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Research Article

Design and Optimization of Nanosponges of Poorly Soluble Voriconazole Using Central Composite Design

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ABSTRACT

Voriconazole loaded nanosponges were prepared by emulsion solvent diffusion method using ethyl cellulose (EC) and polyvinyl alcohol (PVA) as a stabilizer. A 3 level design was employed in this study, requiring 9 experiments. The composition and development process of Nanosponges are heavily influenced by formulation optimization. The 3^2 design for optimization of effect of independent variables was investigated. Selected as a drug: ethyl cellulose ratio (X_1) and stirring rate (rpm) (X_2) on dependent variables i.e. particle size (nm), entrapment efficiency (%) respectively. The prepared nanosponges were characterized for particle size, entrapment efficiency, zeta potential, FTIR and SEM. The optimized nanosponges batch was incorporated into gel using carbopol 934. The particle size of formulated voriconazole Nanosponges ranges from 267.1 nm to 537.5 nm. Zeta potential of optimized formulation was found to be -26.7 mV. The EE (%) ranged from 62.21% to 82.11%. The formulation F6 has shown the maximum cumulative % of drug release, 85.57% for 8 hours.

INTRODUCTION

In the past two decades, there has been an increase in the prevalence of fungal infections, which has raised the morbidity and mortality rates [1,2]. Every year, over 40 million people worldwide-in both developed and undeveloped nations-suffer from fungus infections [3]. There are 600 different fungus species that can infect humans. Fungi can cause allergies as well as infections of the mucosa, nails, skin, and hair [4]. According to a recent estimate, there are 3 million cases of chronic pulmonary aspergillosis, 0.22 million cases of cryptococcal meningitis complicating HIV/AIDS, 0.7 million cases of invasive candidiasis, 0.5 million cases of *Pneumocystis jiroveci pneumonia*, 0.25 million cases of invasive aspergillosis, 0.1 million of disseminated histoplasmosis, 10 million of fungal asthma and 1 million fungal keratitis cases annually [5-7].

Despite the availability of numerous medications, drug resistance, poor absorption, drug interactions, and toxicity problems make antifungal therapy one of the most challenging treatments. Antifungal therapy is difficult to provide as a result of widespread use of antifungal medicines in agriculture, lumber preservation, human and animal health care, and other fields [8-10].

Nanosponges are porous structures with nanometric dimensions that resemble sponges and have a diameter of ≤1µm [11]. By increasing their wetting and solubility, they increase the bioavailability of medicines that are poorly water soluble (hydrophobic). They give medication molecules stability in the face of abrasive physical, chemical, and biological conditions [12, 13]. Because they are a free-flowing powder, they can be added to a variety of dosage forms, including as tablets, capsules, emulgels, hydrogels, and saline water, making it easier to administer them orally, topically, pulmonaryly, and parenterally [14].

The present study aims to design and optimization of nanosponges of poorly soluble voriconazole using central composite design.

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MATERIALS AND METHODS

Materials

Pure Voriconazole was purchased from Dhamtech Pharma, Mumbai. Ethyl cellulose (EC), Dichloromethane (DCM), polyvinyl alcohol (PVA), Triethanolamine, Carbopol 934P and ethanol were purchased from Loba chemicals, Mumbai. All the reagents were analytical grade.

Experimental Design

Design expert software was employed to execute the statistical evaluation of experimental design. The most popular response surface method was the central composite design. A 3 level design was employed in this study, requiring 9 experiments. The composition and development process of Nanosponges are heavily influenced by formulation optimization. The 3^2 design for optimization of effect of independent variables was investigated (Table 1). Selected as a drug: ethyl cellulose ratio (X_1) and stirring rate (rpm) (X_2) on dependent variables i.e. particle size (nm), entrapment efficiency (%) respectively. Table 1 summarizes the coded three different levels low (-1), medium (0), and high (+1) converted to experimental units, experimental runs, and their factor combinations examined in this investigation. The significant model was analyzed by using ANOVA.

