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NANOSPONGES AS NOVEL CARRIER FOR TOPICAL DELIVERY OF LULICONAZOLE -AN ANTIFUNGAL DRUG

B. S. Wakure *, M. A. Salunke, P. T. Mane, S. R. Awale, R. D. Shinde and S. S. Eklinge

Vilasrao Deshmukh Foundation, Group of Institutions, VDF School of Pharmacy, Latur - 413531, Maharashtra, India.

Keywords:

Nanosponges; Luliconazole, Antifungal activity, Topical nanosponge gels

Correspondence to Author:
Dr. B. S. Wakure

Principal,
Vilasrao Deshmukh Foundation, Group of Institutions, VDF School of Pharmacy, Latur - 413531, Maharashtra, India.

E-mail: balaji.wakure@gmail.com

ABSTRACT: The main goal of this work is to formulate nanosponges and access its suitability to deliver Luliconazole for topical application, to improve its therapeutic effect, better dispersion and good storage. Nanosponges are water-soluble, which does not mean that molecules are chemically decomposed in water but that nanosponge particles are mixed with water and used as a carrier fluid. Compared with traditional drug delivery methods, nanosponges have several advantages. Using polyvinyl alcohol as a surfactant, a nanosponge with ethyl cellulose as a polymer was prepared by the emulsion solvent diffusion method. An optimized batch of nanosponges with high entrapment efficiency was used to formulate the gel with Carbopol 940. The prepared gel was evaluated for pH value, viscosity, spreadability, in vitro diffusion study, skin irritation test and *in-vitro* antifungal activity. The nanosponge system is non-toxic, non-irritating, non-allergenic, and non-mutagenic. Nanosponge gel as a local drug delivery system has a huge potential, with better antifungal activity and stability. These small sponges loaded with luliconazole proved to be better scaffold for topical application.

INTRODUCTION: The development of a wide range of nanotechnology has begun to change the basis of disease diagnosis, treatment and prevention. Various nano-devices had a significant impact on medical technology, greatly improving the efficacy of many existing drugs and enabling the construction of brand-new treatment methods. Nanosponge is a new type of material with a cavity of a few nanometers in size, in which various substances can be encapsulated [1]. These particles can carry lipophilic and hydrophilic substances and increase the solubility of poorly water-soluble molecules [2]. Nanosponge is a virus sized, naturally degradable scaffold like structure.

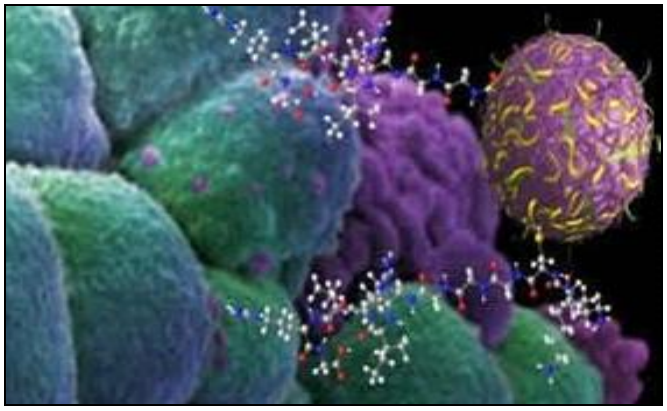

FIG. 1: AN ARTIST'S PERCEPTION OF A NANOSPONGE TARGETING A BREAST CANCER CELL (PEPTIDE LINKERS ARE SHOWN AS BALL AND STICK MODEL) (CREDIT: HARTH LABORATORY)

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The long polymer strands are mixed in solution with small molecules called cross-linking, which have affinity for certain parts of polymer [3]. They have completely changed the treatment of various diseases and early trials have shown that this technology is five times more effective than traditional methods in targeting drugs to breast cancer cells [4]. The artistic perception of nanosponges is shown in **Fig. 1.**

Drug Release Mechanism of Nanosponges: Sponge atoms have an open arrangement and active materials can freely enter and exit the particles and enter the carrier until equilibrium is reached. In the case of topical administration, once the finished product is applied to the target tissue, the active substance already in the carrier will be absorbed into into it, depleting the carrier that will become unsaturated, thus disturbing the balance. This will allow the active substance to flow from the sponge particles into the carrier and from it to the target tissue, until the carrier dries or is absorbed. Even after that, the sponge particles remaining on the surface of the tissue will continue to gradually release the active substance to the tissue, providing a prolonged release over time [3].

Chemicals Used for the Synthesis of Nano-sponges:

Polymer: The type of polymer used will affect the formation and performance of the nanosponge. For complexation, the cavity size of the nanosponge should be suitable for accommodating drug molecules of a specific size. The ability of the polymer to crosslink depends on functional groups and reactive groups to be substituted. The choice of the polymer depends on the desired release and the drug to be encapsulated [5]. Ex- Hyper crosslinked polystyrenes, cyclodextrin and its derivatives like Alkyloxy carbonyl cyclodextrin, Methyl β-Cyclodextrin, 2-Hydroxy Propyl β-Cyclodextrins.

