ejpmr, 2022,9(9), 299-305

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT OF NANOSPONGES BASED ON VORICONAZOLE LOADED β-CYCLODEXTRIN

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Article Received on 15/07/2022 **Article Revised on 05/08/2022** **Article Accepted on 25/08/2022**

ABSTRACT

Aim: To formulate and evaluate of voriconazole loaded β-cyclodextrin based nanosponges. **Methodolgy:** The ratios of ethyl cellulose to polyvinyl alcohol vary while making these nano-sponges. Polyvinyl alcohol (0.2 percent w/v) was dissolved in dichloromethane (20 ml) and slowly added to 150 ml of aqueous continuous phase with the ethyl cellulose and drug-containing dispersion phase. It was agitated for two hours at 1000 rpm. Filtration was used to collect the nanosponges, which were then dried for 24 hours at 400 degrees Celsius in an oven. A vacuum desiccator was used to remove any remaining solvent from the dried nanosponges. It was discovered and analysed which factors in the formulation have an impact on the manufacture and qualities of nanosponges. Polymer (β-Cyclodextrin, PVA) and cross-linker ratio (Dichloromethane), Stirring speed and time are examples of process variables. The particle size and polydispersity index were used as the foundation for the optimization. **Results:** The voriconazole entrapped nanosponge drug entrapment effectiveness in all of its produced formulations. All nanosponge formulations had drug entrapment effectiveness ranging from 81.66 percent to 93.11 percent, with the F4 formulation having the highest, at 93.11 percent. **Conclusion:** Voriconazole nanosponge release tests were conducted in a pH 1.2 buffer diffusion medium for 12 hours. It had a biphasic release with a burst effect at the beginning. F1 to F6 had a total of 19.6 mg of medication released in the first 30 minutes.

KEYWORDS: Voriconazole nanosponge, β-cyclodextrin, Stability, Antifungal.

INTRODUCTION

Approximately $65 billion is wasted each year in the medical research process owing to inadequate bioavailability of promising preclinical treatments that fail to make it into the market because of the possible adverse effects related with nonspecific drug delivery. Because of the intricate chemistry involved, designing a system for long-term and precise medication administration may be difficult. There have been some encouraging outcomes with nanoparticulate medication delivery in terms of enhancing bioavailability and providing more precise treatment. When combined with nanoparticulate drug delivery, Nanosponge (NS) has the potential to provide a longer-acting and more bioavailable drug delivery system. NS are novel, spherical, 150-400 nm-sized nanoparticulate drug carrier systems.[1]

Cross-linked polymers with a cross linker create the little voids that make up these structures.[2] Depending on the cross-linker utilised, they may be produced in either a neutral or acidic state. Because of their ability to form inclusion and non-inclusion complexes with hydrophilic and lipophilic medicines, NS are a marvel. The cross linker binds to certain polyester strands and the scaffold structure that they form. Varied polymers and cross linkers are used in different quantities to regulate pore size. Because of their specificity to the tissue, peptide linkers may be linked to their structure. The biodegradable polymer that releases the medication in a controlled manner. By injecting these nanoparticles straight into the body, the risk of adverse effects associated with other nanoparticulate drug delivery systems that employ a chemical transporter is greatly reduced.[3] Before they can connect to the tissue, these bound N-peptides circulate through the bloodstream. By utilising hydrophilic polymers, the reticulo endothelial system (RES) has a restricted ability to detect the substance. Drugs of various structure and solubility profile are used to test NS9's incorporation efficiency in a variety of ways. Nanocarriers for biomedical applications employ NS to boost the aqueous solubility of weakly water-soluble compounds, and the simple chemistry required in NS formulation should allow them to be commercially produced without the requirement of specialised equipment.[4]

Methodology

Optimization and Formulation of nanosponges

Preparation of nanosponges[5]

The ratios of ethyl cellulose to polyvinyl alcohol vary while making these nano-sponges. Polyvinyl alcohol (0.2 percent w/v) was dissolved in dichloromethane (20 ml) and slowly added to 150 ml of aqueous continuous phase with the ethyl cellulose and drug-containing dispersion phase. It was agitated for two hours at 1000 rpm. Filtration was used to collect the nanosponges, which were then dried for 24 hours at 400 degrees Celsius in an oven. A vacuum desiccator was used to remove any remaining solvent from the dried nanosponges. It was discovered and analysed which factors in the formulation have an impact on the manufacture and qualities of nanosponges.

Polymer (β-Cyclodextrin, PVA) and cross-linker ratio (Dichloromethane), Stirring speed and time are examples of process variables. The particle size and polydispersity index were used as the foundation for the optimization.

