Research Article

Development and Evaluation of Terbinafine Loaded Nanosponges

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ABSTRACT

In the current study, four formulations (TNS1-TNS4) of Terbinafine (TBF) loaded Nanosponges (NS) were fabricated by solvent emulsification method, using different concentration ethyl cellulose (EC) and polyvinyl alcohol (PVA) respectively. Prepared Nanosponges were characterized for particle size (PS), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE) and drug loading (DL). Nanosponges TNS2 was optimized on the particle characterization and drug entrapment efficiency. It was further evaluated for physicochemical characterizations; FTIR, Zeta potential and SEM. Selected NS TNS2 composed of TBF (250mg), EC (200mg), and PVA (300mg) showed, PS (258.4 ± 0.57 nm), PDI (0.268 ± 0.02), ZP (-15.10mV ± 0.91 mV), %EE (83.226± 0.258%) and %DL (27.742± 0.086%), respectively. Fabricated NS also revealed; polymer-drug compatibility, drug-encapsulation, non crystalline state of the drug in spherical NS as per the physicochemical evaluations. Optimized NS (TNS2) with 3% w/v amount of TBF was incorporated into the 1% w/v carbopol gel. TBF loaded NS based gel was then evaluated for visual appearance, pH study, viscosity, drug content, Invitro drug release studies.

Keywords: Terbinafine, Nanosponges, Topical Gel, Zeta Potential, Particle Size.

1. INTRODUCTION

Effective medication amount at the specific target site for a predetermined amount of time can be achieved through an ideal drug therapy, which helps to reduce side effects and ensure a successful therapeutic outcome. The amount at which drug is moved and delivered to the specific site of action and the drug input rate are the two most important factors which help to achieve desirable therapeutic response. Targeted drug delivery refers to the exclusive delivery of a medicine to a receptor, organ, or other portion of the body, as opposed to other tissues, where the distribution of the drug may be wasteful, useless or toxic. For a very long time, such drug delivery systems have been a pipe dream, but the difficult chemistry required for the creation of such systems has mainly frustrated it. Reaching the drug at the targeted place in the body and its controlled release are the major challenges for medical researchers. This issue might be resolved through the creation of novel, intricate molecules called nanosponges.[1]

Nanosponges have the potential to deliver the medication to the targeted area in a controlled manner. They are able to moving both hydrophilic and lipophilic molecules. Due to their tiny particle size and spherical shape, they can be created in a variety of dose forms, including topical, oral, and parenteral preparations. These are a novel kind of tiny sponges the size of a virus that may be filled with medication and then injected into the body after adhering to particular chemical linkers that bind preferentially to a characteristic exclusively present on tumor cells surface. Once tiny sponge is administered, it moves throughout the body and adheres to the tumor cell surface and then it releases potent drug in a predictable and controllable manner.

The body is protected by nanosponges that resemble red blood cells. They are able to contain and stop the transmission of disease. They primarily attack the toxins, and the toxins band together to pierce the membrane of the red blood cell (nanosponges); this process of piercing the membrane is known as pore formation. Cell damage and cell death happen when the toxins are attached to nanosponge.

A lengthy polyester backbone forms the three-dimensional network of nanosponges. Polyester degrades gradually in the body since it is biodegradable. The size of the nanosponge particles can also be managed by adjusting the ratio of crosslinkers to polymers.[1]

Nanosponges are the particles having spherical shapes and holes filled with medicinal molecules, they act as the carriers for lipophilic and hydrophilic which have low aqueous solubility. Nanosponges are targeted to particular sites in the body, with the help of specific peptide linkers, this ultimately results the decrease of side effects and improvement of drug efficacy. They also provide various pharmaceutical applications for future, like enhanced product elegancy and performance, decreased irritation, improved physical, chemical and thermal stability.[2]

Nanosponges help in the formation of complexes and novel molecules, which are able tosolve these problems.[3] Nanosponges appears as either crystalline or in para crystalline form. Various loading capabilities can be displayed via Paracrystalline nanosponges, which primarily depends on the crystallization level. Nanosponges can be produced in a specific size which enables the controlled release of drug molecule.[4] A various variety of substances

can be encapsulated with-in these dosage forms, since they have nanometers sized cavities which is made of microscopic particles.[5] Nanotechnology is also used in the preparation of artificial blood platelets. Platelets are blood components which plays a major role in blood coagulation process. The ability to "tag" cells with colored nanoparticles to study cell biology in an yet another technique evolved from the use of nanoparticles.[6] Antifungal drugs incorporated in nanocarriers showed excellent results compared with conventional drug carriers.[7]

