WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 5, 1184-1204. Research Article ISSN 2277– 7105

FABRICATION AND CHARACTERIZATION OF ITRACONAZOLE LOADED NANOSPONGE GEL

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Article Received on
13 Feb. 2019,

Revised on 05 March 2019,
Accepted on 26 March 2019

DOI: 10.20959/wjpr20195-14701

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ABSTRACT

The present study was carried out to utilize and to easily done by using polymeric 'Nanosponges' as an alternative carrier for targeting and controlled Itraconazole to the skin through topical gel formulation. Itraconazole is a second generation azole antifungal agent. Nanosponges are porous polymeric drug delivery system and they were helped to reduce irritation without reducing efficacy and it can be effectively incorporated into topical gel formulation. These Nanosponges are prepared by using emulsion solvent diffusion method and they were formulated by using polyvinyl alcohol and ethyl cellulose, dichloromethane. The selected Nanosponges are formed into gel by adding carbopol 934 using varying concentrations of permeation enhancers (propylene glycol) and pH neutralizer (triethanolamine) and itraconazole Nanosponges loaded gels (F1-F10) were evaluated by using SEM analysis, particle size determination and dissolution studies, drug entrapment efficiency etc.

KEYWORDS: Itraconazole, Topical gel, Nanosponges.

INTRODUCTION

The drug delivery system has manifested a new curiosity for the drugs by furnishing them new life through their biological targets.[1] Targeting the delivery of a drug is a major problem faced by the medical researchers. The need for development of targeted drug delivery system is as the system upgrade the drugs therapeutic efficacy & controls the release of drugs, deplete the side effects and optimize the therapeutic window.[2]

There developments in targeted drug delivery system leading trends in the area of therapeutics and nanotechnology is improved in pharmacy are nanomaterials/particles which helps in

diagnosing and focusing the drug to the targeted place in the body which provides the controlled release of drugs as it Afford the minimum toxic effects and maximizing therapeutic index[3] of the drug. This System afford excellent topical drug delivery of drugs.[4]

Nanosponges are the new class of matter which are used as carriers and it is made up of the microscopic particles with some nanometer wide cavities. In these cavities a large variety of drug substances are encapsulated. These Nanosponges are capable of carrying both lipophilic and hydrophilic substances and improving the solubility of poorly soluble drugs.[5]

Nanosponges are about the size of virus and acts as a 3D network (or) Scaffold. The back bone is naturally long length degradable polyester. The long length polyester strands are mixed in solution which contains small molecules called cross-linkers. Which has the affinity for certain portions of the polyester. These cross-linkers segments of the polyester forms a spherical shape which contains many cavities/pockets where the drug molecules can be stored. As the polyester is predictably biodegradable. So that the polyester breaks down gradually in the body and they loaded the drug in the cavities are released on a known schedule[6] in a predictable fashion.

The Nanosponges are associate to specific size and to release the drug over time can be done by varying the proportions of cross-linker to polymer. As these Nanosponges represent a novel class of nanoparticles and are usually obtained by natural derivatives when compared to other nanoparticles. As Nanosponges are soluble in both water and organic solvents and are porous, non- toxic, stable at high temperatures up to 300°C.

Advantages

- This technology proffer entrapment of components and deplete side effects.[7]
- These Nanosponges modify the release of drug.
- Can be used to mask unpleasant flavours and to convert liquid substances to solids.[8]
- These can increase the bioavailability of drug.[9]
- Non- mutagenic, non- irritating.[10]
- Non- toxic and biodegradable
- These formulations are steady at the temperature up to 130°C
- Improved stability, increased elegance and enhanced formulation flexibility.
- Improved solubility of both hydrophilic and lipophilic drugs.
- These are self sterilizing, as their average pore size is 0.25m where bacteria cannot penetrate.

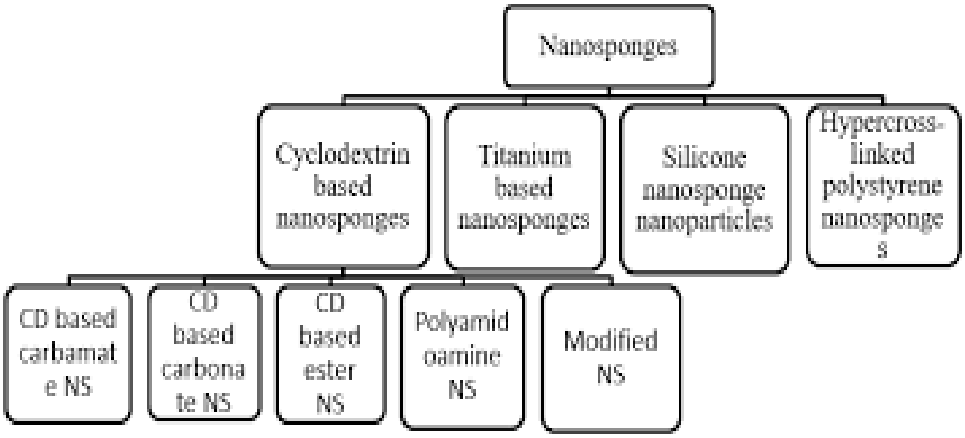
- Formulations are cost effective and easy scale – up.

Disadvantages

- In these Nanosponges loading capacity is very less.[11]
- Dose dumping.