Table 1: 3^2 Full factorial design levels and factors

Coded Values Level	Independent Variables	
	X_1, Drug: Ethyl cellulose (ratio)	X_2, Stirringrate(rpm)
-1	1:1	1200
0	1:2	2400
+1	1:3	3600

Preparation of Voriconazole Nanosponges

Voriconazole nanosponges were prepared by Emulsion solvent diffusion method [15]. Voriconazole and ethyl cellulose were dissolved in dichloromethane (Phase1), and Phase 2 was prepared by adding polyvinyl alcohol to distilled water. Phase 1 and Phase 2 were put separately on a magnetic stirrer for 15 min. then slowly added phase 1 to Phase 2 while stirring and then left them for 15 min on the stirrer at room temperature. The mixture was homogenized at different speeds for 2 hr. after that it filtered. The formed nanosponges were dried at 40°C for 12hr. The different ratios of drug: EC and stirring rate for the nine formulations are presented in Table 2.

Evaluation of Nanosponges

Saturation Solubility

A study on voriconazole's saturation solubility in the nanosponges formulation was conducted. In summary, 10 mg of nanosponges formulations were suspended in 1 mL of distilled water, packed into 2 mL centrifuge tubes, and centrifuged for 1 hour at 15000 rpm. After filtering the supernatant through Whatman filter paper (45µ), the voriconazole content was measured using a spectrophotometer set to λ_{max}256. In order to quantify the effect of nanosponges entrapment on voriconazole solubility, the test was performed in comparison with pure drug, and the saturation solubility of an equivalent amount of voriconazole in 1 mL distilled water was calculated [16].

Particle Size Determination

A Dynamic Light Scattering Instrument (DLSI) with particle sizing software can be used to determine particle size and determine the total mean diameter and size distribution of drug loaded nanosponges.

Table 2: Formulation of voriconazole-loaded Nanosponges

Formulation code	Drug: Ethyl cellulose (ratio)	Polyvinyl alcohol (%w/v)	Dichloromethane (mL)	Distilled water (mL)	Stirring rate (rpm)
F1	1:1	1	10	20	1200
F2	1:1	1	10	20	2400
F3	1:1	1	10	20	3600
F4	1:2	1	10	20	1200
F5	1:2	1	10	20	2400
F6	1:2	1	10	20	3600
F7	1:3	1	10	20	1200
F8	1:3	1	10	20	2400
F9	1:3	1	10	20	3600

To get required light scattering intensity for voriconazole nanosponges, the dried nanosponges were dispersed in water. The mean diameter can be calculated using this information [17-19].

Zeta Potential

A zeta sizer determines the surface charge or zeta potential of prepared nanosponges. In the electrophoretic cell, the nanosponges are diluted with water. To calculate the stability of the nanoparticles, a zeta potential study was done. A measurement of the impact of electrostatic charges is the zeta potential. This fundamental force is what separates nearby particles from one another. Depending on how strong both forces are, the net effect will either be attraction or repulsion. The relationship between zeta potential determination responses of the nanoparticles is described by the thumb rule [20-23].

Drug Entrapment Efficiency

50 mg of the drug-loaded nanosponges that were created using the emulsion solvent diffusion method and the appropriate polymer were suspended in 50 mL of ethanol and ultracentrifuge for 40 minutes. Spectrophotometric analysis was used to determine the percentage of drug incorporation. The amount of free drug was found in the supernatant following centrifugation of the aqueous mixture, and the amount of integrated drug was calculated as a result of the initial drug minutes the free drug. The following calculation is used to compute the percentage of drug entrapment [24- 27].

$$\text{Entrapment Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Fourier Transforms Infrared (FTIR) Spectrophotometer

Test was carried out to identify and check integrity of functional groups of API which is used preparation of nanosponge. The FTIR of pure drug and formulation was measured by FTIR spectrophotometer. (FTIR, Alpha 2 Bruker). The samples were then scanned over a wave number range from 4000-500 cm^{-1} [28-30].

Surface Morphology by Scanning Electron Microscopy

Scanning electron microscopy (SEM) can be used to examine the microscopic aspects of the drug, nanosponges, and the result (drug/nanosponge complex). The contrast in crystallization states of the raw ingredients and the end product, as examined under an electron microscope, demonstrates the development of complex formation. After the material was softly sprinkled over a double adhesive tape attached to an aluminum stub, it was examined under a scanning electron microscope to assess the surface shape of nanosponges. Platinum was then applied to the stubs. The coated sample-containing stub was put into a scanning electron microscope. After a random scan of the samples, photomicrographs were obtained at a 20 kV acceleration voltage [31-34].

