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Formulation and Evaluation of Nanosponges Loaded Hydrogel Using Different Polymers Containing Selected Antifungal Drug

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Abstract

Objectives

Nanosponge is a new concept for the drug delivery system. In the present research work, an attempt was made to develop nanosponge based topical hydrogel containing griseofulvin. Nanosponges are mainly used to provide the sustained release and thereby reduce the side effects caused by conventional dosage form.

Methods

Nanosponges were prepared using emulsion solvent diffusion method by using ethyl cellulose and eudragit S 100 in different concentrations. The prepared nanosponges were evaluated for preformulation parameters. The nanosponges formulation of all batches were evaluated for production yield, entrapment efficiency. Optimized formulation was evaluated for SEM analysis and incorporated into a gel base. The nanosponges containing gel was evaluated for pH determination, spreadability, swelling studies, viscosity determination, and in vitro diffusion study using franz diffusion cell for 10 hrs.

Results and Discussion

The results of FTIR analysis showed that there is no physical and chemical interaction between drug and other excipients. F2, F5 are considered to be the optimized nanosponge formulation. G1 was considered to be the best formulation. The data from the in vitro release study were fitted to various mathematical models. The results of mathematical model of fitting data obtained indicated the best fit in all cases. Stability study for 1 months at various condition shows G1 has good stability.

Conclusion

The study indicates that the rate of drug release can be improved by incorporating drug into the nanosponges and thus it can improve the targeting of the drug at the specific site and thereby reduce systemic toxicity.

INTRODUCTION

Nanosponges are tiny mesh – like nanoporous particular structure in which a large variety of substances can be encapsulated or suspended, and then be incorporated into a dosage form. They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior. Nanosponges have recently been developed and proposed for drug delivery. Nanosponges can solubilize poorly water soluble drug and provide prolonged release as well as improving drugs bioavailability. Nanosponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility .Nanosponges are more like a three- dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called crosslinkers that act like tiny grappling hooks to fasten different parts of the polymer together. The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core[4].

As the nanosponges have an open structure with pores on its surface ie; in the surrounding of nanosponges they do not have any uninterrupted membrane, the active substance is added to the vehicle in an encapsulated form. The encapsulated active substance is able to move freely from the particles into the vehicle until the vehicle gets saturated and the equilibrium is attained. When the product is applied on to the skin, the vehicle containing the active gets unsaturated causing a disturbance in the equilibrium. This will start a flow of the active from the sponge particle into the vehicle and from it to the skin until the vehicle is either dried or absorbed. Even after the withholding of the nanosponge particles on the surface of skin i.e. the stratum corneum, the release of active substance continues to skin for a long period of time.

For prolonged and controlled release of the drug products on the skin the nanosponges technology is the most efficient technology. Antifungal, antibiotics, anti-inflammatory are the common type of drugs used in the topical application. Conventional products release the drug in a relatively high concentration this may led to serious side effects but the nanosponge drug delivery

system release the drug in a sustained and predictable manner. The nanosponges can be formulated into ointments, gels, creams, lotions[8]

MATERIALS AND METHODS

Griseofulvin, Ethyl Cellulose, Eudragit S 100, Dichloromethane, Polyvinyl Alcohol, Carbopol 934, propylene glycol, Triethanolamine were obtained from Yarrow Chem products, Mumbai.

FORMULATION OF NANOSPONGES

and polyvinyl alcohol are used to prepare nanosponges. Two phases are used in this method– dispersed and continuous. The dispersedphase consists of ethyl cellulose and the drug, which is then dissolved in 20 ml of dichloromethane and some amount of polyvinyl alcohol (PVA) is added to 150 ml of the continuous phase (aqueous). Then, the mixture is stirred at the speed of 1000 rpm for about 2 h. The product i.e. the nanosponges are collected by filtration. Finally, the product is dried inanoven at a temperature of 400˚C [Venkateshet al.Int J App Pharm, Vol 10, Issue 4, 2018, 1-5

Materials used in the preparation of nanosponges

Polymer,Copolymer,- Hyper cross-linked polystyrenes, cyclodextrins and its derivatives like methyl β-cyclodextrine, 2-hydropropyl β-cyclodextrine.Ethyl cellulose (EC), polyvinyl alcohol (PVA), Crosslinkers like Di-phenyl Carbonate (DPC), diarylcarbonate, diisocyanates, pyromelliticanhydride, carbonyl diimidazole, 22-bis (acrylamide) acidic acid and dichloromethane. [8, 9]

Ultra-sound assisted synthesis

Polymers are made to react with crosslinkers in a flask without the solvent. The flask is placed in an ultrasound bath which is filled with water and heated up to 90˚C and the mixture is sonicated for 5 h.

