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Statistical Design Approach for the Formulation And Optimization of Nanosponges Using Poorly Water-soluble Candidate

Ritu Verma[1], Pranshul Sethi[2], Sonali Rastogi[3], Vinayak Sahebrao Mundhe[4], Rajesh KS[5], Saurabh Mishra[6], Mayur Rajendra Bhurat[7], Prasanthi Samathoti[8*]

[1]Principal, Venkateshwara Institute of Pharmacy, Department of Pharmacology, Venkateshwara Group of Institutions Jatoli, Meerut, Uttar Pradesh, India
[2]Assistant Professor, Department of Pharmacology, College of Pharmacy, Shri Venkateshwara University affiliation, Gajraula, India
[3]Assistant Professor, Department of Pharmacy, Venkateshwara group of institutions, Nh- 58, Delhi- Roorkee, Bye Pass Road, Jatoli, Meerut (U.P.), India
[4]Dr. Vedprakash Patil Pharmacy College,Chhatrapati Sambhajinagar Maharashtra 431005, India
[5]Department of Pharmacology, Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Mangaluru, India
[6]Amity University, Malhaur Railway Station Road, Gomti Nagar, Lucknow, Nijampur Malhaur, Uttar Pradesh 226010, India
[7]Principal, Shri Prakashchand Jain college of pharmacy and research, Palaas Kheda, Maharashtra 424206, India
[8]Associate Professor, Department of Pharmaceutics, MB school of pharmaceutical sciences (Erastwhile Sree Vidyanikethan college of pharmacy), Mohan Babu University, Sree sainathnagar, A.Rangampet, Tirupati, Andhra Pradesh – 517102, India

***Correspondence Author:**
Prasanthi Samathoti, Associate Professor, Department of Pharmaceutics, MB school of pharmaceutical sciences (Erastwhile Sree Vidyanikethan college of pharmacy), Mohan Babu University, Sree sainathnagar, A.Rangampet, Tirupati, Andhra Pradesh – 517102, India

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Abstract

Background and Objectives: Nanosponges are one of the most innovative ways to use the newest developments in nanodrugs delivery. Nanosponges can catch drugs that dissolve in water or ones that don't. This work uses statistical design to find the best nanosponges for drugs that don't dissolve easily and make them.

Material and Methods: It was looked into how to statistically make the most of the effects of independent factors. The ethyl cellulose ratio and stirring rate were chosen based on how they affected the dependent variables, such as particle size and how well they were trapped. FTIR, SEM, zeta potential, entrapment efficiency, and particle size data were used to test the nanosponges that were made. Using carbopol, the best lot of nanosponges was added to the gel.

Results: Using ethyl cellulose and polyvinyl alcohol as stabilizers in the emulsion liquid diffusion

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method, it was possible to make drug-loaded nanosponges. It was possible to make the nanosponges composition work better by using Central Composite Design. It has been seen that making drug-filled nanosponges improves stability.
Conclusion: The study showcased the enhanced capacity of a formulation with decreased particle size and high entrapment efficiency to disseminate effectively.

Keywords Statistical design, nanosponges, solubility, nano drug delivery

INTRODUCTION

Nanosponges are one of the most innovative ways to use the newest developments in nanodrug delivery. Nanosponges are good at catching both drugs that like water and drugs that don't like water. It's not dangerous, these substances don't dissolve in water or organic solvents, they have small holes, and they don't change when heated up. When these nanosponges are given through an IV, they move through the body until they reach the right place, where they release the drug in a controlled way [1-3].

Nanosponges are made up of very small particles with spaces that are only a few nanometers across and can hold different substances. These particles can carry both hydrophilic and lipophilic chemicals, which makes molecules that don't dissolve well in water dissolve better. Studies in this area show that nanosponges, which are very small structures that look like mesh, could completely change the way many diseases are treated. Based on early tests, this technology may be able to deliver drugs for breast cancer up to five times more effectively than traditional ways [2-6].

In the last twenty years, there has been an increase in fungal diseases, which have led to more illness and death. About 40 million people in both rich and developing countries get fungal diseases every year. There are 600 different kinds of fungus that can affect people. Fungi can cause illnesses and allergies in the skin, nails, hair, and mucosa. Even though there are many drugs available, antifungal therapy is hard to do because of drug resistance, poor absorption, drug interactions, and worries about toxicity [7-10].