Itraconazole (Sporonax)® is the newest triazole antifungal agent. It is structurally related to other agents and has the activity against candidiasis, aspergillus species and other filiform fungi. Randomized, trails have shown for the prevention of invasive fungal infections in immune compromised patients. It has also shown promising results in the treatment of various fungal infections re-factory to other antifungal therapy.[12]

Types of Nanosponge[13]



FORMUALTION OF ITRACONZOLE NANOSPONGE

Itraconazole Nanosponge gel was formulated by incorporated diluents such as polyvinyl alcohol, ethyl cellulose, di-chloro methane etc., it was prepared by emulsion solvent diffusion method. They are further coated with gelling agent. All excipients are mixed for 30mins and gelling agent is added to itraconazole Nanosponges. The formulated gel was evaluated with gelling properties.

METHOD OF PREPARATION OF NANOSPONGES[14]

1. Melt method
2. Solvent diffusion methods:

a. Emulsion solvent diffusion method

b. Quasi-emulsion solvent diffusion

3. Ultra sound assisted method

4. Solvent method.

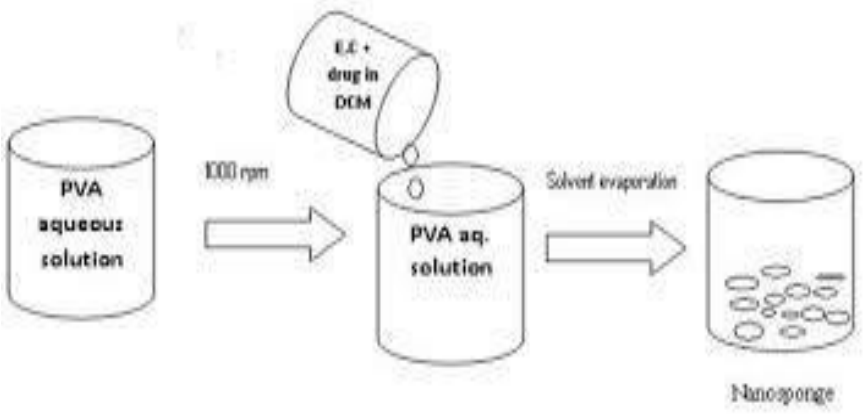
Table 1: Preparation of itraconazole loaded Nanosponge.

Formulations	Drug:polymer ITZ:Ethylcellulose	Polyvinylalcohol (%W/V)	Dichloromethane (ml)	Purifiedwater (ml)
F1	0.5g: 3g	2g	20ml	50ml
F2	0.5g: 2g	**1g**	20ml	50ml
F3	0.5g:3g	**3g**	20ml	50ml
F4	0.5g:0.5g	**1g**	20ml	50ml
F5	0.5g: 1g	**0.5g**	20ml	50ml
F6	0.5g: 1g	**1g**	20ml	50ml
F7	0.5g: 1g	**2g**	20ml	50ml
F8	0.5g: 1g	**3g**	20ml	50ml
F9	0.5g: 2g	**2g**	20ml	50ml
F10	0.5g: 5g	**3g**	20ml	50ml

Drug loading in Nanosponges can takes place in two ways, one-step process or by two-step process; based on physicochemical properties of drug to be loaded. If the drug is typically an inert non-polar material, it will create the porous structure and it is called porogen. Porogen drug, which hinders the polymerization nor becomes activated by it and stable to free radicals is entrapped with one-step process.[15]

Solvent evaporation method[16,17]

Nanosponges using required amount of ethyl cellulose and polyvinyl alcohol were prepared by solvent evaporation method. Disperse phase consisting of itraconazole(1gm) and requisite quantity of ethyl cellulose dissolved in 10ml solvent (di-chloro methane or ethanol) was slowly added to a definite amount of PVA in 100ml of aqueous phase, prepared by using microwave oven. The reaction mixture was stirred at 1000rpm for 3hrs on a magnetic stirrer. The Nanosponges formed were collected by filtration through whatmann filter paper and dried in oven at 50°C for 2 hours. The dried Nanosponges were stored in vacuum desiccator to ensure the removal of residual solvent.



Emulsion solvent diffusion method

This method was used to fabricate itraconazole loaded Nanosponges by using a suitable polymer.

↓

Dispersed phase is added with suitable amount of drug and polymer.

↓

It is dissolved in organic solvent like dichloromethane.

↓

Aqueous phase consists of specific amount of polyvinylalcohol

↓

Dissolved in distilled water Dispersed phase is added drop by drop to the aqueous phase.

↓

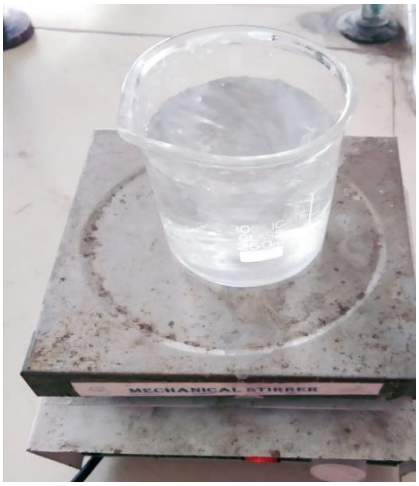
Stirred by magnetic stirrer at 1000rpm for about 2 hours.