Preparation of Nanosponges Based Gel

A quantity of 1.5 g of carbopol 934 was dissolved in 100 mL distilled water. pH was neutralized using triethanolamine. Optimized voriconazole nanosponges equivalent weight was incorporated into the gel to attain 1% w/w voriconazole. A conventional voriconazole gel was also prepared. The prepared gel was packed in a suitable vessel and kept in the refrigerator overnight to ensure complete dissolution of carbopol 934 for further use.

Evaluation of Nanosponges Based Gel

Physical Properties

The physical properties of the formulated voriconazole nanosponges based gel were examined, including texture, colour, appearance, odour, transparency, homogeneity [35].

Viscosity

The viscosity of formed gel was measured by using Brookfield Viscometer. It is measured using spindle number S64 with an optimal speed of 0.6 rpm [35, 36].

pH Determination

The pH of gel formulation was noted using digital pH meter. 5 mg of voriconazole nanosponge loaded gel was uniformly dispersed in 5 mL of distilled water and kept for 2 hr at room temperature then the pH of the dispersion was measured [37, 38].

Spreadability Test

A 0.5 gm sample of nanosponge based gel compound was placed between two slides and left for 5-10 minutes, with no further spreading expected. The circle diameters were measured in centimetres and used as a comparison for spreadability. The test was repeated three times

and the average spreadability value was determined. It is calculated by using the formula:

$$S = \frac{M \times L}{T}$$

Where,
M= weight tied to upper slide
L=length of glass slide
T= time taken to separate the slide

In Vitro Drug Release Study

In vitro drug release study was carried out using dialysis bag. The dialysis membrane was soaked for 24 hr in phosphate buffer pH 7.4. The nanosponge gel equivalent to 18 mg of drug placed in dialysis bag and tied at both the end. The bag was suspended in a beaker containing 100 mL release media (phosphate buffer pH 7.4). The temperature was maintained at 37°C and stirring kept at 100 rpm on magnetic stirrer. 5mL sample was withdrawn at predetermined intervals of time 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12 hr and replaced with the freshly prepared phosphate buffer solution to maintain the sink condition. Samples were analyzed UV spectroscopy at 256 [37, 39-41].

RESULTS AND DISCUSSION

Saturation Solubility

According to the findings, voriconazole nanosponges significantly increased voriconazole's solubility in distilled water when compared to the drug in its pure form. When compared to the pure medication, the produced nanosponge formulations enhanced the solubility of voriconazole. Voriconazole's solubility in water was 0.5mg/mL whereas the solubility of voriconazole in nanosponges formulation increases nearby 10 times.

The results confirm that voriconazole solubility can be increased by nanosponges by forming a polymer network or mesh with nanoscale channels that can absorb drug molecules and increase solubility upon cross-linking.

Experimental Design

ANOVA for Quadratic Model

Response 1: Particle Size

The Model F-value of 5230862.83 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2, B^2 are significant model terms.

The Predicted R^2 of 1.0000 is in reasonable agreement with the Adjusted R^2 of 1.0000; i.e. the difference is less than 0.2.Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 6504.070 indicates an adequate signal.

Table 3: Response 1: Particle Size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	67807.48	5	13561.50	5.231E+06	< 0.0001	significant
A-Drug : Ethyl cellulose	57135.04	1	57135.04	2.204E+07	< 0.0001	
B-Stirring rate	8490.08	1	8490.08	3.275E+06	< 0.0001	
AB	0.0100	1	0.0100	3.86	0.1443	
A^2	1870.68	1	1870.68	7.215E+05	< 0.0001	
B^2	311.67	1	311.67	1.202E+05	< 0.0001	
Residual	0.0078	3	0.0026			
Cor Total	67807.49	8				

Table 4: Fit Statistics for response 1

Std. Dev.	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
0.0509	416.61	0.0122	1.0000	1.0000	1.0000	6504.0696