Terbinafine belongs to the group of medicines called antifungals. It is used to treat fungus infections of the scalp, body, groin (jock itch), feet (athletes foot), fingernails, and toenails. It is a highly lipophilic antifungal medication. By blocking the enzyme fungal squalene monooxygenase, which is a step in the creation of the fungal cell wall, Terbinafine prevents the production of ergosterol. The chemical structure of Terbinafine is given in Figure 1.

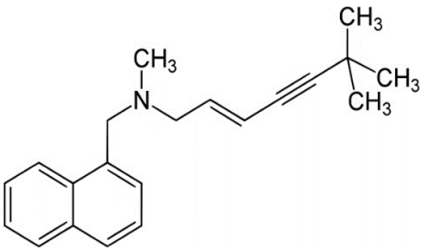

Figure 1: Chemical Structure of Terbinafine

2. MATERIALS AND METHODS

2.1 Chemicals and Solvents

Terbinafine (TBF) was obtained as a gift sample from GSK. Other ingredients used were ethyl cellulose (Fisher Scientific India Pvt. Ltd.), Polyvinyl alcohol, Dichloromethane, Triethanolamine, Propylene glycol and Carbopol 934. All other ingredients used were of analytical grade.

2.2 Development of TBF loaded NS

Different amount of ethyl cellulose and polyvinyl alcohol were used to create terbinafine nanosponges using the emulsion solvent diffusion process. Two phases, the continuous phase and the scatter phase, were employed in this procedure. The dispersion phase contains 20 mL of dichloromethane, 250 mg of terbinafine, and the amount of ethylcellulose indicated (Table 1) in the disperse phase. The continuous phase then contained 100 mL of water and a specific volume of polyvinyl alcohol (Table 1). At 35°C, the aqueous continuous phase was gently mixed with the organic dispersed phase while being agitated at 1000 rpm for three hours using a magnetic stirrer. To guarantee that all remaining solvent was removed, the produced Vacuum filtration was used to extract terbinafine nanosponges, which were subsequently dried at 40°C for 24 hours before being stored in dessicators.[8] The Schematic Presentation of Method of Preparation of Nanosponge is shown in Figure 2.

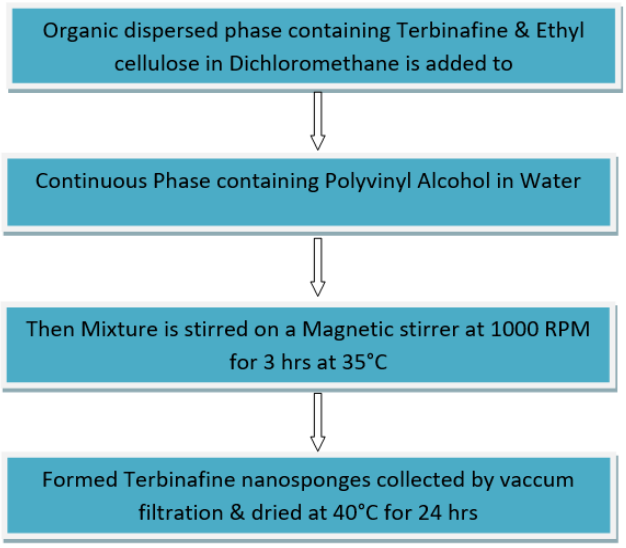

Figure 2: Schematic Presentation of Method of Preparation of Nanosponge

Table 1: Composition of Different Nanosponge Formulations

Formulation code	TNS1	TNS2	TNS3	TNS4
Terbinafine (mg)	250	250	250	250
Polyvinyl alcohol (mg)	200	300	200	300
Ethyl cellulose (mg)	200	200	300	300
Dichloromethane (mL)	20	20	20	20
Distilled water (mL)	q.s	q.s	q.s	q.s