↓

The Nanosponges formed are filtered by filtration and dried in oven at 40°C for about 24hours.

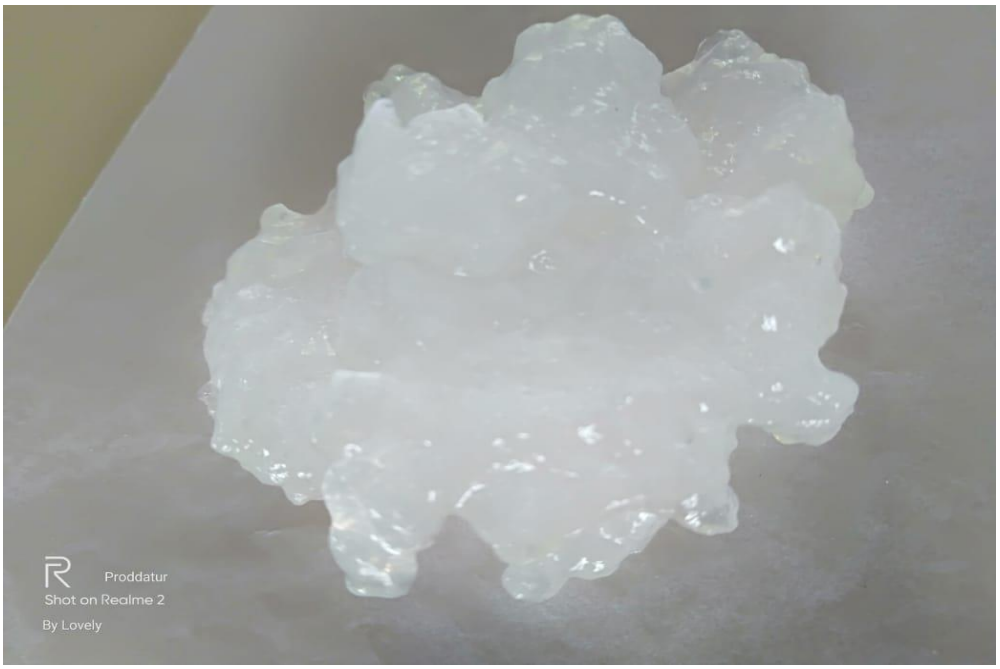
↓

They are kept in vacuum desiccators to remove the residual solvent and Nanosponges are obtained by emulsion solvent diffusion method.[18]



Development of Nanosponge loaded gel

Intially, accurately weighed amount of drug with polymer carbopol-934 (100mg) was moistened in water (5ml) to get a gel for 2-3hours and it is dispersed by constant stirring at 600rpm with the aid of magnetic stirrer to get a smooth dispersion. Then the above dispersion added triethanolamine or ethanol to neutralize pH. The previously prepared Nanosponge suspension the permeation enhancers like propylene glycol was added and make up to 100ml with distilled water.[19]



EVALUATION OF NANOSPONGES

Particle size determination[20]

The particle size of Nanosponge is an important criteria in the optimization process. Particle size can be determined by laser light diffractometry or Zeta sizer. Cummulative percentage drug release from Nanosponges of different particle size can be plotted against time. World Journal of Pharmacy and Pharmaceutical Sciences effect of particle size on drug release. Particle size larger than 30 m can show gritty feeling and particle size range from 10 –25 m can be preferred for topical drug delivery.

Polydispersity index

Polydispersity index was resolute, which is the measurement of the width of the particle size distribution is given by d0.9, d0.1 and d0.5 are the particle size dogged at 90th, 50th and 10th percentile of particle undersized. The particle size and polydispersity index of nanosponges was measured by Photon Correlation Spectroscopy using a Zeta-sizer Malvern, Version 7.03. Samples were diluted suitably with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 208.2–602.0 for measurements and the pH of diluted samples ranged from 6.9 to 7.2. The measurements were carried out in disposable sizing cuvette at 25°C, in 75%-100% intensity. The samples were analyzed at IIT, Roorkee and Troikaa Pharmaceuticals Limited.

Scanning Electron Microscopy (SEM) analysis

SEM analysis significant for determination of surface characteristics and size of the particle. Scanning electron microscopy was operated at an acceleration voltage of 15kV. A concentrated aqueous suspension was spread in an equipment cell receiver and dried under vacuum. The sample was shadowed in a gold layer 20 mm thickened cathodic evaporator attached with a monitor which represents the images of the sample. The processed images were recorded and individual formulated Nanosponges particle diameter were measured to obtain average particle size.

DETERMINATION OF LOADING EFFICIENCY

The loading efficiency (%) of the Nanosponges can be calculated according to the following equation,[21,22]

$$L.E = \frac{\text{loading efficiency actual drug in Nanosponges}}{\text{Theoretical drug concentration}} \times 100$$

Evaluation of Gel

Estimation of a Nanosponge based gel was performed by evaluating the formulation for measurement, clarity examination, spreadability, homogeneity, viscosity, skin irritability, drug content %, In-vitro drug diffusion profile. Such mentioned estimation was measured in triplicate and average values were projected. Such characterization performed by the following process.