Antifungal drugs are used a lot in many fields, like gardening, preserving wood, and caring for people and animals' health. This makes giving antifungal therapy very difficult. Nanosponges are porous structures that look like sponges. They are measured in nanometers, with a width of less than 1 micrometer. They improve the absorption of drugs that don't dissolve well by making them wetter and more soluble [4-6]. Even when bad physical, chemical, or biological conditions happen, pharmaceutical chemicals stay stable. Because they can flow easily as a powder, it is easier to give them by mouth, on the skin, through the lungs, or as an injection when they are in different dosage forms, like pills, capsules, emulgels, hydrogels, and saline solution [11-17]. The goal of this study is to use central composite design to make voriconazole nanosponges, which don't dissolve easily, and improve them.

MATERIAL AND METHODS

Alkem Labs Ltd. in India provided us with a complimentary sample of the drug. We purchased ethanol, polyvinyl alcohol, triethanolamine, dichloromethane, carbopol, and ethyl cellulose from Loba Chemicals in Mumbai. All reagents used were of analytical grade.

Experimental Design

The statistical analysis of the experimental plan was done by professionals in the field using special software. A design with three levels needed a total of twelve tests for this question. Getting the mixture just right has a big effect on how nanosponges grow and are structured. A study was done to look into the 32-design for making the effects of independent factors stronger. The way drugs were chosen was based on two factors that affected each other: particle size (measured in nanometers) and entrapment efficiency (given as a percentage). The percentage of ethyl cellulose (A1) and the rate of stirring (rpm) (A2) were chosen based on the requirements. The three levels that were coded—low (-1), middle (0%), and high (+1)—were turned into experimental units, runs, and combinations of factors that were studied in this study [18-20]. These specifics can be seen in Table 1. The ANOVA test was used to look at how statistically significant the model was.

Preparation of Nanosponges

Voriconazole nanosponges were made by diffusing

an emulsion fluid. Voriconazole and ethyl cellulose were dissolved in dichloromethane to make phase 2. Polyvinyl alcohol and pure water were then mixed together to make phase 3. Phases 1 and 2 were put on a magnetic mixer one at a time for 12 minutes each. After that, Phases 1 and 2 were slowly mixed together while the mixer was kept at room temperature for 15 minutes. The mixture was mixed together at different speeds for two hours and then it was cleaned. The nanosponges that were made were dried at 40°C for 12 hours. For each of the nine versions, Table 1 shows the drug:EC and stirring rate ratios [21-23].

Evaluation of Nanosponges

Saturation Solubility

The researchers looked at how much voriconazole could dissolve in the nanosponge mixture at most. For the full story, 10 mg of nanosponge formulations were mixed with 1 mL of pure water. After that, these mixtures were put into 2 mL centrifuge tubes and spun at 18000 rpm for one hour. A spectrophotometer set to measure at the longest wavelength (λmax) of 256 was used to find out how much voriconazole was present. Before it was analyzed, the supernatant was passed through Whatman filter paper. The test was done to see how entrapping voriconazole in nanosponge changed its ability to dissolve by comparing it to the drug itself. It was worked out how much voriconazole dissolves in 1 mL of pure water [24-28].

Particle Size and Zeta Potential Evaluation

Using a Dynamic Light Scattering Instrument with particle sizing software, you can find out how the sizes of drug-loaded nanosponges are spread out and what their average diameter is. The dry nanosponges were mixed with water to get them to the right amount of light scattering for voriconazole nanosponges. With these numbers, you can figure out the average width. A zeta sizer is used to find the zeta potential, also known as the surface charge, of the nanosponges that are made. The nanosponges inside the electrophoretic cell are weakened by water. To find out how stable the nanoparticles were, the zeta potential was looked at. The effect of electric charges can be measured by zeta potential. When particles are close to each other, the basic force keeps them apart. The combined forces will either push something together or pull it away, based on how strong they are compared to each other. The thumb rule shows how the processes that determine the zeta potential of nanoparticles are related [29,30].