Then the mixture is cooled down to room temperature and then the product isbroken into rough pieces. At last, the non-reacting polymer is removed by washing the product with water and refining is done using soxhlet apparatus (ethanol) to obtain nanosponges[10].

Emulsion solvent diffusion method

In this method, different proportion or amount of ethyl cellulose and polyvinyl alcohol are used to prepare nanosponges. Two phases are used in this method– dispersed and continuous. The dispersedphase consists of ethyl cellulose and the drug, which is then dissolved in 20 ml of dichloromethane and some amount of polyvinyl alcohol (PVA) is added to 150 ml of the continuous phase (aqueous). Then, the mixture is stirred at the speed of 1000 rpm for about 2 h. The product i.e. the nanosponges are collected by filtration. Finally, the product is dried inanoven at a temperature of 400˚C [11Venkateshet al.Int J App Pharm, Vol 10, Issue 4, 2018, 1-5

The nanosponges are prepared by emulsion solvent diffusion method. In this method two phases are used in different proportions. The dispersed phase having ethyl cellulose or eudragit S 100 and drug(griseofulvin) get dissolved in dichloromethane (20 ml) and a definite amount of polyvinyl alcohol added to 100 ml of aqueous continuous phase. Then, the mixture was stirred properly at 1000 rpm for 2hr. The required nanosponges were collected by the process of filtration by using membrane filter (pore size 0.45 µm) and kept for drying in oven at 40ºc for 24hr. Nanosponge which are dried and stored in dessicator are ensured of removal of residual solvents[16].

COMPOSITION OF GRISEOFULVIN NANOSPONGES

Table a: Composition of Griseofulvin Nanosponges

INGREDIENTS	FORMULATIONS					
	F1	F2	F3	F4	F5	F6
Griseofulvin(mg)	100	100	100	100	100	100
Ethyl Cellulose(mg)	200	400	600	_	_	_
Eudragit S 100(mg)	_	_	_	200	400	600
Dichloromethane(ml)	20	20	20	20	20	20
Polyvinyl alcohol(mg)	500	500	500	500	500	500
Distilled water(ml)	100	100	100	100	100	100

FORMULATION OF NANOSPONGE LOADED GEL

The polymer Carbopol 934 was initially soaked in water for the gel for 2 hrs and dispersed by agitation at 600rpm by using magnetic stirrer to get smooth dispersion. Triethanolamine was added to neutralise the pH. The previously prepared optimized nanosponge suspension was thereby added and permeation enhancers Propylene glycol was added as ethanolic solution to the aqueous dispersion[16]

Table b: Composition of nanosponges loaded gel

Ingredients	Quantity
Griseofulvin loaded nanosponges(g)	1
Carbopol(g)	1
Propylene Glycol(ml)	5
Triethanolamine(ml)	q.s
Distilled water(ml)	100

EVALUATION OF GRISEOFULVIN LOADED NANOSPONGES

PHYSICAL EXAMINATION

The prepared griseofulvin loaded nanosponges were inspected visually for their colour and appearance.

PRODUCTION YIELD

The prepared nanosponges were collected and weighed. Production yield of nanosponges was determined by formula mentioned below

Production yield = $\frac{Practical\ mass}{Theorectical\ mass} X100$

SURFACE MORPHOLOGY

Scanning electron microscopy was used to analyze particle size and surface topography was operated at 15kV accelaration voltage. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer 20nm thick. Photographs were elaborated by an image processing program and individual diameters were measured to obtain mean particle size[16].

ENTRAPMENT EFFICIENCY

To calculate the entrapment efficiency, accurately weighed quantity of nanosponges (10mg) with 5ml of methanolic HCl (HCl: Methanol-10:1) in a volumetric flask was shaken for 1min using vortex mixer. The volume was made upto 10ml with Methanolic HCl. Then the solution was filtered and diluted and the concentration of drug was determined spectrometrically at 295nm[16].

Entrapment Efficiency = $\frac{Actual\ drug\ content\ in\ nanosponges}{Theorectical\ drug\ content}$ **X 100**

EVALUATION OF PREPARED NANOSPONGE LOADED GEL

VISUAL INSPECTION

The organoleptic properties, such as colour, odour, homogeneity, and physical appearance of gel containing nanosponges were checked by visual inspection.

pH DETERMINATION

The pH of the prepared nanosponge loaded hydrogel formulations were determined by using a digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and stored for two hours. Then, pH measurement was performed. The measurement of pH of each formulation was done in triplicate and average values were calculated[16].