In vitro diffusion studies[23]

The abdominal skin of goat, weighing 20–25 gm of 8–10 w old was shaved using hand razor and clean the skin with hot water cotton swab. 5 gm of gel was applied uniformly to the skin. The skin was mounted between the compartments of the Frantz diffusion cell with stratum corneum facing the donor compartment. Reservoir compartment was filled with 900 ml phosphate buffer of pH 6.8. The study was carried out at 37±5°C and the speed was adjusted until the vortex touches the skin and it carried out for 12 h. 5 ml of the sample was withdrawn from the reservoir compartment at 30 min interval and absorbance was measured spectrophotometrically at 259 nm. Each time the reservoir compartment was replenished with the 5 ml volume of phosphate buffer pH 6.8 solution to maintain a constant volume.

Percentage Yield

The empty container was weighed in which the gel formulation was stored then again the container was weighed with gel formulation. Subtract the empty container weighed and again with the gel container formulation then it gives the practical yield. Hence the percentage yield was calculated by using the formula,

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Drug content

Weighed 10 gm of each gel formulation were transferred in 250 ml of the volumetric flask containing 20 ml of alcohol and stirred for 30 min. The volume was make up to 100 ml and filtered. 1 ml of the above solution was further diluted to 10 ml with alcohol and again 1 ml of the above solution was further diluted to 10 ml with alcohol. The absorbance of the solution was measured spectrophotometrically at 259nm. Drug content was calculated by the following formula,

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} \times \text{Dilution factor} \times \frac{1}{1000}$$

Determination of pH

Weighed 50 gm of each gel formulation were transferred in 10 ml of the beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 3–9 to treat the skin infection.

Extrudability

The gel formulations were filled into a collapsible metal tube or aluminium collapsible tube. The tube was pressed to extrude the material and the extrudability of the formulation was checked.

Viscosity estimation

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand.

a) **Selection of spindle**: Spindle T 95 was used for the measurement of viscosity of all gels.
b) **Sample container size**: The viscosity was measured using 50 gm of gel filled in a 100 ml beaker.
c) **Spindle immersion**: The T-bar spindle (T95) was lowered perpendicular in the centre taking care that spindle does not touch the bottom of the jar.

d) Measurement of viscosity: The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. Which affect the viscosity was maintained during the process. The heli-path T-bar spindle was moved up and down giving viscosity at a number of points along the path. The torque reading was always greater than 10%. The average of three readings taken in one minute was noted as the viscosity of gels.

Drug Content or Entrapment Efficiency (%)[24]

50 mg from the prepared drug loaded Nanosponges by emulsion solvent diffusion method using suitable polymer were suspended in 50 ml of methanol and were subjected for ultracentrifugation for 40 minutes. The percentage of incorporated itraconazole was determined spectrophotometrically at 259nm. After centrifugation of the aqueous suspension, amount of free drug was detected in the supernatant and the amount of incorporated drug was determined as a result of the initial drug minus the free drug. The drug entrapment efficiency (EE) of itraconazole Nanosponges[25] was determined using the formula,

$$\text{Entrapment efficiency} = \frac{\text{Total drug (assay)} - \text{Free drug}}{\text{Total drug}} \times 100$$

In-vitro drug release study

For drug diffusion release study of itraconazole loaded Nanosponge based topical gel formulation modified Franz diffusion cell was used and for simulation cellophane membrane was used. Such process performed in following steps: The membrane was soaked in 0.1 N HCl

for 18 hr. In receptor compartment of Franz diffusion cell was filled with 6.8 pH phosphate buffer solution about 7 ml. In donor compartment, a measured quantity about (1g of gel contains 100mg of drug loaded Nanosponges) was applied uniformly on the cellophane membrane surface. The prepared membrane mounted over the modified Franz diffusion cell cautiously to evade air bubbling underneath the membrane. The entire assembly was conserved at 37ºC for 08 hrs and stirring also be at constant speed about 600-700 rpm. Then, from the receptor compartment, 1ml sample was withdrawn every 1hr and equal amount 1 ml fresh sample was deposited. Such procedure follows for 5-6 times for 8 hrs. Maintain the drug diffusion graph.[26]

SPREADABILITY

It is assessed by wooden base block and glass slide apparatus. For detection of spreadability, sample spread in between two slides and was compressed to a uniform thickness by employing 1000 gm weight for a time period of 5 minutes. Weighed about 50 gm added to the pan. Then quantify the time required to separate the slides from each other. So, spreadability was dignified as a time that was taken to separate upper glass slide moves over the lower plates. Following formula used to measured spreadability of gels:

S=ML/T

Where, S= Spreadability,

M= Weight applied upon the upper slide,

L= Length moved on the glass slide,

T= Time utilized to separate the slide

RESULTS AND DISCUSSION

RESULTS: All the analysis results were computed in following way.

Physical properties analysis data for quality determination of drug given below in following figure report:

Table 1.2: Tabulated representations of physical properties of Itraconazole.

S.NO.	Parameters	Inferences
1)	Appearance (color, nature and taste)	Unpleasant taste. White, Crystalline powder
2)	Melting point	166^0C
3)	Solubility	Insoluble in water, soluble in Methanol

UV Spectrometric measurement

Table 1.3: Tabulated and graphical representation of the standard curve of Itraconazole in Phosphate Buffer (Ph 6.8).