Optimization of formulation variables

For the optimization of ethyl cellulose and cross-linkers, the NS formulation were prepared with verifying ratio of cross linkers i.e. β-Cyclodextrin, PVA, dichloromethane in the following ratio like (1:1 1:2 1:3 1:4 1:5 1:6 1:7 1:8) keeping other parameter constant. Based on average particle size and polydispersity index (PDI) of NSs, which was evaluated by Malvern zeta-Sizer, optimization was carried out.

Formulation parameters of nanosponges[6]

Nanosponge formulation F1 through F6 were used for the optimization investigations. Table 1 shows the parameters utilised in the creation of this optimised formulation. After the optimization of all parameters final six formulation i.e. NS1, NS2, NS3, NS4, NS5, NS6, were prepared using different ratio of cross-linker keeping amount of polymer constant.

Loading of drug

The Emulsion solvent diffusion technique was used to create the nanosponge containing Voriconazole. The final formulation NS1 NS2 NS3 NS4 NS5 NS6 (w/w) included nanosponge and powdered voriconazole in the following ratios: 1:1, 2:1, 4:1, 6:1, 8:1, 10:1. Afterwards, the resulting combination was put into the distilled water. After 24 hours of stirring, the mixture was filtered to eliminate any remaining drug particles. It was then lyophilized to produce a powdered form of the voriconazole-loaded nanosponge. In order to conduct biological tests, some of the product was kept as a solid and some was suspended in water or DMSO.[7]

Evaluation of formulated nanosponges

- The prepared nanosponges of different formulation were evaluated for various physiochemical parameters such as particle size, polydispersity ratio, drug entrapment, morphological examination and in vitro drug release studies, ex-vivo antifungal studies and stability studies.[8]
- Surface morphology by TEM
- Drug entrapment efficiency
- In vitro drug release study
- Ex-vivo antifungal studies
- Stability studies

Particle morphology (TEM): Nanosponges were studied using an electronic transmission microscope (JEOL model JEM-1230, Tokyo, Japan) at 70 kV to study their morphology. A 1 percent (w/v) EDTA stain was used to observe the samples after a 50-fold dilution in the preparation's original dispersion solution.[9] Results are shown in Figure 2.

Drug entrapment: Nanosponges (10mg) and 5ml methanolic HCl (10:1) in a volumetric flask were agitated with a vortex mixer for 1 minute to determine the drug entrapment effectiveness. Methanolic HCl was used to get the volume to 10 ml. The voriconazole concentration was then measured spectroscopically at 256nm after the solution had been filtered and diluted.[6] Table 3 shows the results.

In-vitro drug release (diffusion studies): The voriconazole release rates from the nanosponges under investigation were evaluated using a dialysis membrane with a pore size of 2.4 nm and a molecular weight cutoff of 12,000-14,000 Da.Cells were set up using membranes that had been steeped in distilled water for 12 hours. As a receptor fluid, phosphate buffered saline (pH 7.4) was employed. A suitable amount of phosphate buffer saline was added to dilute the nano sponges. Temperature was maintained at 37–5°C in the upper donor chamber by applying 1 ml of diluted nanosponges to the upper chamber. Over the course of 48 hours, an aliquot of 100 l of samples was taken from the receiver compartment using a side tube. In order to keep a consistent volume, the fresh medium was replenished each time The quantity of voriconazole in the receiver medium was used to compute the release percentage.[10]

Using a UV-VIS Spectrophotometer, we measured the voriconazole concentration in the receiving medium (UV-1800, 240V, Shimadzu, Japan). The results are shown in Table 4.

Evaluation of voriconazole loaded nanosponges gel

A) "Determination of drug content, Spreadability, and pH."

a) Drug content: In 100-milliliter volumetric flasks, 1 gramme of gel was weighed and dissolved in ethanol.diluted, and examined using a UV-VIS Spectrophotometer to determine the drug concentration (UV-1800, Schimadzu, Japan).

b) Spreadability: It was determined by applying this method: the spreadability of this test sample The 0.5 gramme test formulation was put inside a pre-marked circle of 1cm diameter on a glass plate.

Resting on the top glass plate for 5 minutes, a 500g weight was permitted to rest. The spread of the test formulation was seen to increase the diameter.

c) **pH:** In order to acquire the pH of a 5% (w/w) water dispersion of the test sample, we used a Digital pH metre Model EQ610, which was firstly calibrated with buffers of pH 4 and pH 7.