2.3 Entrapment Efficiency & Drug Loading

The quantity of entrapped Terbinafine in the nanosponges was calculated in order to estimate the entrapment capacity and drug loading of the nanosponges. Calculated amounts of nanosponges were added to 10 ml of methanol in order to assess the Terbinafine entrapment efficiency and drug loading. The dispersion was centrifuged at 15000 rpm for 15 minutes. After centrifugation, the supernatant was collected, and the amount of free terbinafine with respect to the percentage of drug entrapment was calculated spectrophotometrically (max = 223 nm). The entrapment efficiency & drug Loading has been determined according to the following equation:

$$\%EE = \frac{(Initial\ amount\ of\ drug\ added - drug\ amount\ in\ supernatant)}{Initial\ amount\ of\ Drug\ added} \times 100$$

$$\%DL = \frac{(Initial\ amount\ of\ drug\ added - drug\ amount\ in\ supernatant)}{Total\ amount\ weight\ of\ nanosponges} \times 100$$

2.4 In-Vitro Drug Release Study

Terbinafine nanosponges were used in an in-vitro release research in a USP paddle equipment (Type II dissolution test apparatus). For the analysis, a sample of a nanosponge formulation containing 250 mg of the medication was

employed. A temperature of 37±0.5°C and a paddle rotation speed of 50 rpm were both maintained. As a dissolving media, 900 mL of phosphate buffer pH 6.8 (the pH of normal skin) were used for a release research. To maintain the sink condition, samples (5 mL) were removed from the dissolution apparatus at intervals of 30, 60, 120, 180, 240, 300, and 360 minutes and replaced with an equivalent volume of new dissolution media. The withdrawn aliquots underwent filtering and 223nm analysis. Calculated and compared with pure drug release was the cumulative proportion of drug released from nanosponges. Drug percentage cumulative analysis was done in triplicate.[9, 10]

2.5 Particle Size and Zeta Potential Determinations

Dynamic light scattering (DLS) was used to analyze the particle size and polydispersibility index of nanosponges at 250°C and a scattering angle of 90. Prior to testing, the terbinafine nanosponge sample was diluted in distilled water.[11]

2.6 Study of nanosponge morphology by scanning electron microscopy:

The surface morphology of the selected formula of TBFNS-2 was examined by SEM. In this investigation, prepared nanosponges were coated with gold-palladium at room temperature while being kept in an argon environment. After being randomly scanned, photomicrographs were acquired at a 20 kV acceleration voltage. The average particle size was computed from the generated image.

2.7 Preparation of Transdermal gel of optimized NS (TNS2)

Based on the size, PDI, ZP, EE and DL evaluation, TNS2 was optimized and selected to incorporate into carbopol gel. By mixing 1% of carbopol 934 with 10 ml of distilled water for 24 hours to allow the polymer to completely swell, nanosponge gels were created. Terbinafine nanosponges were evenly distributed throughout the weighed amount of carbopol gel basis. Propylene glycol was then used to improve penetration. To change the pH to 6.8, triethanolamine was added drop wise while being gently stirred in a homogenizer. The Terbinafine nanosponges sans penetration enhancer gel and the drug-loaded control gel were also made in the same manner.[12]

2.8 Characterization of Gel

Based on the physical and physiochemical characteristics TNS2 was selected and incorporated into the polymeric gel. The developed gel was further evaluated.

2.8.1 Visual Appearance

The color, texture, and appearance of the nanosponges produced gel formulation were examined visually.

2.8.2 pH Determination

Using a digital pH meter, the pH of the produced gel formulation was determined. In 100 ml of distilled water, one gram of gel was dissolved before being let to stand for two hours. It was done to measure the formulation's pH.[13]

2.8.3 Viscosity Determination

The viscosity of Nanosponge Gel was estimated to determine the mechanical properties of gel. Viscosity was analyzed by means of Brookfield viscometer (Brookfield Engineering, Middleboro, MA) by using spindle-C, S-96. A T-bar spindle was dropped perpendicularly through gel that had been placed in a beaker, being careful not to let the spindle hit the beaker's bottom. After 30 seconds, when the gel level settled, the values were taken while the spindle was rotating at a speed of 50 rpm. [14]