Drug Entrapment Efficiency

We made 50 mg of drug-loaded nanosponges and the right polymer using the emulsion liquid diffusion method. After the mixture was made, it was mixed with 50 mL of ethanol and ultracentrifuged for 40 minutes. Spectrophotometric analysis was used to find out what amount of the medicine was mixed in. After centrifuging the watery mixture, the supernatant showed that there was medicine that wasn't bound to anything else. The amount of integrated drug was found by taking the original drug amount and subtracting the amount of free drug. Use the following formula to find the drug entrapment rate [31].

Fourier Transforms Infrared

Table 1: Formulation Batches

Batches code	Drug Polymer Ratio	PVA (%w/v)	DCM (mL)	Vehicle (ml)	Stirring rate (rpm)
B1	1:1	1.0	10.5	25	1000
B2	1:1	1.0	10.5	25	1500
B3	1:1	1.0	10.5	25	1800
B4	1:2	1.0	10.5	25	2000
B5	1:2	1.0	10.5	25	2200
B6	1:2	1.0	10.5	25	2400
B7	1:3	1.0	10.5	25	2600
B8	1:3	1.0	10.5	25	2800
B9	1:3	1.0	10.5	25	3000
B10	1:1	1.0	10.5	25	3200
B11	1:2	1.0	10.5	25	3400
B12	1:1	1.0	10.5	25	3600

A spectrophotometer test was done to check the functional groups in the active pharmaceutical ingredient (API) that was used to make the nanosponge and make sure they were intact. The FTIR spectra of both the pure medicine and its mixture were looked at with a Fourier Transform Infrared (FTIR) spectrophotometer. After that, the samples were scanned over a range of wave numbers, from 400 to 500 cm-1 [31].

Surface Morphology Study

Scanning electron microscopy (SEM) can be used to look at the tiny parts of the medicine, like the nanosponges and the drug/nanosponges complex that forms. When looked at through an electron microscope, the difference in crystallization state between the finished result and the basic parts shows how complex things are made. To check the shape of the nanosponges' surfaces, the material was carefully spread out on double-sided tape that was stuck to a metal stub. After that, a scanning electron microscope was used to look at the object. After that, platinum was added to the stubs. A scanning electron microscope was used to look at the material, which had stubs in it. After scanning the samples randomly, photomicrographs were taken using a voltage of 20 kV acceleration [32].

Preparation of nanosponges based gel

To dissolve 1.5 grams of carbopol, 100 milliliters of pure water were used. To change the pH, triethanolamine was used. So that the voriconazole concentration was 1% w/w, the gel was made stronger by adding the right number of tailored voriconazole nanosponges of the same weight. It was also possible to make a regular voriconazole gel. The gel that was made was put in a suitable container and put in the fridge overnight to make sure that carbopol 934 was completely broken down [32].

Characterization

Viscosity and pH Determination

The Brookfield Viscometer was used to find out how thick the gel was. As long as the speed stays at 0.6 rpm, spindle number S64 is used to measure. To find out what the gel's pH was, a digital pH meter was used. The pH of the mixture was found by spreading 5 mg of voriconazole nanosponge-filled gel equally into 5 mL of distilled water and leaving it at room temperature for two hours [33].

Spreadability Test

After putting a 0.5-gram sample of a gel mixture containing nanosponge between two slides, it was thought that the gel would stop spreading after five to ten minutes. Millimeters were used to compare the sizes of the rings based on how much they could spread. Three times of the test were needed to get the average number for spreadability [34].

In Vitro drug release study

A dialysis bag was used in an in vitro medicine release trial. A phosphate buffer solution with a pH of 7.4 was put over the dialysis membrane and left there for 24 hours. There is a dialysis bag with 18 mg of medicine in it that is sealed on both ends and filled with nanosponge gel. The bag was floating in a beaker with 100 mL of release media in it. A magnetic stirrer was used to keep the temperature at 37°C and the speed of the stirring at 100 rpm. At set times of 0, 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 hours, a 5 mL portion of the sample was taken out to keep the sink state. The removed volume was then filled back up with a phosphate buffer solution that had just been made. UV spectroscopy with a frequency of 256 was used to look at the samples [35-41].