B) Anti-fungal activity of nanosponges gel with marketed voriconazole preparation

a) **Zone of inhibition:** Study results showed that both the improved formulations and the commercially available preparation (Flucos® Mfg. by Cosme pharma labs ltd.) have antifungal efficacy against the Candida albicans pathogen. Sabouraud dextrose agar diffusion tests were used to determine this, utilising the usual paper disc diffusion technique with previously sterilisedpetridish. It was necessary to enable the test organism (0.5mL) to settle on Nutrient Agar (20 mL) in the Petri dish. At a sufficient distance from the surface of the dextrose agar, sterile paper discs (Whatman filter no. 42, diameter 4mm) were carefully placed with the use of sterilised pointed forceps. At 27 °C for 48 hours, the plates were incubated They were able to identify the areas where inhibition occurred. The sizes of the inhibitory zones were measured. Table 6 shows the results of the measurements.

Stability studies

The goal of stability testing is to offer proof of how the quality of a formulation changes over time as a result of external conditions including temperature, humidity, and light. Under tropical circumstances with high ambient temperature and humidity, degradation is likely to take place. Ten, twenty, thirty and sixty days later, the nanosponges that had been generated using the solvent diffusion approach were held at 4°C, 25°C and 45% room temperature. Efficacy of Entrapment and average particle size have been calculated.

RESULTS AND DISCUSSION

Formulation and Evaluation of nanosponge

Formulation of nanosponge

Table 1 and Table 2 provide the formula and results of the evaporation of Emulsion-solvent emulsion to generate Nanosponge. The particles were determined to be less than microns in diameter, with a spherical form and no agglomeration.

Table 1: Formulation of nanosponge without drug.

Content	F1	F2	F3	F4	F5	F6
Ethyl cellulose (mg)	100	200	300	100	200	300
polyvinyl alcohol (ml)	10	10	10	20	20	20
Dichloromethane (ml)	20	20	20	20	20	20
β-Cyclodextrin(mg)	10	15	20	10	15	20
Water (ml)	150	150	150	150	150	150

Table 2: Formulation of nanosponge with drug.

Content	F1	F2	F3	F4	F5	F6
Voriconazole (mg)	200	200	200	200	200	200
Ethyl cellulose (mg)	100	200	300	100	200	300
polyvinyl alcohol (ml)	10	10	10	20	20	20
Dichloromethane (ml)	20	20	20	20	20	20
β-Cyclodextrin(mg)	10	15	20	10	15	20
Water	150	150	150	150	150	150

Surface Morphology by TEM analysis

The SEM image indicated that nanosponge are found to be abundant, spherical in shape and smooth. The TEM report is given in Figure 1 and 2.

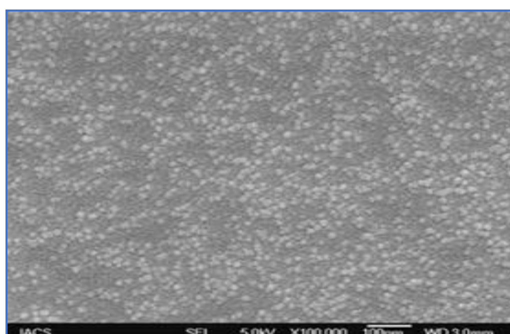

Figure 1: Transmission electron microscopy report of nanosponge.

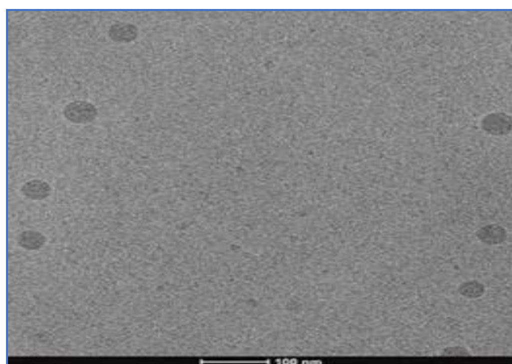

Figure 2: SEM Report of nanosponge.

Drug entrapment efficiency

Table 3 show the drug entrapment effectiveness of Voriconazole entrapped nanosponge in all of its various formulations. Nanosponge drug entrapment effectiveness ranges from 81.66% to 93.11%, with F4 formulation having the highest percentage of drug entrapment, 93.11%.

Table 3: Entrapment efficiency of various drug loaded nanosponge formulations.