2.8.4 Drug Content Estimation

The Terbinfine Nanosponges gel samples were weighed out and then dissolved in methanol using ultrasonication for one hour at 30°C. The samples were filtered using a 0.2um membrane filter, and a UV double-beam spectrophotometer was used to detect absorbance at 223nm.[15]

2.8.5 *In-Vitro Permeation Studies*

Utilizing a fake dialysis membrane, in vitro release tests were carried out. A Franz diffusion cell and a reservoir were employed in this experiment. The artificial membrane was firmly positioned in the gap between the diffusion cell's two sides. The receptor compartment contained phosphate buffer (pH 6.8) and was constantly stirred at 100 rpm with a magnetic stirrer at a temperature of 37±0.5ºC. One gram of gel formulation (control gel, terbinafine nanosponge gel, and terbinafine nanosponge gel including propylene glycol) was placed inside the donor cell. At certain intervals, 1ml of the samples in total were taken out of the receptor compartment and replaced with an equivalent volume of brand-new receptor fluid. The aliquots were appropriately diluted with receptor media before being examined with a UV spectrophotometer. The means of the measurements, which were carried out in triplicate, were reported.[15]

2.8.5 In-Vitro Drug Release Kinetic

The release profile is described by many mathematical functions that form the foundation of model dependent approaches. Once a suitable function has been selected, the model parameters that were acquired are used to evaluate the release profiles. The following models of data treatment were used to visualize the data from ex vivo permeation studies:

a) Zero Order Kinetics Model
b) First Order Kinetics Model
c) Higuchi's Model
d) Korsmeyer-Peppas Model

3. RESULTS AND DISCUSSION

3.1 Entrapment Efficiency & Drug Loading

Percentage Drug Entrapment of developed NS

formulations (TNS1-TNS4) was found to be in range of (71.039±0.124% to 83.226±0.258%), the results are given in Table 2. Amount of the drug entrapped was found to be increased with the higher particle size and EC polymer concentration. In contrast, less particle size would be responsible for low entrapment efficiency, as the small particles could have more surface area to give more prospect for drug escape from the porous nanocage.[16] Terbinafine loading in NSs was found to be in range of (23.680±0.041% to 27.742±0.086%). TNS2 showed the maximum drug loading. The results of Drug Loading are given in Table 2.

Table 2: Percentage Entrapment Efficiency & Drug Loading of Nanosponge Formulations Containing Terbinafine

Sr. No.	Formulation Code	Entrapment efficiency (%)	Drug Loading (%)
1	TNS1	71.039±0.124	23.680±0.041
2	TNS2	83.226±0.258	27.742±0.086
3	TNS3	78.160±0.180	26.053±0.060
4	TNS4	73.620±0.072	24.540±0.024

3.2 Particle Size and Zeta Potential Determinations:

Selected NS TNS2 showed PS (258.4 ± 0.57 nm), PDI (0.268 ± 0.02) & ZP (-15.10mV ± 0.91 mV) respectively. The data of Zeta potential and PDI exhibits that the TNS2 were negatively charged with sufficient inter-particle repulsive force with the narrow size distribution as the PDI value was found to be less than 0.7.

3.3 In-Vitro Drug Release Study

Utilizing a USP paddle apparatus, in vitro release tests were performed. The phosphate buffer had a pH of 6.8. The TBF2 formulation had the largest drug release of any of the formulations tested.

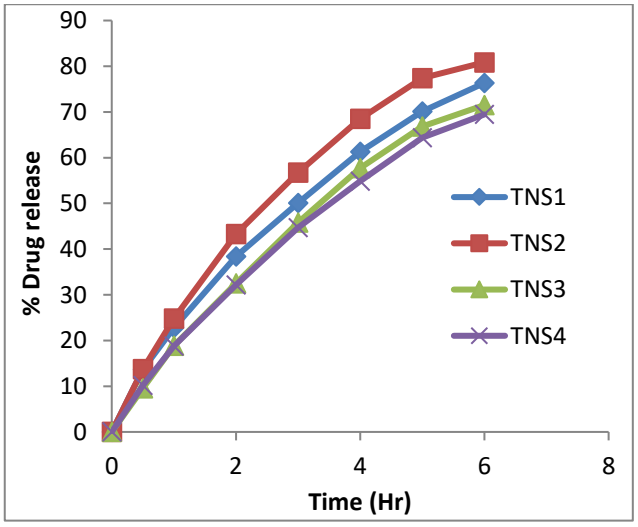

Figure 3: % Drug release of Nanosponges Formulations

At the end of the sixth hour, the TBF2 formulation displayed a drug release of 80.860±0.986%. The data from the in vitro release were displayed in Figure 3. Based on physical and chemical characteristics and medication release in vitro, TBF2 was selected as an optimized formulation for incorporation into topical gel.