Concentration(µg/ml)	Absorbance
0	**0**
2	**0.1316**
4	**0.2419**
6	**0.3416**
8	**0.4315**
10	**0.5221**

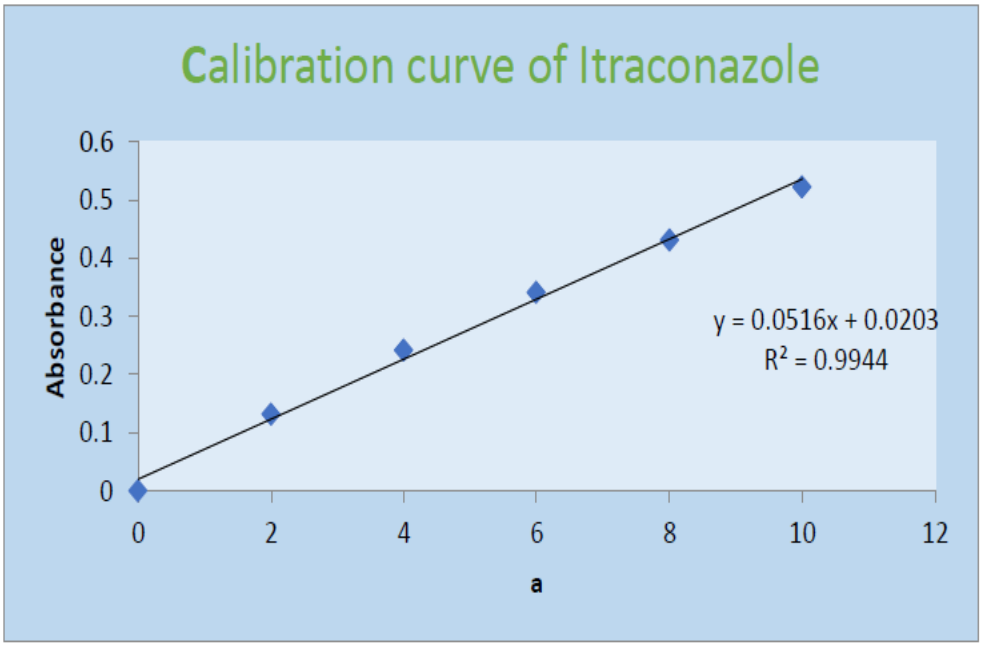


Fig: 1: Graphical Representation of the standard curve of Phosphate buffer pH.

Drug Content

Formulation	Drug content (%)
F1	38.5
F2	25.9
F3	43.8
F4	55.2
F5	51.8
F6	70.6
F7	60.4
F8	58.9
F9	65.7
F10	69.3

Table 1.4: Tabulated and graphical representation of Entrapment efficiency.

Formulation	Drug content (%)
F1	38.5
F2	25.9
F3	43.8
F4	55.2
F5	51.8
F6	70.6
F7	60.4
F8	58.9
F9	65.7
F10	69.3

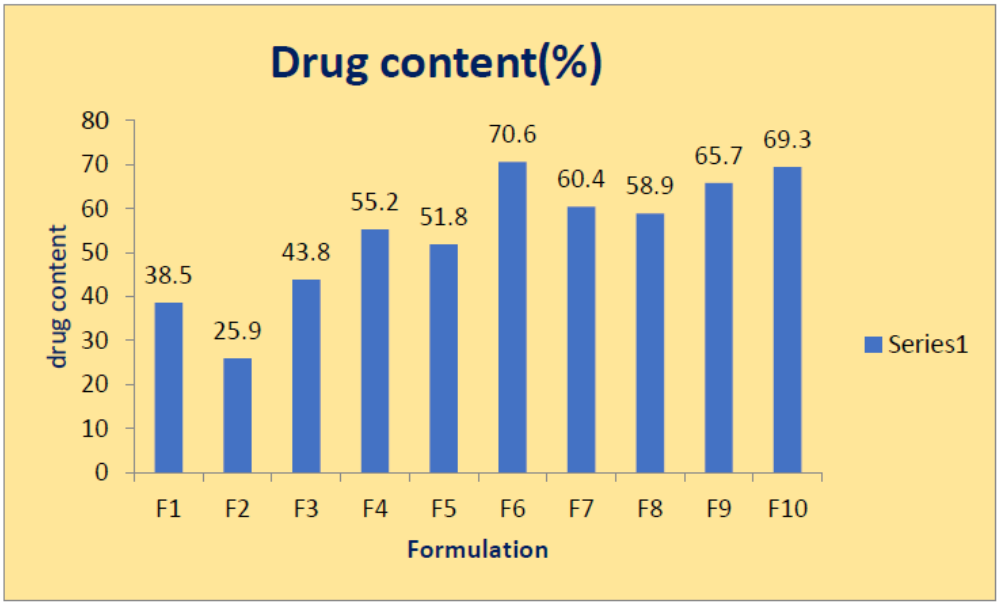


Fig. 2: Graphical representation of Drug content.

Table 1.5: Graphical representation of kinetic study of drug release data.