S. No.	Formulation code	Drug entrapment efficiency
1	F1	91.86
2	F2	83.35
3	F3	90.06
4	F4	93.11
5	F5	86.26
6	F6	81.66

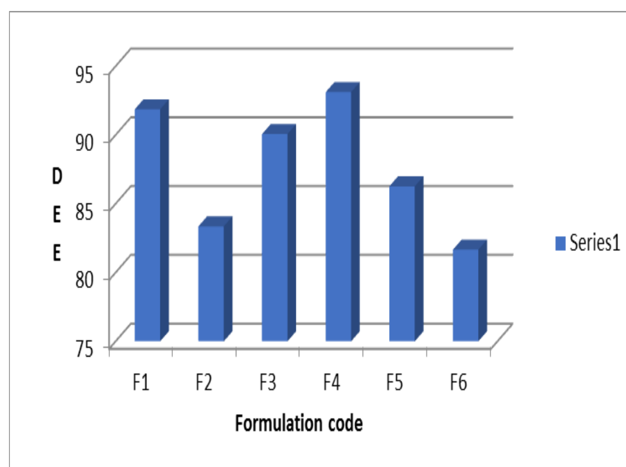

Figure 3: Entrapment efficiency of drug loaded Nanosponge formulations.

***In-vitro* drug release study**

Using pH 1.2 buffer as a diffusion medium, the in-vitro release tests of the produced Voriconazole nanosponge were carried out for 12 hours. It had a biphasic release with a burst effect at the beginning. F1 to F6 had a total of 19.6, 16.48, 16.55, 16.12, 17.74, and 15.59 mg of medication released in the first 30 minutes. There are two possible explanations for the burst release: the microspheres are laden with a medication, or the encapsulation is faulty. At the conclusion of the 12th hour, the cumulative percent release for F1, F2, F3, F4, F5, and F6 was 94.5, 85.00, 88.08, 96.27, 84.04, and 81.35. (Table 4 and Figure 4).

Table 4: *In vitro* release data of Voriconazole nanosponge.

Time (h)	% Cumulative drug release					
	F_1	F_2	F_3	F_4	F_5	F_6
0	0	0	0	0	0	0
0.5	19.6	16.48	16.55	16.12	17.74	15.59
1	31.44	22.23	25.90	25.87	24.64	19.69
2	40.12	31.24	33.49	34.97	33.31	27.24

3	45.93	37.13	41.22	39.21	38.73	30.28
4	53.44	42.37	47.13	43.60	43.02	37.20
5	58.29	48.58	54.92	51.28	48.08	43.33
6	62.57	51.86	58.29	58.56	53.36	48.44
7	67.08	57.99	62.03	63.23	59.27	53.67
8	73.48	60.24	68.61	69.41	62.46	58.55
9	79.91	68.89	75.79	73.80	68.28	68.04
10	83.55	73.61	78.33	79.68	72.66	73.69
11	91.87	78.67	82.46	85.38	81.25	76.64
12	94.5	85.00	88.08	96.27	84.04	81.35

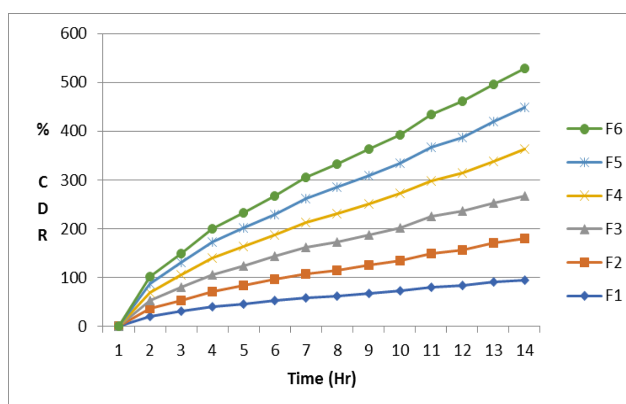

Figure 4: Comparative in vitro release profiles of voriconazole nanosponge.

Evaluation of voriconazole loaded nanosponges gel: "Determination of drug content, spreadability, and pH:"

Spirophotometric methods were used to measure the drug's concentration. In order to get the gel's equivalent of 50mg, methanol was used to dissolve the gel and filter it. In order to increase the volume to 10ml, methanol was used. Measurement of absorbance at 427 nanometers was made using the diluted solution.The F4 formulation shows maximum % Drug content i.e., 93.11% and thus selected as a final formulation. The results are shown in Table 5.

Nanosponge agent exhibited spreadability values ranging from 11.60-14.75 g.cm/s. The spreading coefficient of various nanosponge gel formulations are given below in table 5.

In order to prevent the possibility of skin irritation, the pH of formulations ranged from 6.29 to 6.93, which was regarded appropriate. Table 5 shows the results. The pH of the improved formulation (F4) was 6.25. All formulations showed no significant change in pH levels over time.

Table 5: Determination of drug content, Spreadability and pH.