Table 5: Response 2: Entrapment Efficiency

Source	Sum Of Squares	Df	Mean Square	F-Value	P-Value	
Model	337.57	5	67.51	4612.87	< 0.0001	significant
A-Drug : Ethyl cellulose	259.12	1	259.12	17704.21	< 0.0001	
B-Stirring rate	70.38	1	70.38	4808.91	< 0.0001	
AB	0.0552	1	0.0552	3.77	0.1474	
A^2	4.18	1	4.18	285.33	0.0005	
B^2	3.84	1	3.84	262.12	0.0005	
Residual	0.0439	3	0.0146			
Cor Total	337.62	8				

Table 6: Fit Statistics for response 2

Std. Dev.	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
0.1210	72.30	0.1673	0.9999	0.9997	0.9984	202.4035

Response 2: Entrapment Efficiency

The Model F-value of 4612.87 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2, B^2 are significant model terms.

The Predicted R^2 of 0.9984 is in reasonable agreement with the Adjusted R^2 of 0.9997; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 202.403 indicates an adequate signal.

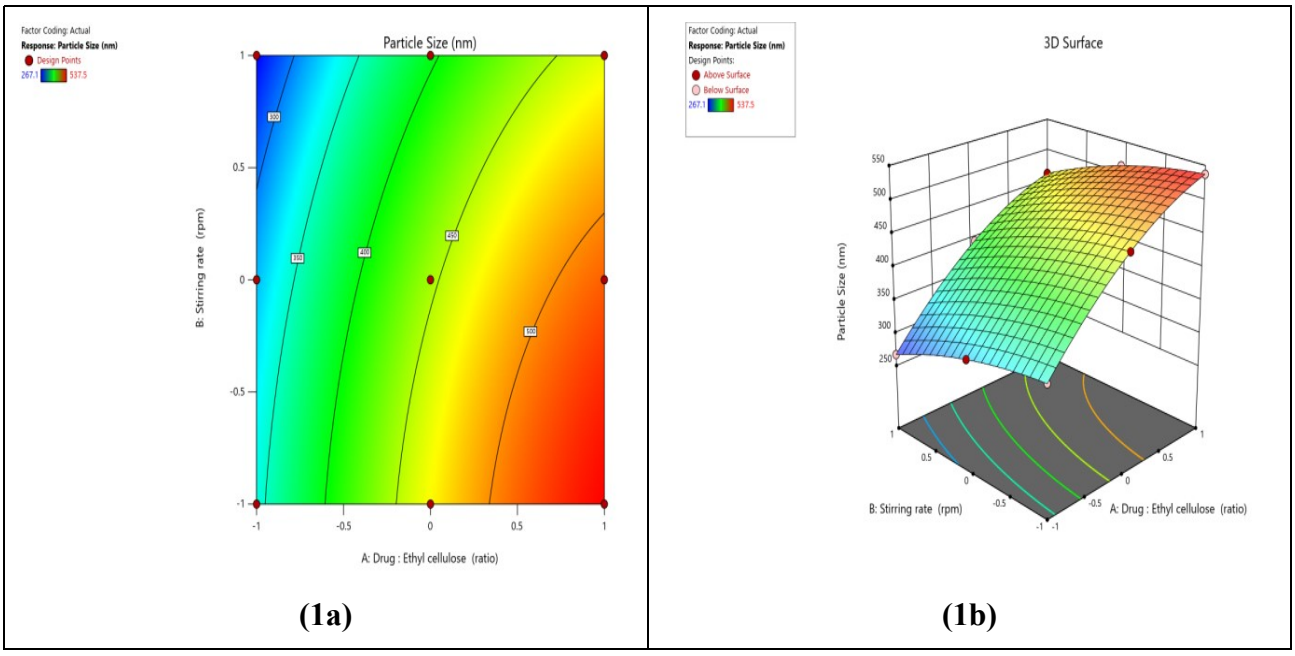
Final Equation in Terms of Coded Factors

Response 1: Particle size, Y_1

$$Y_1 = 445.32 + 97.58X_1 - 37.62X_2 - 0.0500X_1X_2 - 30.58X_1^2 - 12.48X_2^2 \quad \text{.... Eq. (1)}$$