S. No	Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	0	8.1	1.7	2.8	0.9	0.8	0.4	1.3	9.0	2.7	3.6
2	2	17.1	8.1	11.6	11.3	9.0	11.6	13.8	12.7	11.4	13.0
3	4	29.8	15.3	21.6	32.3	23.4	24.3	24.0	26.2	30.7	32.3
4	6	34.4	19.8	34.4	42.3	36.8	42.7	33.5	44.4	51.7	51.8
5	8	45.4	36.8	53.6	51.5	45.2	50.2	40.4	56.2	61.3	60.6
6	10	65.6	70.4	69.8	79.6	83.1	74.9	69.6	80.4	70.0	88.6

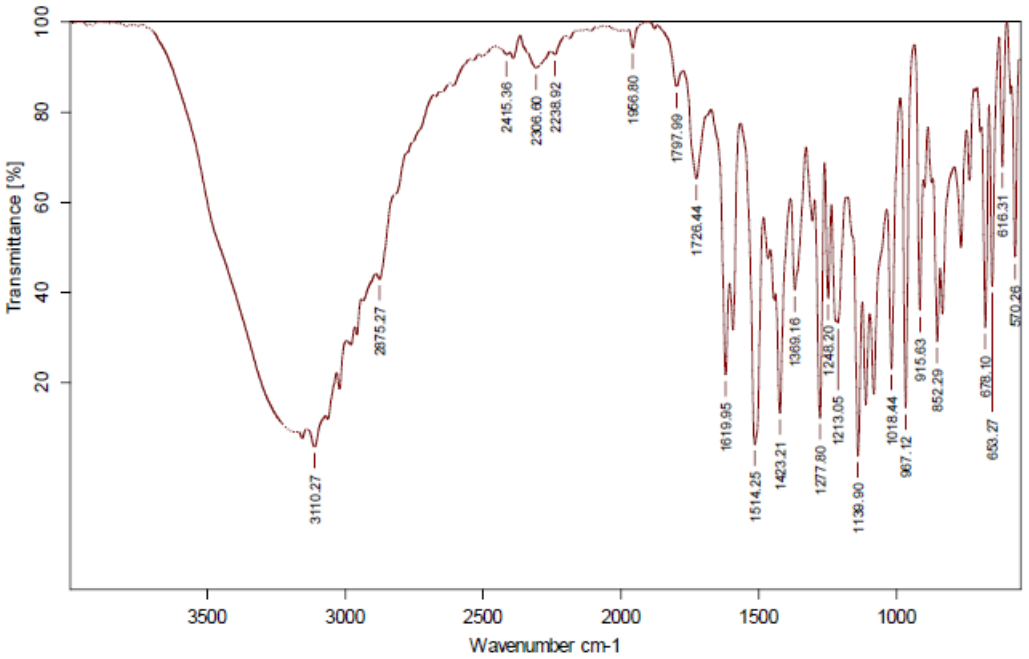


Fig 3: IR SPECTRA OF ITRACONAZOLE.

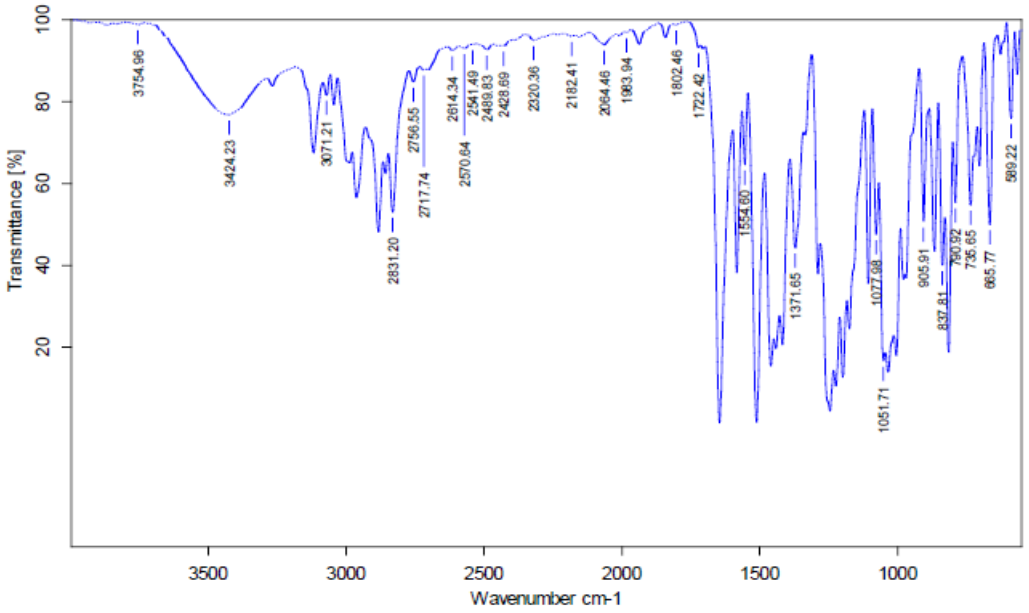


FIG 4: IR SPECTRA OF ITRACONAZOLE WITH ETHYLCELLUOSE.