VISCOSITY MEASUREMENT

The viscosity of prepared hydrogels was measured using Brookfield viscometer. Viscosity was measured at 25^{o}C at 100 rpm using spindle no.LV- 61[38].

SPREADABILITY STUDIES

Spreadability is a term expressed to denote the extent of the area to which the gel readily spreads on application to the skin. The therapeutic efficacy of a semisolid formulation also depends on its spreading value.1 g of the formulation was placed within a circle of 1cm diameter pre-marked on a ground glass slide. The gel formulation was sandwiched between this slide and the second slide having the same dimension. A weight of 500 g was allowed to rest on the upper glass slide for 5 min. The increase in the diameter due to gel spreading was noted. The spreadability was then calculated from the following formula[21].

S= M × L/T

S= Spreadability

M = Mass in grams

L=Length of the slide

T =Time

DRUG CONTENT ESTIMATION

1 g of prepared griseofulvin nanosponge loaded hydrogel formulation containing drug equivalent to 100 mg was extracted with 30 ml of ethanol. The volume was made up to 100 ml with phosphate buffer 7.4. The solution was filtered. The absorbance of the resulting solution was measured at 295 nm using a UV spectrophotometer after suitable dilutions. The drug content of the formulation was determined using the following equation.

% Drug content = $\frac{\text{Actual concentration of drug in the formulation}}{\text{Theorectical concentration of drug}} X100$

***IN VITRO* DRUG RELEASE STUDIES**

In vitro release study of griseofulvin nanosponges loaded hydrogel was carried out by using Franz diffusion cell. The formulation was taken in the donor compartment and phosphate buffer saline was taken in the receptor compartment. The cellophane membrane previously soaked overnight in the diffusion medium (phosphate buffer 7.4) was placed between the donor and the receptor compartment. 1 g of the formulation was spread uniformly on the cellophane membrane, which is in contact with the receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37± 0.5^{o}C. At specific intervals, 1 ml of sample was withdrawn from the receptor compartment and replaced with an equal volume of Phosphate buffer 7.4. After suitable dilutions, the absorbance of the sample was determined at 295 nm by UV-visible spectrophotometer[21].

DRUG RELEASE KINETICS

Release kinetics of drug from the dosage form was determined by various mathematical models such as zero order, first order, korsemeyer-peppas and higuchi model.

- Zero Order Plots(Cumulative percentage drug released) v/s time

- First Order Plots(Log cumulative percent drug remaining) v/s time
- Higuchi Plots(Cumulative percentage drug release) v/s square root of time
- Korsemeyer-Peppas Plots(Log cumulative percentage drug release) v/s log time

RESULTS AND DISCUSSIONS

FTIR analysis was carried out for pure drug and drug excipient mixtures. Spectrum of drug showed the prominent peaks with respect to functional groups. The spectrum of physical mixture of drug with excipients showed that there is no significant interaction between the drug, polymer and excipients. In the spectrum of drug polymer mixtures the characteristic peak of drug was not altered.