3.4 Scanning electron microscopy:

SEM indicated uniform and spherical shape, discrete particles without aggregation and appear to be smooth in surface morphology with nano-size range. The nanosponges are spherical, uniform, sponge like structures are shown in the Figure 4a and Figure 4b.

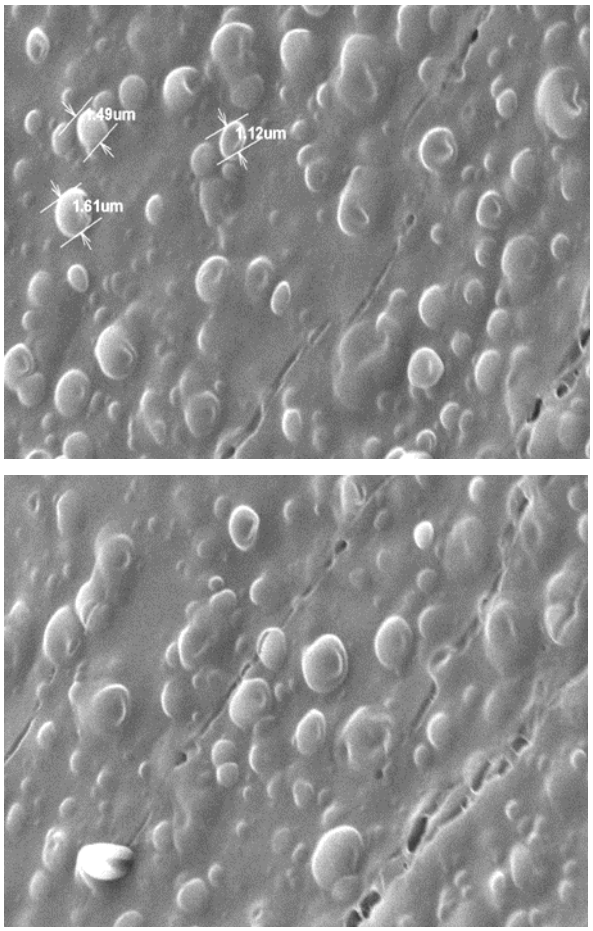

Figure 4 a) & 4 b): SEM of Nanosponge Formulation TBF2

3.5 Evaluation of topical gel impregnated with TNS2 nanosponges

3.5.1 PH, Viscosity & Drug content estimation:

The optimized TBF loaded NS based topical gel showed a PH 6.839±0.008, meet the benchmark PH for skin application. Drug content estimation TBF was found to be 97.491±0.621. Viscosity of TBF NS loaded gel was 4352±20.84cps.

3.5.2 Drug diffusion and release kinetics:

The fabricated TNS2 subjected to in vitro drug release showed an initial burst release followed by sustained drug release. The porous matrix formed by EC was conferred for sustained and progressive release of TBF. Slow diffusion of water inside the hydrophobic EC lead to a release of drug for prolonged period. The cumulative % drug data of the TNS2 was fixed in four mathematical release kinetics

models and regression analysis was executed. The results revealed that release fitted into higuchi diffusion kinetic model based on the coefficient of correlation (R^2 values 0.992), Korsmeyer-Peppas model with a diffusion exponent (R^2 values 0.969) represents anomalous non-Fickian release kinetics.

CONCLUSION

The nano based gel formulation is ideal for the effective treatment of fungal infections, since the nanocarrier could permeate the drug deeper into skin layer where other topical semisolid preparations may not reach. Nanocarriers improve therapeutic effect by channelizing drug into target site deeper into skin layers for complete eradicated of fungal infections. The developed TBF loaded NS impregnated carbopol polymeric gel could be efficient drug delivery system of antifungal agent for the effective treatment of fungal infections by sustaining the drug release, thereby reducing the dosing frequency.

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