certain concentration, the ratio of drug to polymer was increased the particle size decreased. This may be because of the high drug to polymer ratio; the amount of the polymer available was less. The result of the average particle size of optimized batch F4 was observed 488.59 nm. The particle size of griseofulvin nanosponges batch (F4) is decreased as compared to the previous studies as we adopted another method of preparation in which the stirring time was extended upto 2hrs with 1000 rpm. Also it is concluded that particles size varies with the concentration of the drug-polymer ratio.[27]

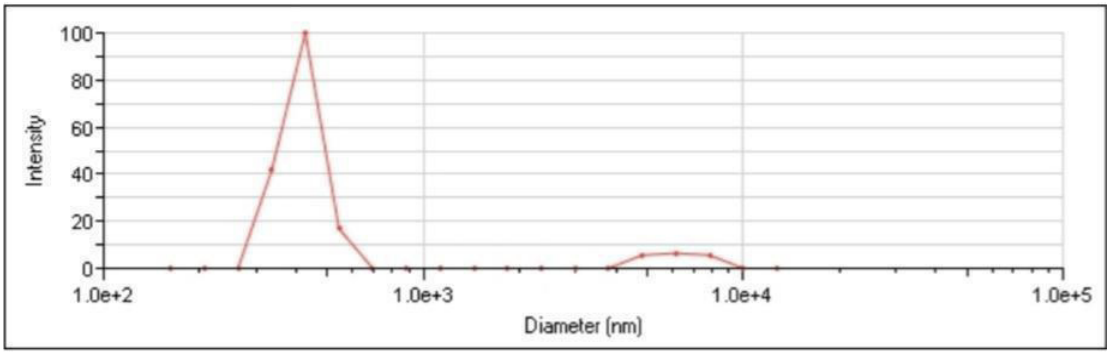

Fig.7 *particle size distribution of optimized batch F4*

Figure 7 illustrates the graphical representation of particle size distribution of optimized batch F4. Particle size were determined by a dynamic light scattering particle size analyser Malvern zetasizer.The average particle size of optimized batch was found to be 488.59nm which is in the range of standard paticle size of nanosponges.

3.3.4. Zeta potential

Table.8: *Zeta potential of all formulation Batches*

Batch code	Zeta potential
F1	-20.32
F2	-20.71
F3	-21.94
F4	-20.77
F5	-26.93
F6	-21.08

Table. 8 illustrates the zeta potential analysis of the griseofulvin loaded nanosponges. Zeta potential is the measure of magnitude of the electrostatic or charge repulsion or attraction between particles and it is one of the fundamental parameters known for affecting the stability of formulation. Zeta potential of all formulation batches was not much affected as compared to previous studies. So it can be concluded that increase in stirring time, drug and polymer ratio, and crosslinking agent did not affects the zeta potential of griseofulvin nanosponges The zeta potential of the prepared nanosponges was found to be in the range of -20.32 to -26.93. The zeta potential of the optimized batch was found to be -20.77.[27]