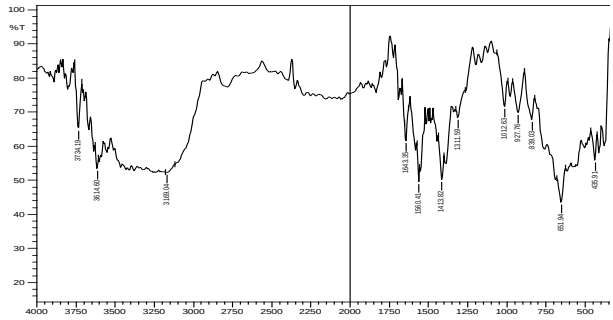

Fig 1: FTIR Spectrum of the Griseofulvin

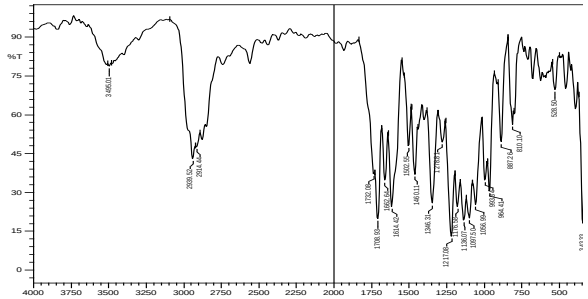

Fig 2: FTIR Spectrum of Griseofulvin and Ethyl Cellulose

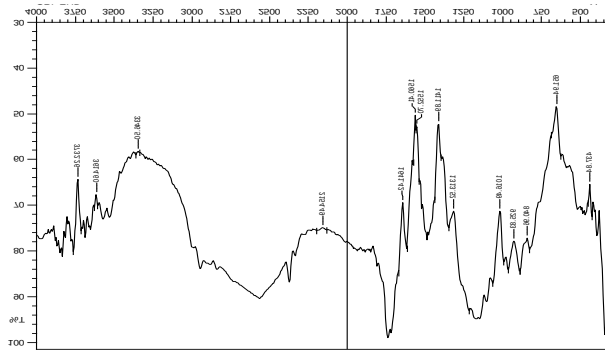

Fig 3: FTIR Spectrum of Griseofulvin and Eudragit S 100

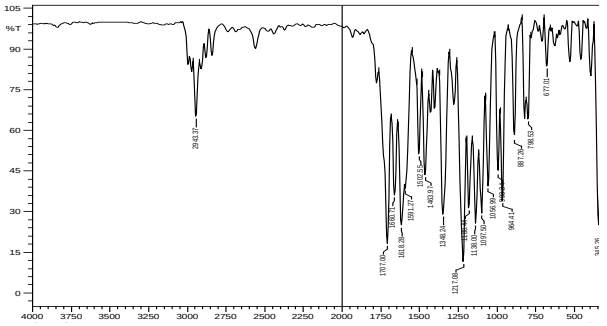

Fig 4: FTIR Spectrum of Griseofulvin and Dichloromethane

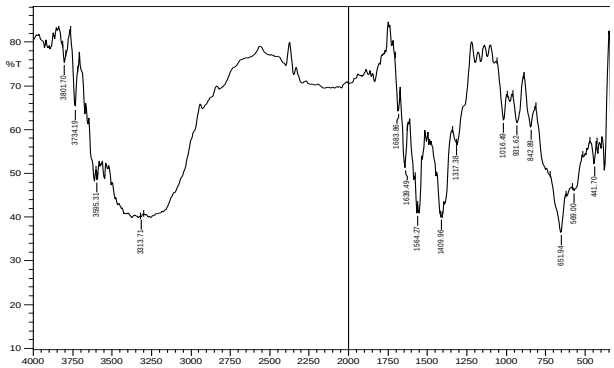

Fig 5: FTIR Spectrum of Griseofulvin and Polyvinyl Alcohol

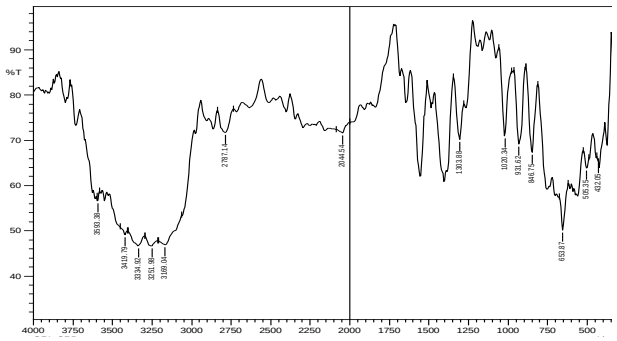

Fig 6: FTIR Spectrum of Griseofulvin and Carbopol 934.