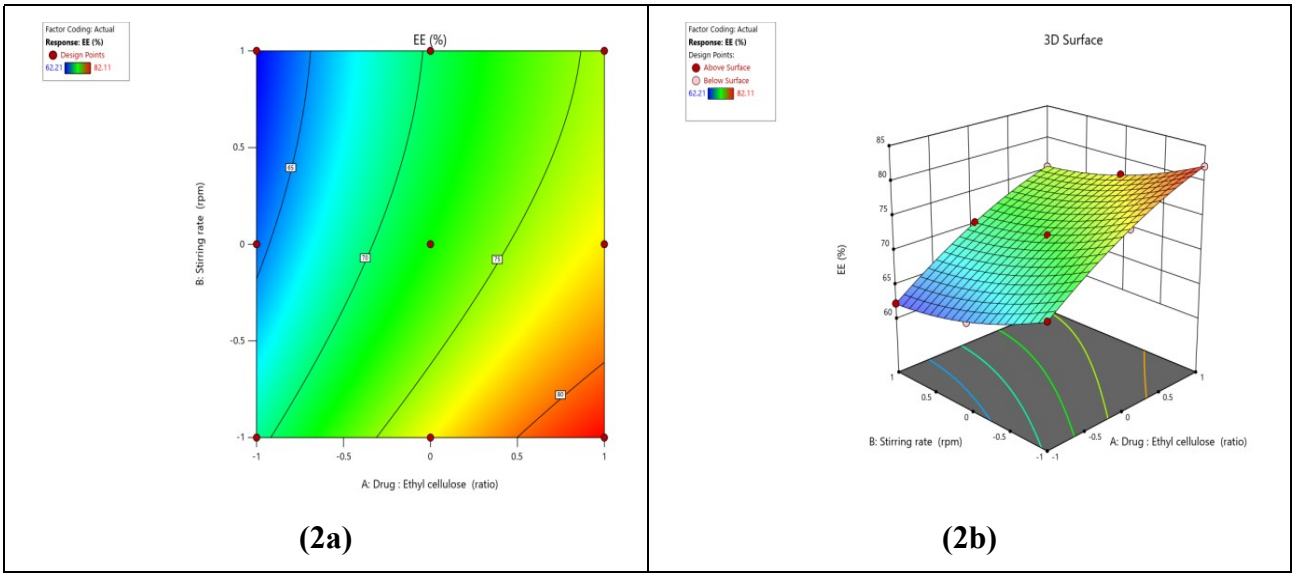
Response 2: Entrapment Efficiency, Y_2

$$Y_2 = 72.34 + 6.57X_1 - 3.42X_2 + 0.1175X_1X_2 - 1.45X_1^2 + 1.38X_2^2 \quad \text{.... Eq. (2)}$$



(1a) **(1b)**

Figure 1: 2D Contour plots for evaluating influence of Drug: Ethyl cellulose (ratio) (X_1) and Stirring rate (rpm) (X_2) on Particle size (Y_1) and Entrapment efficiency (Y_2)



(2a) **(2b)**

Figure 2: 3D Response surface plots for evaluating influence of Drug: Ethyl cellulose (ratio) (X_1) and Stirring rate (rpm) (X_2) on Particle size (Y_1) and Entrapment efficiency (Y_2)

Particle Size Determination and Polydispersity Index (PDI)

The particle size of formulated voriconazole nanosponges ranges from 267.1 nm to 537.5 nm. And the smaller particle size of nanosponges is F3 was 267.1 nm. And highest particle size of nanosponges is F7 was 537.5nm. Their size distribution was provided in Fig. 3 and Table 7.