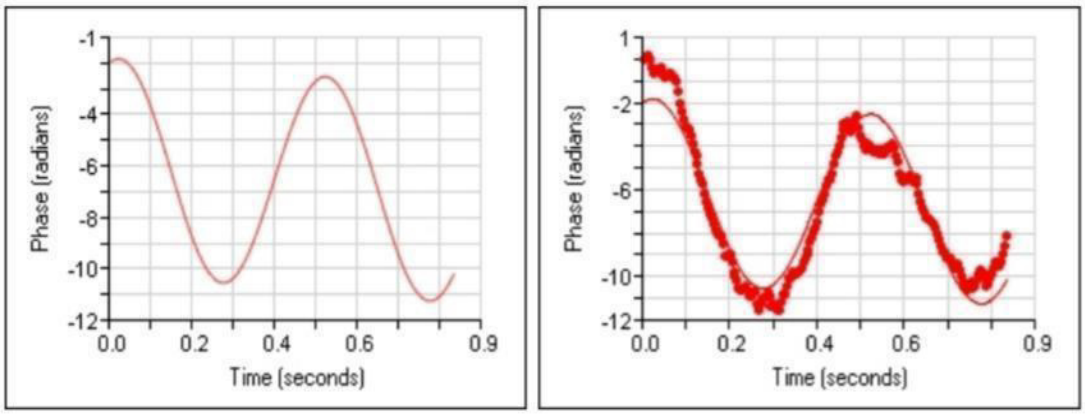

Fig.8 *zeta potential of optimized batch (F4)*

Figure.8 illustrates the zeta potential of optimized batch (F4) which was found to be -20.77 which was considered quite sufficient for keeping the particles well- separated from each other as a result of electric repulsion. The negative charge to value was to be attributed to the carbonyl group and free hydroxyl group of β -cyclodextrin.[27]

3.3.5. Polydispersity index (PDI)

Table.9: *Polydispersity index of all formulation Batches*	
Batch code	**Polydispersity index**
F1	0.203
F2	0.061
F3	0.364
F4	0.207
F5	0.315
F6	0.333

Table. 9 illustrate the polydispersity index of the griseofulvin loaded nanosponges. PDI is an index of width, spread, or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas the PDI of the higher value indicates a wider particle size distribution and polydisperse nature of the sample. The polydispersity index is measure of heterogeneity of sample based on size. The PDI value of formulation batches were not much affected as compared to previous studies. So it can concluded that PDI were not affected with change in drug and polymer ratio, stirring time, type of crosslinking agent used and the method of preparation for griseofulvin nanosponges .The polydispersity index of the optimized batch was found to be 0.207. Therefore, it can be stated that the β -cyclodextrin based nanosponges prepared exhibited a homogeneous size distribution.[28]

3.3.6. Scanning electron microscopy

The SEM technique is used to study microscopic behavior of drug-nanosponges composition.

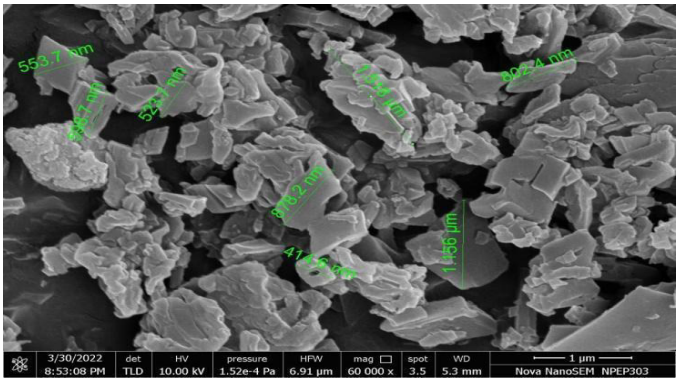

Fig 9: *SEM of nanosponges of optimized batch(F4)*

Figure.9 illustrates the microscopic behavior of griseofulvin loaded nanosponges. The result in the SEM image of nanosponges of optimized batch (F4) are in agreement with results of previous studies. Above figure revealed the highly porous structure of griseofulvin loaded nanosponges formulation batch (F4) indicating sponges-like shape. The porous texture of nanosponges facilitate the infiltration of a drug into an interpenetrating network of nanosponges.[29]

3.3.7. *In-vitro* drug release study

Table.8 cumulative percentage drug released.						
Time (hrs.)	**Cumulative percentage drug released %**					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	2.6±0.001	2.5±0.012	1.3±0.31	3.5±0.23	2.9±0.03	3.1±0.04
2	9.34±0.12	8.45±0.06	9.36±0.002	10.1±0.11	9.34±0.13	11.23±0.15
3	12.67±0.11	11.87±0.16	10.89±0.003	15.7±0.005	11.56±0.001	14.3±0.014
4	17.43±0.42	19.78±0.01	16.97±0.04	30.45±0.12	20.43±0.07	19.31±0.04
5	19.56±0.003	25.9±0.007	23.76±0.008	45.67±0.002	25.43±0.005	24.12±0.01
6	27.45±0.10	30.98±0.01	36.21±0.05	65.56±0.03	28.34±0.01	29.1±0.04
7	30.57±0.01	41.87±0.002	45.14±0.11	70.51±0.01	31.5±0.03	35.43±0.05
8	55.67±0.12	47.21±0.02	56.87±0.03	75.56±0.07	49.6±0.03	40.87±0.01