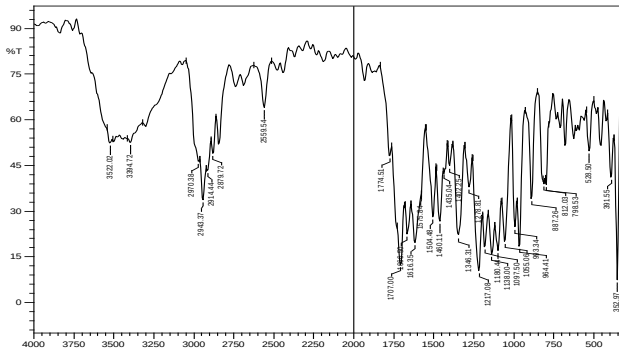

Fig 7: FTIR Spectrum of Griseofulvin and Ethanol

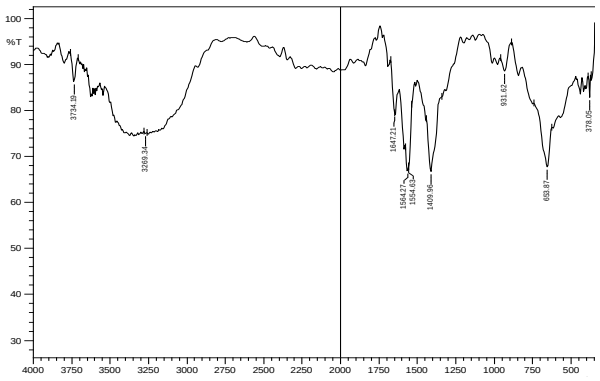

Fig 8: FTIR Spectrum of Griseofulvin and Triethanolamine

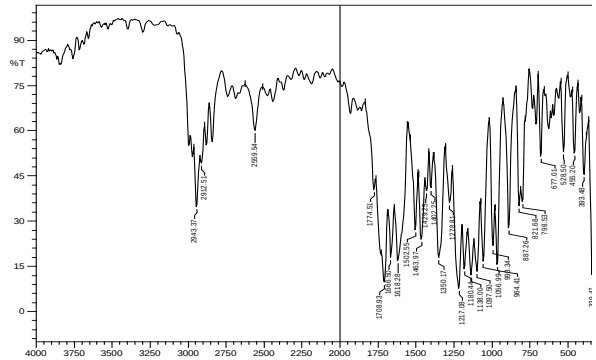

Fig 9: FTIR Spectrum of Griseofulvin and Propylene Glycol

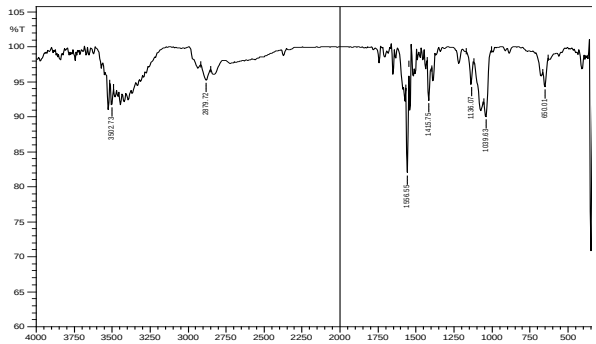

Fig 10: FTIR Spectrum of Griseofulvin and Mixture 1

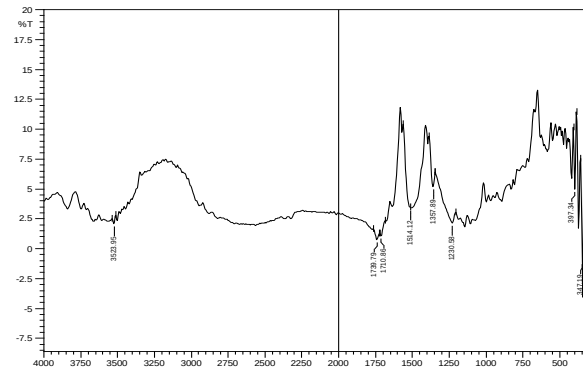

Fig 11: FTIR Spectrum of Griseofulvin and Mixture 2

FORMULATION DEVELOPMENT

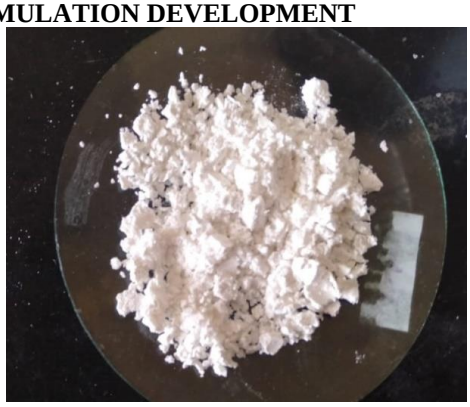

Fig 12: F2 Formulation

F13: G1 Formulation

Fig 14: F5 formulation

Fig 15: G2 formulation

EVALUATION OF GRISEOFULVIN LOADED NANOSPONGE

PHYSICAL EXAMINATION

Physical evaluation of nanosponges were shown in the table c. From the physical evaluation of all the batches formulated. It was concluded that the nanosponges of all batches had desirable physical properties.

Table c: Physical Examination

Sl. No	Formulation Code	Colour	Appearance
1	F1	White	Powder
2	F2	White	Powder
3	F3	White	Powder
4	F4	White	Powder
5	F5	White	Powder
6	F6	White	Powder

PRODUCTION YIELD

The production yield was calculated. Production yield of nanosponges are shown in the table d. The production yield of the prepared nanosponges of griseofulvin ranges from of 74% to 89%. It revealed that all formulation have good production yield.