RESULTS AND DISCUSSION

Solubility Study

We found that voriconazole nanosponges made it much easier for voriconazole to dissolve in clear water compared to when it was just the drug itself. Voriconazole was easier to dissolve in the nanosponge formulations than in its pure form. Voriconazole was soluble in water at a concentration of 0.5 mg/mL, but it dissolves ten times better when it is made into nanosponges. The results show that nanosponges can improve the solubility of voriconazole. Nanosponges are made by weaving a polymer network or mesh with nanoscale openings that can soak up drug molecules and make the solubility better through cross-linking.

Experimental Design

The model is important because it has a high F-value of 5140751.32. As little as 0.01% of the time, an F-value this big could be due to random change or noise. When the P-value for a model term is less than 0.0500, it is statistically significant. In this case, A, B, A^2, and B^2 are all important model words. The disagreeing number between the Adjusted R2 of 1.0000 and the Predicted R2 of 1.0000 is less than 0.2, which means they agree enough.The signal-to-noise ratio is found by Adeq Precision. The number should be higher than 4 if possible. In this case, the percentage of 6504.070

means that the signal is good.

The model is thought to be important because its F-value is 4612.87. As little as 0.01% of the time, an F-value this big could be due to random change or noise. Model terms are statistically important if the p-value is less than 0.0500. In this case, A, B, A^2, and B^2 are all important model words. There isn't much difference between the Adjusted R^2 of 0.9997 and the Predicted R^2 of 0.9984, which means that both are pretty close. The signal-to-noise ratio is found by Adeq Precision. The number should be higher than 4 if possible. In this case, the percentage of 202.403 shows that the signal is strong.

Characterization Studies

Particle Size PDI and Zeta Potential

A zeta potential test was used to check how stable the nanosponges were. The effect of electric charges is worked out. When two particles are close to each other, they push against each other. The shown zeta potential value is good for the physical safety of nanosponges because it stops particles from sticking together by repelling them electrostatically. It was found that the zeta potential of the improved mixture was -26.7 mV. The voriconazole nanosponges are made so that the particles are between 267.1 nm and 537.5 nm in size.