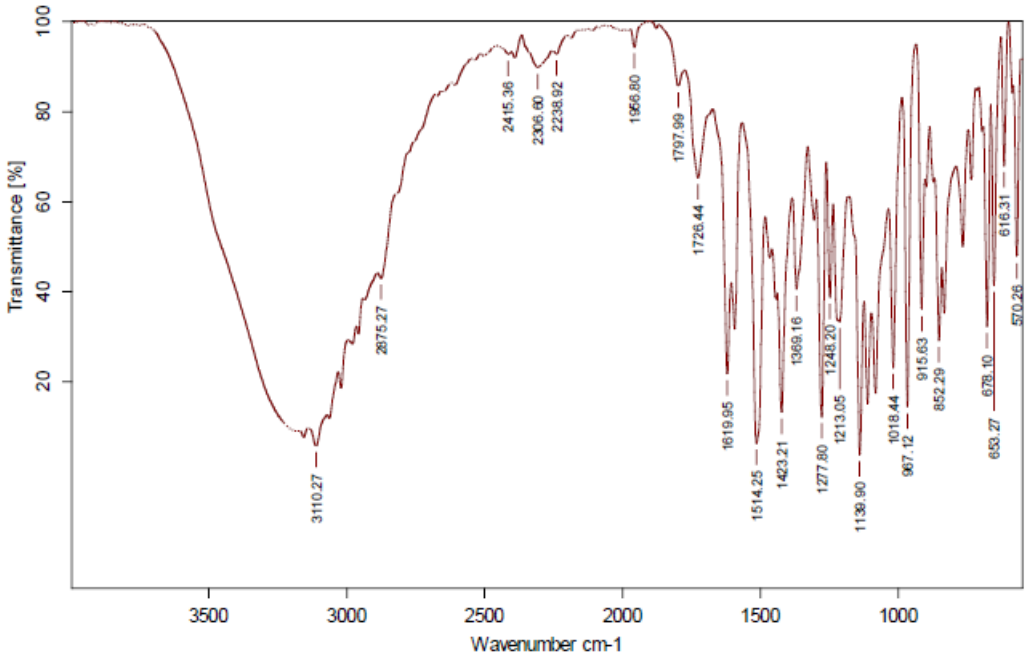


FIG 5: IR SPECTRA OF ITRACONAZOLE WITH ETHYLCELLULOSE AND POLYVINYLALCOHOL.

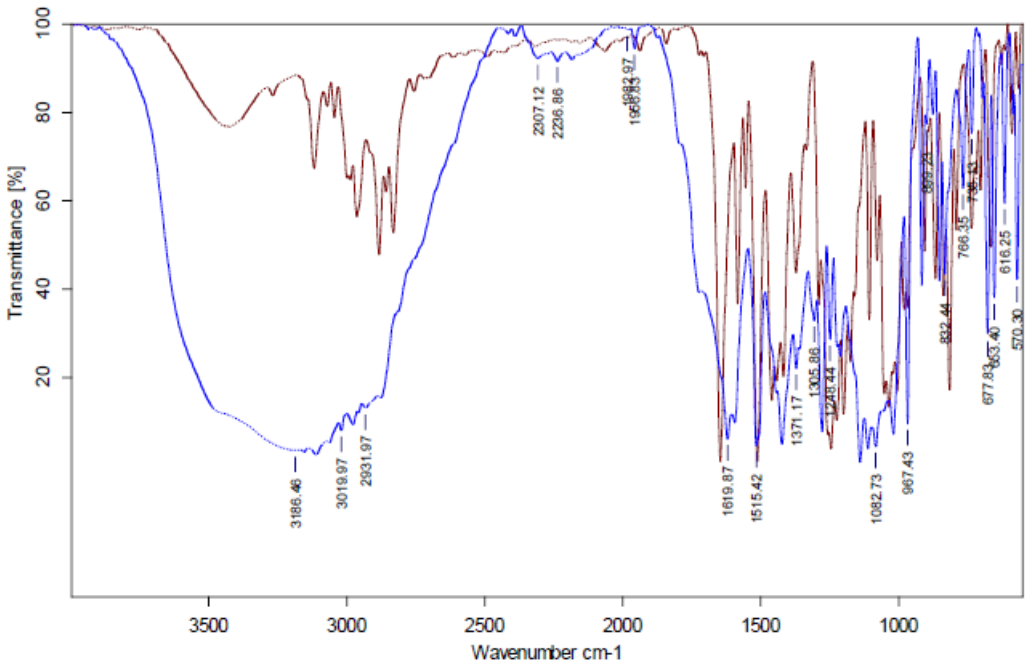


FIG 6: IR SPECTRA OF ITRACONAZOLE WITH POLYMERS AND ITRACONAZOLE PURE.

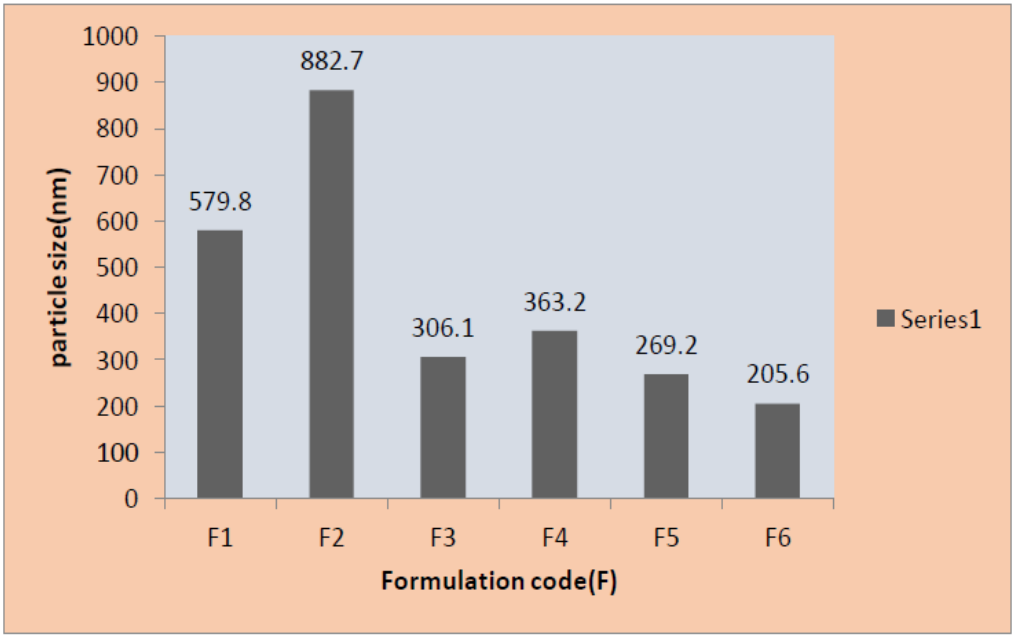
Estimation and discussion of in-vitro release study