Table d: Production Yield

Sl No	Formulation code	Production yield (%)
1	F1	77
2	F2	85
3	F3	87
4	F4	74
5	F5	82
6	F6	89

SURFACE MORPHOLOGY

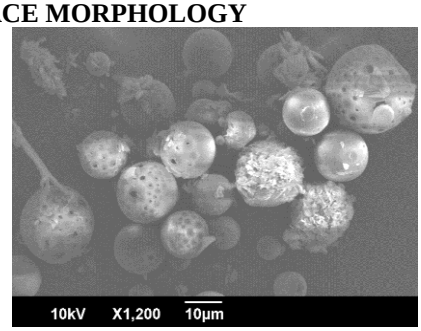

Fig 16: SEM image of F2

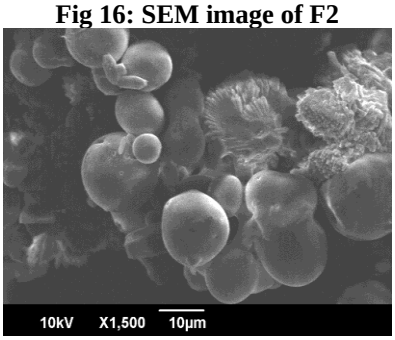

Fig 17: SEM image of F5

ENTRAPMENT EFFICIENCY

The entrapment efficiency of all batches were tested. The results were shown in the table e. The results show that the entrapment efficiency were in the range of 62.9±1.55% to 87.7±1.16%. The entrapment efficiency was higher in F2 and F5.

Table e Entrapment Efficiency

Sl No	Formulation Code	Entrapment Efficiency(%)(*±SD)
1	F1	81.8 ± 1.77
2	**F2**	**87.7± 1.16**
3	F3	68.66± 0.84
4	F4	62.9± 1.55
5	**F5**	**78.13 ± 0.65**
6	F6	66.3± 0.57

***Average of three determinants, SD=Standard deviation**

EVALUATION OF PREPARED NANOSPONGE LOADED GEL

G1: Gel containing F2 formulation

G2: Gel containing F5 formulation

VISUAL INSPECTION

Table f Visual Inspection

Formulation Code	G1	G2
Colour	White	White
Odour	Odourless	Odourless
Appearance	Transparent	Transparent
Homogeneity	Homogeneous	Homogeneous

pH DETERMINATION

The pH determination was done and the results were shown in the table g. The pH of the formulations were found to be satisfactory. The pH of G1 was found to be 7.02 ±0.29 and the pH of G2 was found to be 6.76±0.16.

Table g: pH Determination

Sl No	Formulation code	pH (*±SD)
1	G1	7.02±0.29
2	G2	6.76±0.16

***Average of three determinants, SD=Standard deviation**

VISCOSITY MEASUREMENT

The viscosity of the gel was determined. The viscosity was measured by the Brookfield viscometer spindle no. 61 at 100rpm. The result was shown in the table h. The viscosity of G1 and G2 was found to be 7285 centipoise and 8154 centipoise respectively.

Table h: Viscosity Measurement

Sl No	Formulation code	Viscosity (cps) (*± SD)
1	F1	7285±0.32
2	F2	8154±0.26

***Average of three determinants, SD=Standard deviation**

SPREADABILITY

The spreadability of the formulations were done and the result was shown in the table i. Spreadability of G1 and G2 was found to be 7.56gm-cm/s and 6.24gm-cm/s respectively.

Table i: Spreadability

Sl No	Formulation Code	Spreadability (gm-cm/s) (*± SD)
1	G1	7.56±0.17
2	G2	6.24±0.21

***Average of three determinants, SD=Standard deviation**

DRUG CONTENT

Drug content was calculated and the results were shown in the table j. G1 shows high drug content 98.09%.

Table j: Drug Content

Sl. No	Formulation code	% Drug content (*± SD)
1	G1	98.09±0.13
2	G2	95.27±0.53

***Average of three determinants, SD=Standard deviation**

IN VITRO DRUG RELEASE OF G1 and G2

The *in vitro* drug release of the nanosponges loaded gel was carried franz diffusion cell apparatus with phosphate buffer 7.4 for 10 hrs the results were shown in the table k. The plot of percentage drug release v/s time (hrs) was shown in figure 18. Gel 1 shows high percentage drug release.