Table.8 illustrates the comparative drug release profile of all 8 formulation batches of griseofulvin loaded nanosponges. The *in-vitro* release of griseofulvin from nanosponges was determined by using dissolution test apparatus (USP I I). The griseofulvin released from all batches were found to be 75.56% within 8 hrs. The F4 formulation batch shows the highest drug released and hence considered an optimized formulation batch.[30]

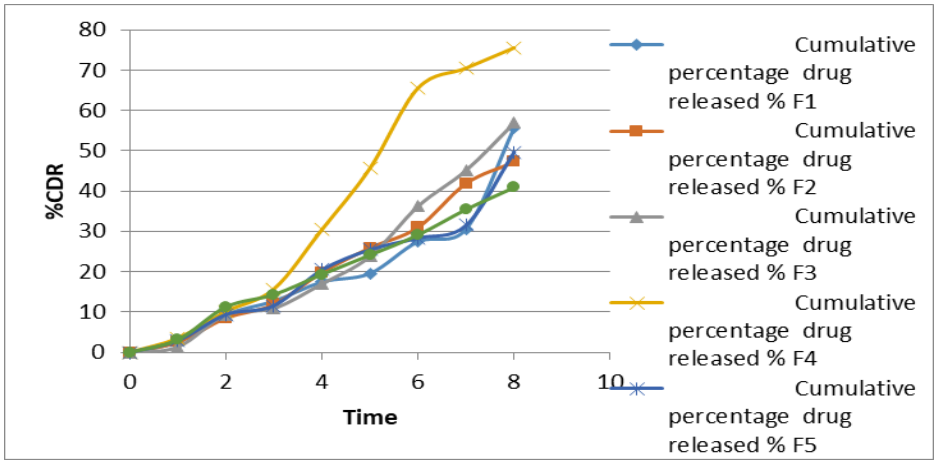

Fig.10 *in-vitro drug release profile of Griseofulvin Nanosponge*

Figure.10 illustrates the comparative in-vitro drug release profile of all formulation batches of griseofulvin loaded nanopsonges. The F4 formulation batch shows the highest drug released and hence considered an optimized formulation batch.[30]

5. CONCLUSION

From all observations and results obtained it can be concluded that the prepared formulation batches of griseofulvin loaded nanosponges show satisfied organoleptic properties. A characterization of the drug and excipient was performed and no immeasurable peaks were observed in FT-IR analysis, so characterization confirmed that there was no interaction between the drug and polymers. It was found that the formula (F4) composed of 1:4 w/w (griseofulvin: β -CD) provided highest entrapment efficiency and showed promising in-vitro release with increase in dissolution efficiency. All results were compared to the standard, which concluded that the drug and excipient were of pure and standard quality. The particle size of the optimized batch (F4) is 488.59 nm. Zeta potential of the optimized batch (F4) is -20.77 shows good stability of formulation. The *in -vitro* drug release study confirmed the release of griseofulvin for a prolonged period of time. Finally, from this overall study we concluded that griseofulvin loaded nanosponges formulation can be used in a successful griseofulvin dosage form for pediatrics in the form of dry suspension for reconstitution.

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7. AUTHOR CONTRIBUTION STATEMENT

All authors conceived and designed the study. Ashish Pawar and Khanderao Jadhav conceptualized and gathered the data with regard to this work. Jyoti rao and Ashvini Tapkir performed the research work and discussed methodology. Prashant Malpure, Rishikesh Bachhav contributed in writing final draft of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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