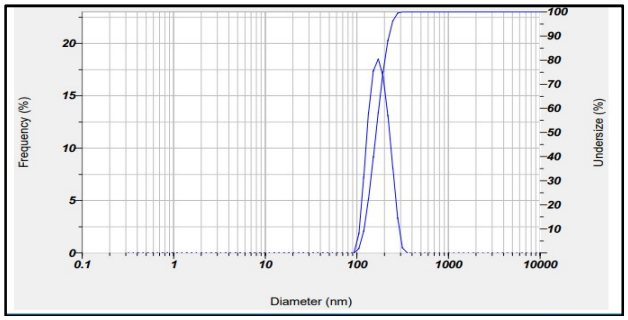


Figure 3: Particle size distribution of optimized Nanosponges

Table 7: Particle size and EE (%) of voriconazole-loaded Nanosponges

Formulation code	Particle size (nm)	EE (%)
F1	342.2	69.32
F2	317.2	64.20
F3	267.1	62.21
F4	470.5	77.12
F5	445.3	72.34
F6	395.2	70.32
F7	537.5	82.11
F8	512.3	77.58
F9	462.2	75.47

Zeta Potential

Zeta potential analysis was performed to estimate the stability of the nanosponges. It measures the effect of electrostatic charges. The force causes the repulsion between adjacent particles. The exhibited zeta potential value is beneficial to nanosponges physical stability as it prevent aggregation between particles to electrostatic repulsion. Zeta potential of optimized formulation was found to be -26.7 mV. The graph of zeta potential is shown in Fig. 4.

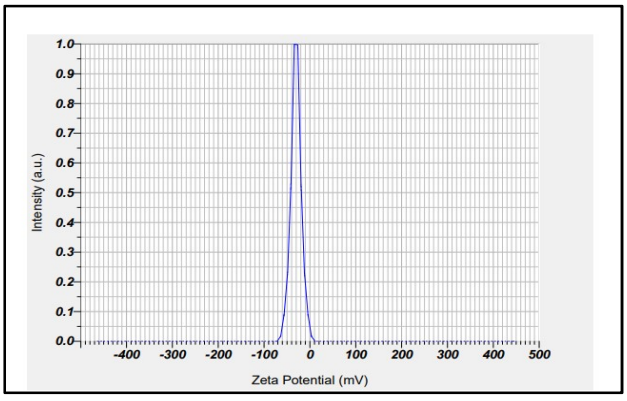


Figure 4: Zeta potential of optimized nanosponge

Drug Entrapment Efficiency

The entrapment efficiency of all formulations of nanosponges results are given in Table 7. The entrapment efficiency results of nanosponges were obtained in ranged from 62.21% to 82.11%. The entrapment efficiency was affected by drug: polymer ratio as well as internal and external phases. The highest value was found to be F7 formulation is 82.11% where a greater

amount of drug was encapsulated. Higher the entrapment efficiency, the greater amount of drug was encapsulated. Percentage entrapment was depends on Internal phase and external phase volume.

Fourier Transforms Infrared (FTIR) Spectrophotometer

The absorption bands of pure voriconazole drug were shown in Fig. 5. FTIR spectrum showed OH stretching at 3195.73 cm^{-1}, C-N stretching at 1492.72 cm^{-1}, and C-F stretching at 1587.66 cm^{-1}, respectively. The absorption bands of Nanosponges formulation were shown in Fig. 6. Voriconazole-loaded Nanosponges FT-IR spectrum showed characteristic bands as follows; OH stretching at 3217.68 cm^{-1}, C-N stretching at 1501.41 cm^{-1}, and C-F stretching at 1629.74 cm^{-1}.