Table k: Percentage Drug Release

Sl.No	TIME (h)	PERCENTAGE OF DRUG RELEASE (*± SD)	
		Gel 1	Gel 2
0	0	0	0
1	1	24.08 ± 0.50	16.54±0.74
2	2	32.96±1.00	22.13±1.69
3	3	38 ±0.36	32.65±0.96
4	4	45.73± 0.65	38.9±0.31
5	5	52.46 ±1.60	42.43±1.51
6	6	67.06± 1.11	58.87±1.01
7	7	75 ± 1.11	67.76±1.62
8	8	84 ± 1.22	72.76±1.63
9	9	91± 1.24	78.9±1.69
10	10	97.6± 0.46	84.6±1.24

***Average of three determinants, SD=Standard deviation**

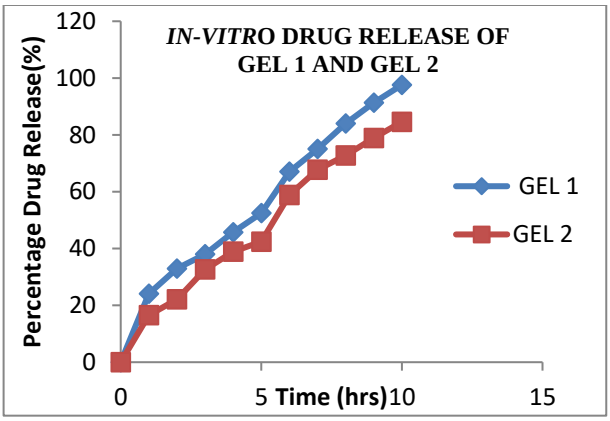

Fig 18: *In vitro* drug release of Gel 1 and Gel

SWELLING STUDIES

Table l: Swelling Studies

Sl. No	Formulation code	Percentage Swelling (%)
1	G1	74%
2	G2	68%

Swelling study was performed and the results were shown table l. From analyzing the percentage swelling, we can conclude that G1 shows high swelling percentage 74%.

KINETIC MODEL GEL 1

The diffusion profile of optimized formulation G1 was fitted to various kinetic models like zero order, first order, Higuchi model and Korsemeyer peppas model.

Zero Order Plot

Graph was plotted between cumulative % drug released v/s time.

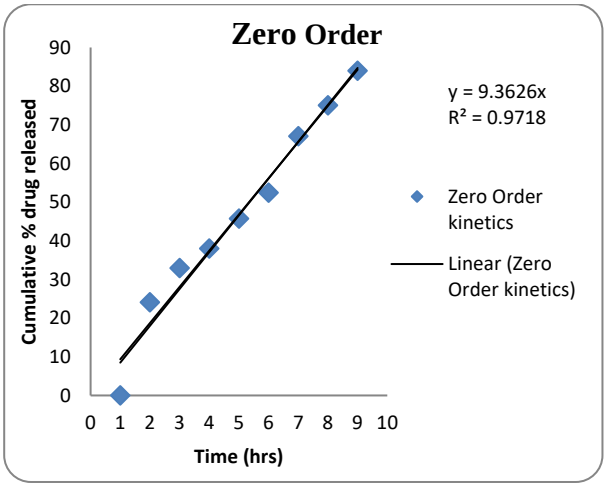

Fig 19: Zero order plot for drug release kinetics of G1 formulation

Korsemeyer peppas plot

Graph was plotted between log cumulative % drug release v/s log time.