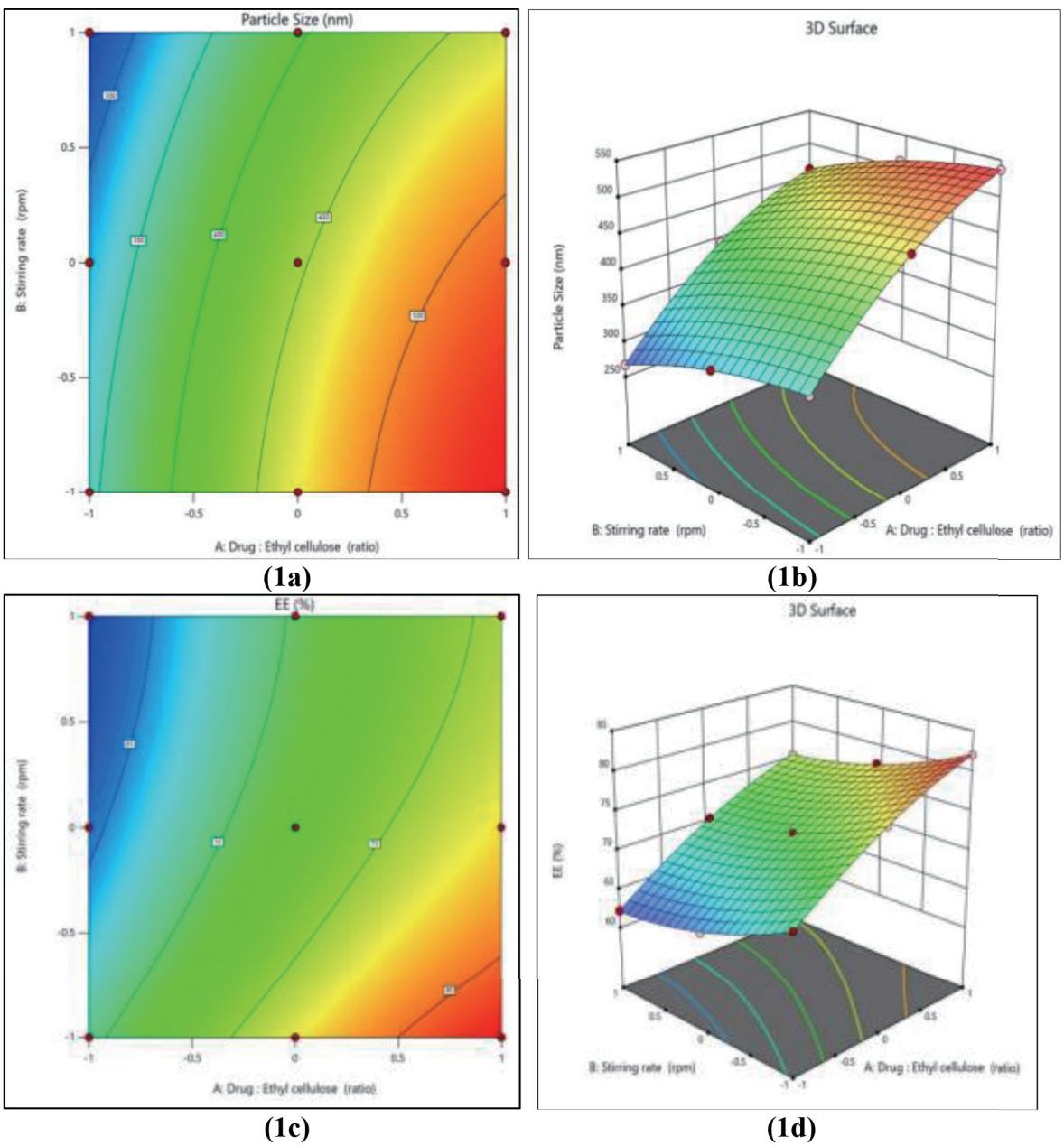

Figure 1: Contour plots in two and three dimensions to assess the impact of the drug:polymer ratio and stirring speed

B3 also had nanoparticles that were 267.1 nm smaller. The B5 nanosponges had the biggest particles, with a size of 537.5 nm. Figure 2 shows how their sizes are spread out.

A zeta potential test was used to check how stable the nanosponges were. The effect of electric charges is worked out. When two particles are close to each other, they push against each other. The shown zeta potential value is good for the physical safety of nanosponges because it stops particles from sticking together by repelling them electrostatically. It was found that the zeta potential of the improved version was -26.7 mV.

Drug Entrapment Efficiency

In Table 2, you can see how well each type of nanosponges traps things. The nanosponges' ability to catch things ranged from 61.81% to 83.68%. How well the drug was trapped depended on the amount of polymer used, as well as the internal and external phases. With an encapsulated drug level of 83.68%, the formulation marked as B5 had the best value. A bigger amount of drug was caught when the entrapment rate was higher. The amount of entrapment is based on the

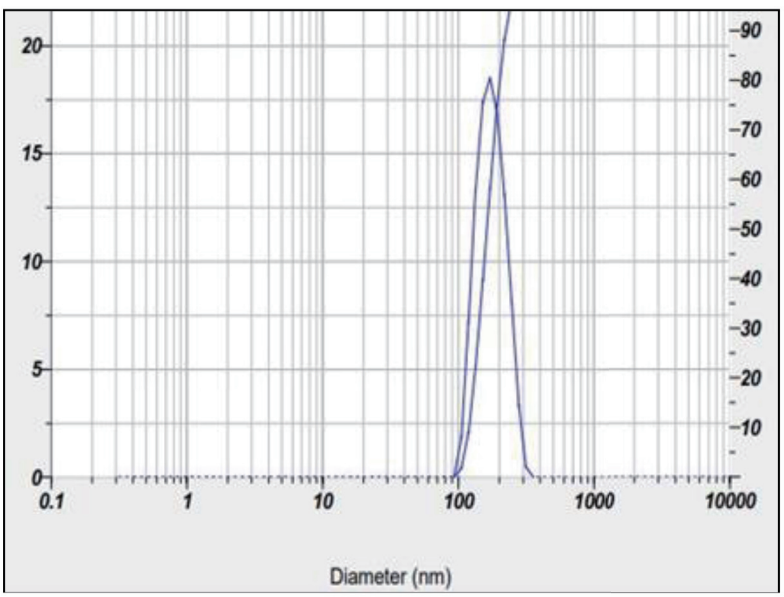

Figure 2: Particle size distribution of optimized Nanosponges

Table 2: Particle size and EE of voriconazole-loaded Nanosponges

Sr. No.	Batches	Particle size (nm)	EE (%)
1	B1	339.1	66.13
2	B2	321.3	65.34
3	B3	270.2	61.81
4	B4	461.0	78.19
5	B5	380.1	83.68
6	B6	441.3	71.14
7	B7	540.4	75.25
8	B8	560.2	72.30
9	B9	474.3	74.34
10	B10	567.0	70.70
11	B11	602.3	68.13
12	B12	712.3	73.01

sizes of the internal and external stages.