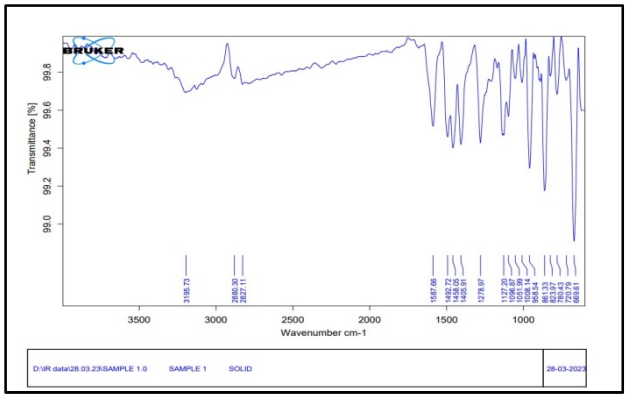


Figure 5: FTIR spectrum of voriconazole

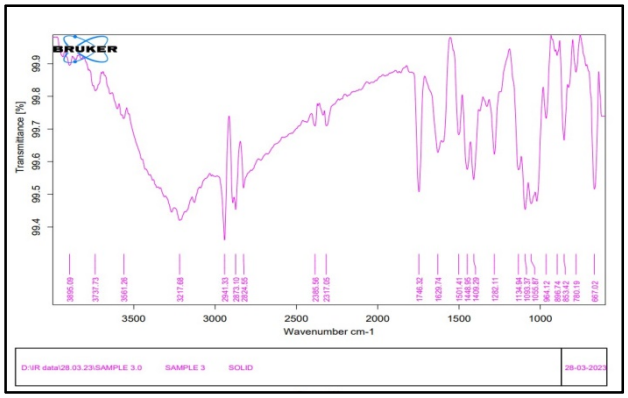


Figure 6: FTIR of voriconazole-loaded nanosponges

Surface Morphology by Scanning Electron Microscopy

SEM analysis of the optimized voriconazole loaded Nanosponges was performed to evaluate the surface morphology of Nanosponges. The SEM image of optimized formulation is shown in Fig. 7, and observed Nanosponges are uniformly porous particles and spherical in shape, smooth surface morphology. The smooth surface observed in the images of Nanosponges reveals complete removal of solvent from the formulation.

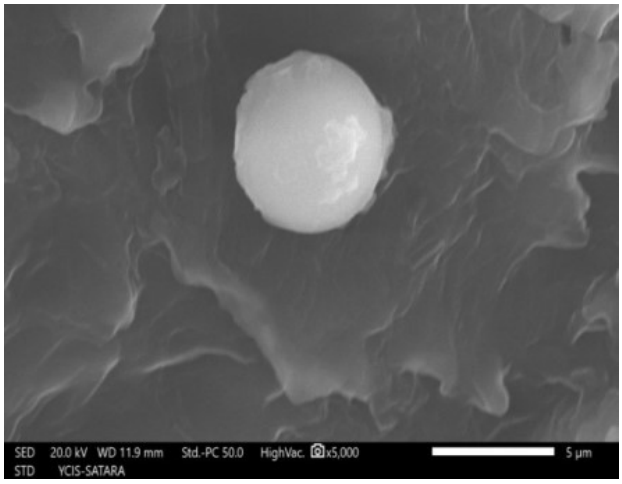


Figure 7: SEM of optimized nanosponge

Preparation of Nanosponges Based Gel

On the basis of data obtained from response parameter F5 formulation selected as optimized formulation. The formulation shows entrapment efficiency is 80.02% and particle size is 547.1 nm. The optimized formulation F5 was used for preparation of nanosponge based gel.

Evaluation of Nanosponges Based Gel

Physical Properties

Physicochemical properties of nanosponges based gel are shown in Table 8.

Table 8: Physical properties of nanosponges based gel

Sl. No.	Physical Properties	Observation
1	Appearance	Viscous jelly like
2	Colour	Off white
3	Odour	Odourless
4	texture	Lump free and smooth
5	transparency	Clear

Viscosity

A Brookfield viscometer was used to evaluate the viscosity of the manufactured nanosponges-based gel at 0.6 rpm. The dial reading revealed that the viscosity of the gel formulation increased as the concentration of polymers in the nanosponges increased. All formulations' viscosities were displayed in Table 9.