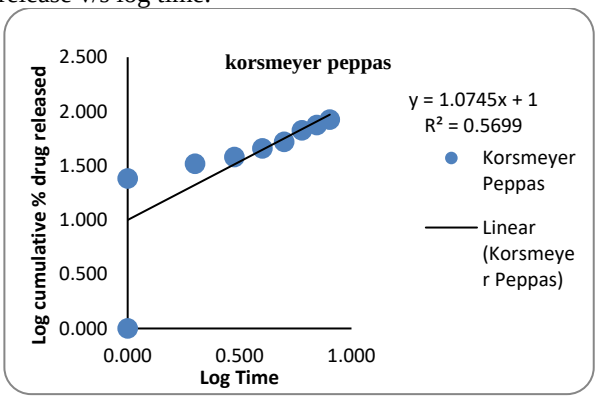

Fig 20: Korsemeyer peppas plot for drug kinetics of G1 Formulation

Higuchi plot

Graph was plotted between % cumulative drug released v/s square root of time

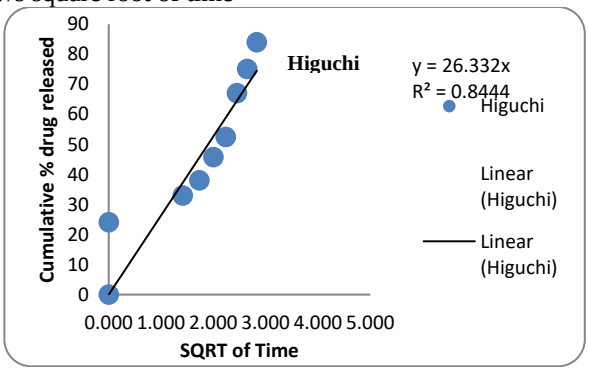

Fig 21: Higuchi plot for drug release kinetics of G1 Formulation

First order plot

Graph was plotted between log cumulative % drug remaining v/s time

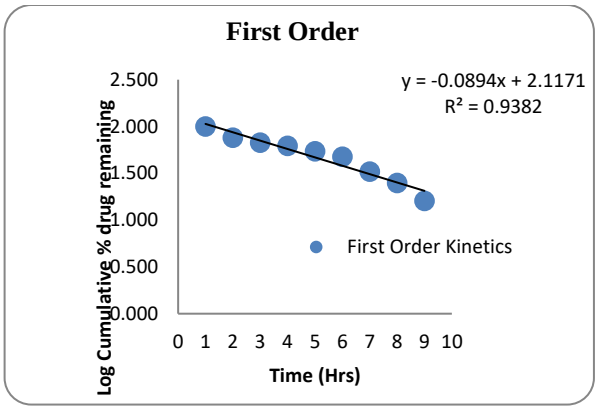

Fig 22: First order plot for drug release kinetics of G1 formulation

Table m: R^2 Values of various kinetics release data of optimized nanosponge gel

G1	Zero order	First order	Korsmeyer-peppas model	Higuchi model
R^2	0.975	0.938	0.532	0.844

The diffusion profile of optimized formulation G1 was fitted to zero order, first order, higuchi model and korsemeyer peppas model to ascertain the kinetic modelling of the drug releasing mechanism showed in figure 19-22. The correlation coefficient (R^2) for all the formulations using different kinetic equation is listed in table m. It was found that the in vitro drug release of optimized formulation G1 was best explained by zero order as the plot show highest linearity (R^2= 0.975) followed by first order. (R^2 =0.938) The R^2 was used to accuracy of fit. The formulation G1 provide best fit to the zero order model.

STABILITY STUDIES

Stability studies were performed as per ICH guidelines. The formulation G1 was selected for the stability studies. After 1 month storage the nanosponge loaded gel were evaluated for various parameters like physical appearance, pH, drug content, percentage drug release.

Table n: Stability study data at 5^0C±2^0C

Days	pH	Physical stability	Drug content	Percentage of drug release
0	7.02±0.29	No Change in appearance	98.09±0.13	97.6± 0.46
15	7.11±0.14	No change in appearance	97.45±0.23	96.51±1.13
30	6.93±0.27	No change in appearance	96.14±0.34	96.34±1.21

Table o: Stability study data at 25^0C ±2^0C

Days	pH	Physical stability	Drug content	Percentage of drug release
0	7.02±0.29	No Change in appearance	98.09±0.13	97.6± 0.46
15	7.22±1.12	No change in appearance	97.25±0.18	96.45±1.18
30	6.95±0.29	No change in appearance	96.13±0.19	96.30±1.10

Formulation G1 after 30 days stability study at different conditions shows that there is no major change in the formulation after the storage as initial. The study shows no major difference was found before and after the storage and all are in satisfactory range. Therefore formulation remains stable for sufficient range. Therefore formulation remains stable for sufficient time after the storage of 30 days.

CONCLUSION

In the present study, nanosponge formulation were presented as a new attempt to enhance the bioavailability of the drug griseofulvin there by provide a sustained delivery to the targeted site for the treatment of several fungal diseases. Nanosponge was prepared by emulsion solvent diffusion method using ethyl cellulose and eudragit S 100 at different concentration. FTIR studies showed that absence of incompatability between drug and excipients. Formulation F2 and F5 was found to be the best formulation based on the entrapment efficiency. F2 and F5 were selected to formulate as gel, G1 and G2 respectively. G1 was found to be the best formulation based on swelling study(74%), viscosity (7285±0.32cps), spreadability (7.56 gm-cm/s), drug content(98.09±0.13%) and in vitro study shows 97.6% of drug release at 10th hour of study. In vitro follows zero order kinetics in drug release kinetic analysis. The optimized formulation G1 was found to be stable during stability study.

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