The drug release data was formfitting in different kinetic models using BIT-SOFT software where data was premediated for zero order, first order and Higuchi kinetics. It was concluded that the drug discharge data followed the zero-order kinetics more closely in comparison to first order and Higuchi kinetics. Further, the data were best fitted to Peppas-Korsmeyer equation as the correlation coefficient was found to be 0.9958. the exponent value was 1.1581 which revealed that drug diffusion followed super case II transport mechanism.

Particle Size and polydispersity Index of different prepared Nanosponge

Table 1.6: Tabulated representation of Particle size and polydispersity Index.

Formulation Index	Particle Size (nm)	Polydispersity
F1(50:100)	579.8	0.682
F2(50:150)	882.7	0.777
F3(50:200)	306.1	0.487
F4(50:250)	363.2	0.599
F5(50:300)	269.2	0.403
F6(50:350)	205.6	0.585



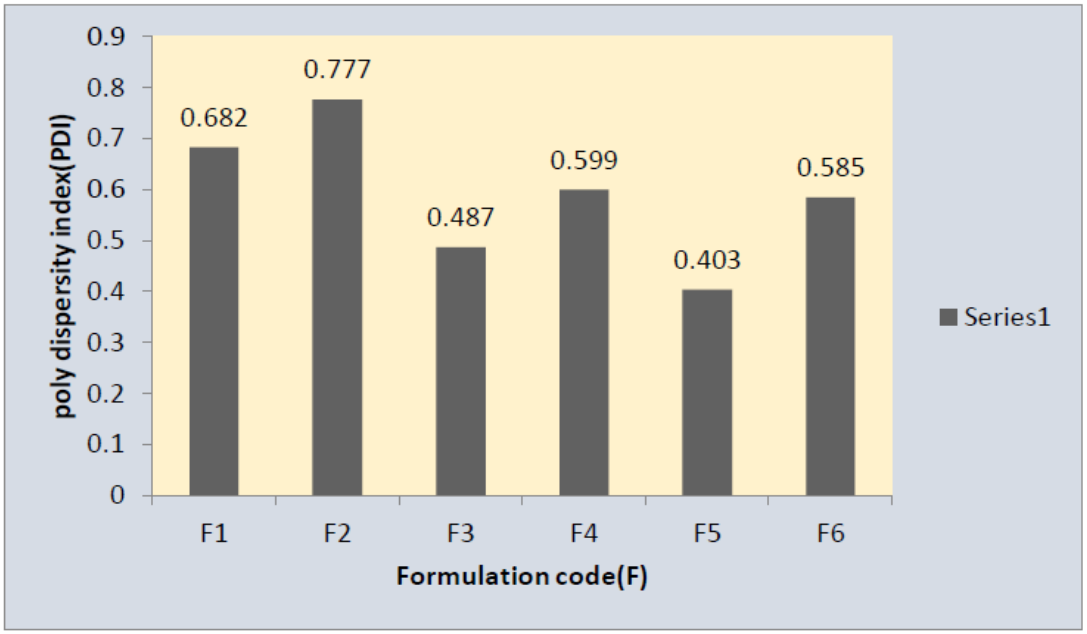


Fig. 7: Bar Diagram of particle siza analysis & polydispersity index Scanning Electron Microscope (SEM) analysis of the different proportion of nanosponge formulation.

Fig. 8: SEM of different proportion of Nanosponge based topical gel formulation (F1,F2,F3,F4,F5 and F6 respectively).

Drug - polymer Compatibility Studies

Compatibility studies of pure drug Itraconazole with polymers were carried out prior to the formulation of Nanosponges. IR spectra of pure drug and polymers were taken, which are

depicted in Figures 2.0-2.3. All the characteristic peaks of Fluconazole were present in spectra at respective wavelengths. Thus, indicating compatibility between drug and polymers. It shows that there was no significant change in the chemical integrity of the drug.

CONCLUSION

In the present study the formulated nanosponge loaded with Itraconazole an Anti-fungal agent resulted in targeted & controlled release. Among all the formulated batches starting from F1through F10 the F6 batch is considered as the best entrapped (70.6%) nanosponge with greater percentage drug release (80.6%) in F10. The characterization by SEM finally concluded the appearance as "Nanosponge".

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