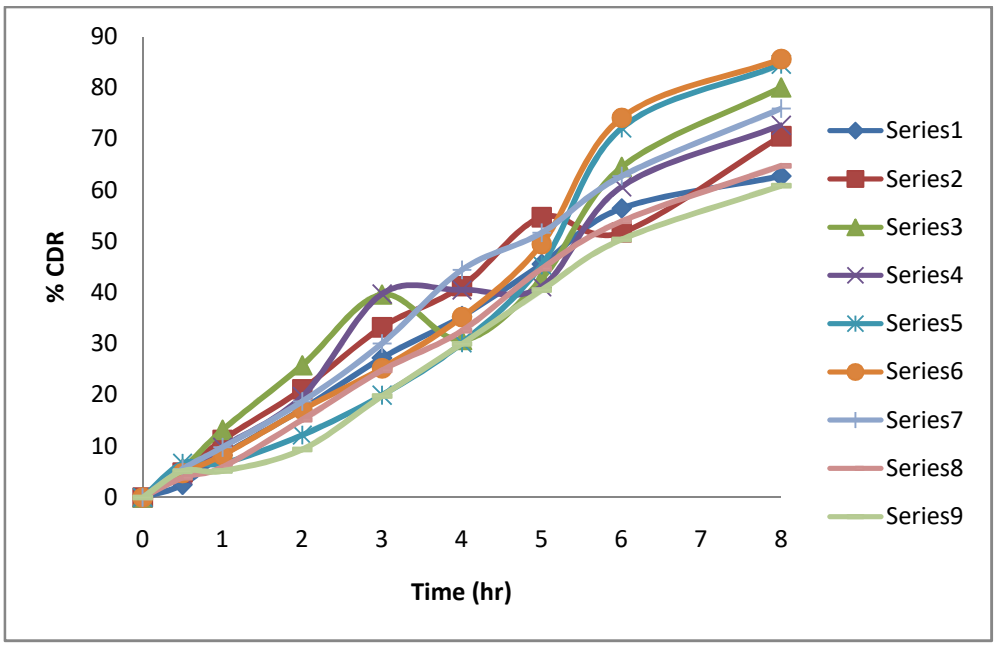


Figure 8: *In vitro* drug release of nanosponges based gel

Table 9: Viscosity of nanosponge based gel

Formulation	Viscosity (Cp)
F1	4608.2
F2	4066.4
F3	5142.5
F4	5202.8
F5	5282.2
F6	5041.1
F7	5375.9
F8	5152.5
F9	5289.1

pH Determination

The pH of the generated gel compositions was measured using a digital pH meter. After dispersing 1g of gel in 100 mL of distilled water, it was kept for two hours. Each formulation's pH was measured three times, and the average results were ascertained. The pH values of the developed formulations varied from 6.02 to 6.80. Applying this to skin will help prevent skin irritation.

SpreadabilityTest

The formulation's spreading coefficient determines how healing it is. As indicated in Table 11, the spreadability values of all gel formulations based on nanosponges varied from 1.1 cm to 2.3 cm. The viscosity and gelling properties of the polymers utilized in the formulation affect spreadability. The formulations with the highest viscosity also have a high coefficient of spreading. According to the spreadability principle, gel with a higher viscosity will spread more readily and require fewer shears. The homogeneous, smooth topical preparations with respectable spreadability were visible upon visual evaluation of the prepared formulations.

Table 10: pH of nanosponges based gel

Formulation code	pH
F1	6.12
F2	6.32
F3	6.02
F4	6.27
F5	6.40
F6	6.80
F7	6.08
F8	6.30
F9	6.10

Table 11: Spreadability of nanosponges based gel

Formulation Code	Spreadability (cm)
F1	1.3
F2	1.1
F3	1.5
F4	1.8
F5	2.2
F6	1.4
F7	2.3
F8	1.6
F9	2

In Vitro Drug Release Study

In vitro drug release study was done by using phosphate buffer pH 7.4. All the formulations of nanosponges gel were subjected to *in vitro* drug release studies by using a dialysis membrane. The temperature was maintained at 37°C and stirring kept at 100 rpm on magnetic stirrer. The cumulative drug release profile was obtained by plotting time on the x-axis and the percent cumulative of drug release on the y-axis. The formulation F6 has shown the maximum cumulative % of drug release 85.57% for 8 hours, compared to all other formulations. This may be attributed because of the high solubility of the drug. The concentration of the polymer played an important role in the release characteristics.

CONCLUSION

Voriconazole loaded nanosponges were successfully prepared by emulsion solvent diffusion method using ethyl cellulose (EC) and polyvinyl alcohol (PVA) as a stabilizer. Central Composite Design (CCD) was employed to optimize the nanosponges formulation. It was observed that solubility can be enhanced by formulating drug into nanosponges. The study showed that formulation having smaller particle size and high entrapment efficiency with excellent spreadability.

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