FT-IR Study

Figure 3 shows the absorption bands of the medicine voriconazole that has not been reduced. There were OH stretching at 3195.73 cm−1, C-N stretching at 1492.72 cm−1, and C-F stretching at 1587.66 cm−1 in the FTIR band. In Figure 4, the absorption bands of the Nanosponges mixture were shown. The voriconazole-containing Nanosponges' FT-IR spectrum showed three clear bands: OH stretching at 3217.68 cm−1, C-N stretching at 1501.41 cm−1, and C-F stretching at 1629.74 cm−1.

Surface Morphological study

A study used scanning electron microscopy to look at the shape of the improved voriconazole-loaded Nanosponges' surfaces. Figure 5 shows a SEM picture of the mixture that worked best. The surface of the nanosponges is smooth, they are spherical, and their porosity is constant. The pictures of the Nanosponges show a smooth, level surface, which suggests that the solvent has been completely taken out of the mixture.

Preparation of nanosponges based gel

It was found that the B5 formulation is the best choice after looking at the reaction parameter data.

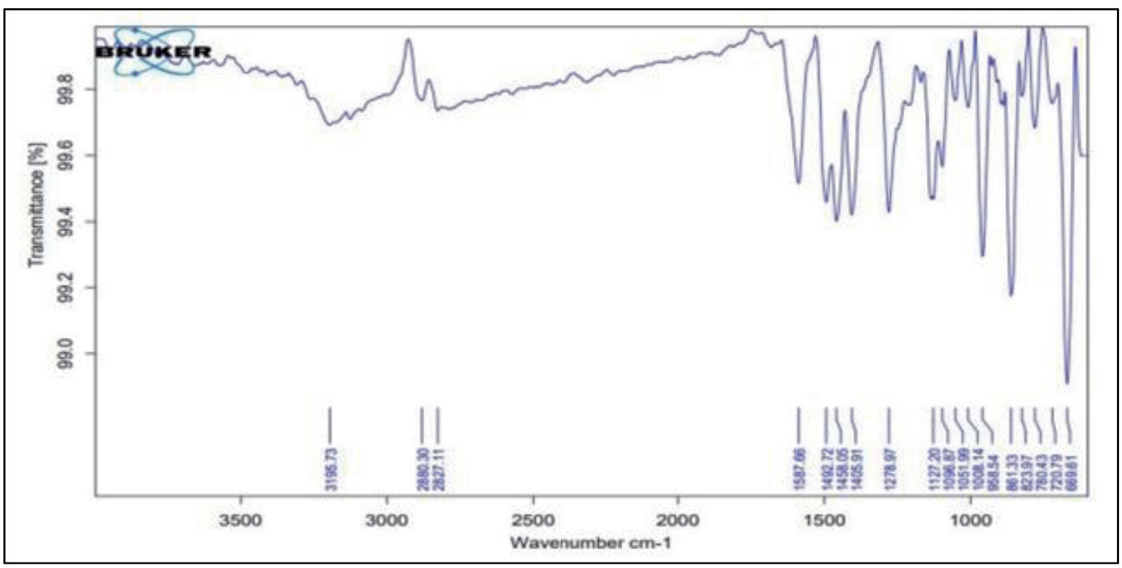

Figure 3: FTIR spectrum of drug

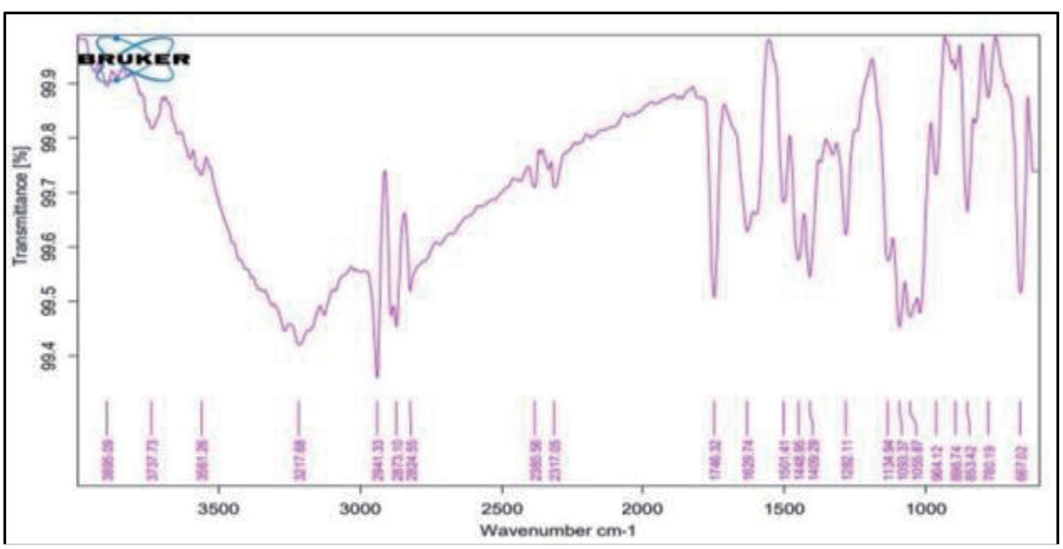

Figure 4: FTIR of Drug-loaded nanosponges

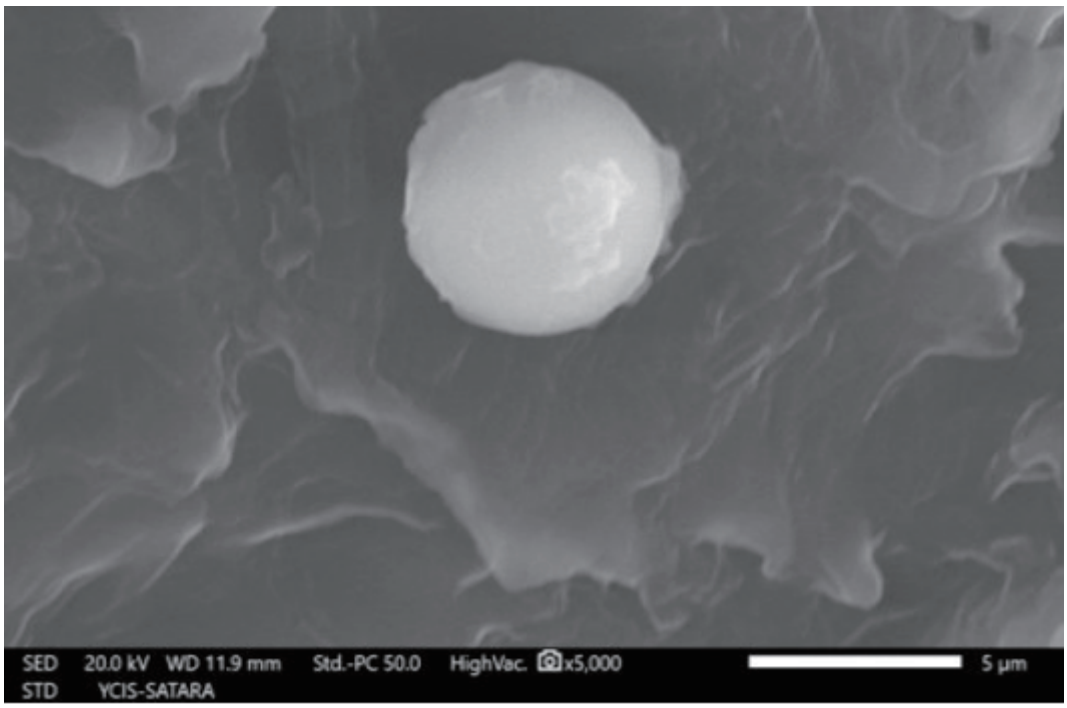

Figure 5: SEM images of optimized nanosponges

The particle size is 547.1 nm, and the formula says that 83.68% of the particles are completely trapped. Nanosponge technology was used to make the gel, which was made using the best formulation B5.