S. No.	Formulation code	Drug content	Spreadibility	pH
1	F_1	85.26 ± 1.44	13.2	6.65
2	F_2	90.64 ± 1.36	11.6	6.54
3	F_3	83.41 ± 1.24	11.9	6.99
4	F_4	91.46 ± 1.54	14.75	6.25
5	F_5	82.71 ± 1.56	13.5	6.54
6	F_6	86.12 ± 1.72	12.5	6.55

Anti fungal studies

Select formulations were evaluated for antifungal activity in a cup plate approach utilising a commercially available gel solution as the reference solution. Figure 5 depicts anti-fungal activity investigations. Formulation F4 showed 24mm inhibition which was high when compared to the zone of inhibition of free Voriconazole gel which showed 19 mm and marketed cream showed 21mm (Figure 5and Table 6).

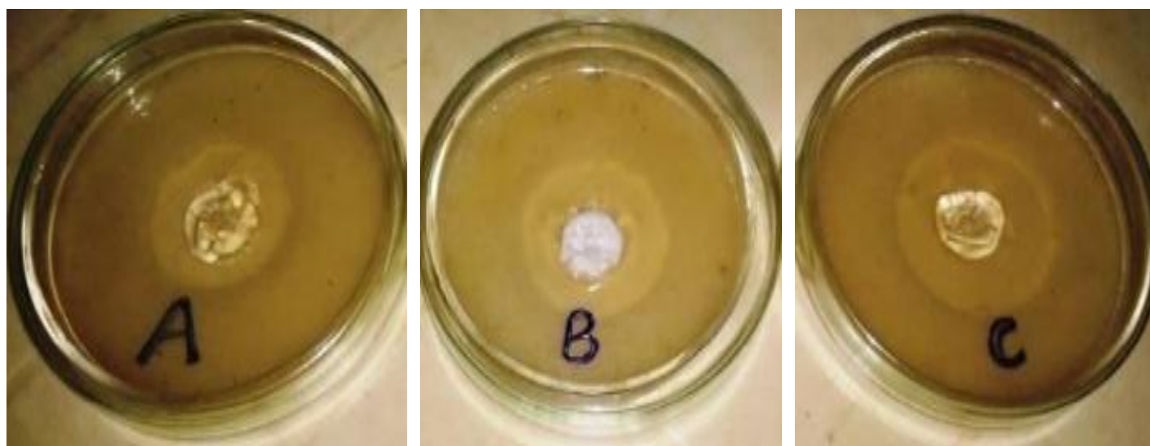

Figure 5: Zone of inhibition of Free voriconazole gel (A) compared with marketed cream (B), voriconazole nanosponge gel F4 (C)

Table 6: Zone of inhibition of Free voriconazole gel (A) compared with marketed cream (B), voriconazole nanosponge gel F4 (C)

Organism (Fungal strains)	Formulation	Zone of inhibition(mm)
Candida albicans	F4	24mm
Candida albicans	Plain Voriconazole gel	19mm
Candida albicans	Marketed cream	21mm

Stability study: To ensure physical and chemical stability, the formulation was held at 40 2°C and 75% RH for one month, according to the stability study. – An insignificant change was found in all optimization parameters with a bias of less than 5 percent. Before and after the stability investigation, there was no noticeable variation in the findings of the optimised batch.

CONCLUSION

In order to create nanosponges, varying amounts of -cyclodextrin and polyvinyl alcohol were used. It was necessary to dissolve the dispersed phase of the medication in 20ml dichloromethane before it could be added to the 150ml of continuous phase of polyvinyl alcohol with a certain concentration of polyvinyl alcohol (0.2 percent by weight). 2 hours of 1000rpm stirring in the reaction mixture yielded good results. Filtration was used to collect the nanosponges, which were then dried in an oven at 40 degrees Celsius for 24 hours. They were kept in vacuum desiccators in order to remove any remaining solvent.

The voriconazole entrapped nanosponge drug entrapment effectiveness in all of its produced formulations. All nanosponge formulations had drug entrapment effectiveness ranging from 81.66 percent to 93.11 percent, with the F4 formulation having the highest, at 93.11 percent.

Voriconazole nanosponge release tests were conducted in a pH 1.2 buffer diffusion medium for 12 hours. It had a biphasic release with a burst effect at the beginning. F1 to F6 had a total of 19.6, 16.48, 16.55, 16.12, 17.74, and 15.59 mg of medication released in the first 30 minutes. There are two possible explanations for the burst release: the microspheres are laden with a medication, or the encapsulation is faulty. At the conclusion of the 12th hour, the cumulative percent release for F1, F2, F3, F4, F5, and F6 was 94.5, 85.00, 88.08, 96.27, 84.04, and 81.35.

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