Viscosity

A Brookfield viscometer was used to test the gel's viscosity at 0.6 rpm. The gel was made from nanosponges. The reading on the scale showed that there was a direct link between the amount of polymers in the nanosponges and how thick the gel mixture was. The viscosities of all the mixtures were found to be between 4066.4 and 5375.9 centipoise (Cp).

pH Determination

A digital pH meter was used to find out what the pH of the gel mixtures were. The gel was mixed with 100 mL of pure water and then left to sit for two hours. The pH of each mixture was checked three times, and the mean number was found. The pH range of the chemicals that were made was from 6.0 to 6.90. Putting this on the skin can help lessen swelling.

Spreadability Test

What makes the healing qualities of the formulation work depends on how well it spreads. According to Table 3, the gel mixtures that had nanosponges had spreadability values between 1.1 cm and 2.3 cm. The viscosity and gelling qualities of the polymers used to make a substance affect how easily it can be spread. The versions with the highest viscosity are also the ones with the highest coefficient of spreading. From the point of view of spreadability theory, gels with higher viscosity will spread more easily and with less force. When the made formulations were looked at visually, it was clear that the topical treatments were smooth, even, and easy to spread.

Table 3: Spreadability test

Sr. No.	Batches	Spreadability
1	B1	1.2
2	B2	1.3
3	B3	1.6
4	B4	1.7
5	B5	2.1
6	B6	1.5
7	B7	2.2
8	B8	1.8
9	B9	2.0
10	B10	2.2
11	B11	1.8
12	B12	2.1

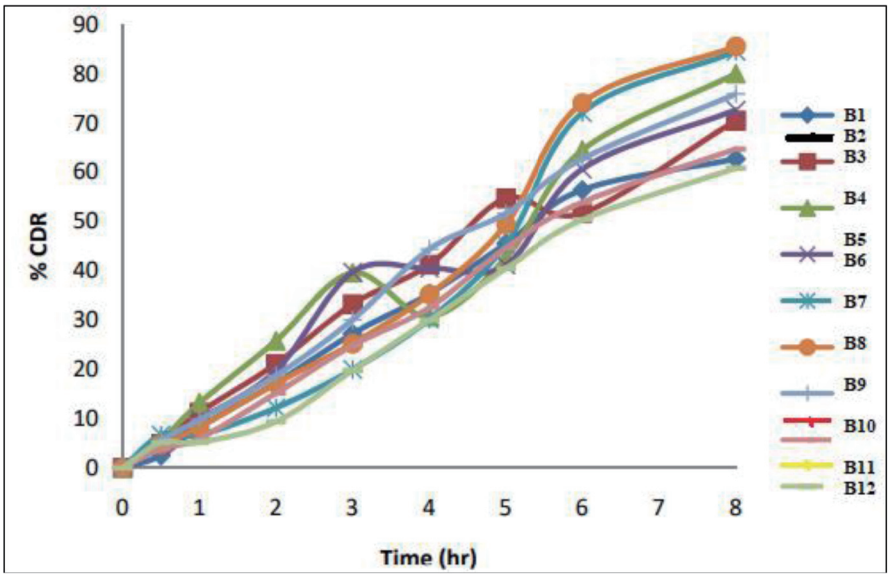

Figure 6: In-vitro Drug release study

In-vitro drug release study

A phosphate buffer solution with a pH of 7.4 was used in an in vitro test to look at how a drug would be released. A dialysis membrane was used to test the nanosponge gel mixtures for drug release in a lab setting. For the magnetic stirrer, the temperature was kept at 37°C and the speed was set to 100 rpm. Putting time on the x-axis and the percent total drug release on the y-axis made it easier to make the cumulative drug release profile. Formulation B5 had the highest total percentage of drug release over 8 hours, at 86.00%. This might be because the drug dissolves very easily. Figure 6 shows that the releasing qualities changed a lot depending on how much polymer was used.

CONCLUSION

We were able to make drug-loaded nanosponges by using ethyl cellulose and polyvinyl alcohol as stabilizers in the emulsion liquid diffusion method. It was possible to make the nanosponges composition work better by using Central Composite Design. It has been seen that making drug-filled nanosponges improves stability. The study showed how a mixture with smaller particles and high entrapment effectiveness could be used to spread information more efficiently.

Conflict of interest

None to declare for all authors.

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N/A

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