Co-polymers: Poly (Valerol-actone-allylvalerol-actone), Poly (Valerolactone-allylvalerolactone oxepanedione), ethylcellulose, polyvinyl alcohol.

Cross-linkers: The choice of crosslinking agent depends on the structure of the polymer and the drug to be formulated. Ex: Carbonyl diimidazoles, Carboxylic acid dianhydrides, Diphenyl Carbonate, Diaryl carbonates, Diisocyanates, Pyromellitic anhydride, Carbonyl diimidazoles, Epichloridrine, Glutaraldehyde, 2, 2-bis (acrylamido) Acetic acid, and dichloromethane.

Drug Molecule Compatibility Properties:

- The drug molecules formulated into nanosponges should have certain characteristics.
- The molecular weight is between 100 and 400 Daltons.
- The drug molecule consists of less than 5 fused rings.
- The solubility in water is less than 10 mg/ml.
- The melting point of the substance is below 250 °C [3].

Preparation of Nanosponges:

Solvent Method: The polymer is mixed with a suitable solvent, especially in polar aprotic solvents such as dimethylformamide, dimethyl sulfoxide. This mixture is then added to an excess of cross-linkers, preferably with a crosslinker/polymer molar ratio of 1:4. The reaction is carried out at a temperature from 100 °C to the reflux temperature of the solvent and the time is 1 to 48 h. The preferred cross-linking agents are carbonyl compounds (dimethyl carbonate and carbonyl diimidazole). After the reaction is complete, let the solution cool at room temperature, then add the product to a large amount of excess double-distilled water, vacuum filter to recover the product, and then extend the Soxhlet purification [2].

Emulsion Solvent Diffusion Method: Nano-sponges can be prepared using ethyl cellulose and polyvinyl alcohol in different concentrations. Different ratios of drug to the polymer are used to improve drug loading and obtain tailored release. The dispersed phase containing the drug and polymer dissolved in 20 ml of dichloromethane is slowly added to a certain amount of polyvinyl alcohol in 100 ml of aqueous external phase, and a magnetic or mechanical stirrer is used at a stirring speed of 1000-1500 rpm for 3-5 h. The formed nanosponges are collected by filtration and dried in an oven at 40 °C for 24 h and packaged in a container [3].

Ultrasound-assisted Synthesis: In this method, the polymer reacts with the cross-linking agent under solvent-free and ultrasonic treatment. Here, the polymer and cross-linking agents are mixed in the flask. Place the flask in an ultrasonic bath filled with water, heat it to 90 ºC and sonicate it for 5 h. Let it cool and wash with water to remove unreacted polymer. Purification was performed by prolonged Soxhlet extraction with ethanol. Dry the product under vacuum and store at 25 ºC [6].

Quasi-Emulsion Solvent Diffusion: Nanosponges can also be prepared by the quasi-emulsion solvent diffusion method using different polymer amounts. To prepare the internal phase, eudragit RS100 is dissolved in a suitable solvent. Then, the drug can be added to the solution and dissolved under ultrasound at 35 °C. Pour the inner phase into the PVA aqueous solution (outer phase) and stir for 1 h and then filter the mixture to separate the nanosponges. The nanosponge is dried in a 40 ºC air heating oven for 12 h [7].

From Hyper Cross-Linked B-Cyclodextrin: Here, β-cyclodextrin can be used as a vehicle for drug delivery. Nanosponges can be obtained by reacting cyclodextrin with a crosslinking agent. Due to the formation of this 3D network, it can be a roughly spherical structure approximately the size of a protein, with channels and pores inside. Cyclodextrin reacts with crosslinking agents such as diisocyanate, dicarbonate, *etc.* The size of the sponge is controlled according to porosity and surface charge density to connect different molecules. Nanosponges can be synthesized in neutral or acidic form. The average diameter of the nanosponge is less than 1µm, but fractions of size less than 500 nm can be selected. They are used to increase the water solubility of poorly water-soluble drugs. They are composed of solid particles and transformed into crystalline form [8].

Polymerization: A non-polar drug solution is prepared in the monomer and an aqueous phase, which usually contains a surfactant and a dispersing agent, is added to it to promote suspension.

Once the monomer is activated by catalysis or elevated temperature to establish a suspension of discrete droplets of the desired size.

The polymerization process leads to formation of a reservoir-type system that opens on the surface through pores [3].

Loading of drug into Nanosponges: Nanosponges used for drug delivery should be pre-treated to obtain an average particle size below 500 nm. The nanosponge is suspended in water and sonicated to avoid aggregates and then the suspension is centrifuged to obtain a colloidal part. The supernatant is separated and the sample is dried by freeze-drying. Prepare an aqueous suspension of nanosponges, disperse the excess drug, and keep the suspension compounded for a specific time under continuous stirring. After compounding, the undissolved drug is separated from the complexed drug by centrifugation. Then, solid crystals of nanosponges are obtained by solvent evaporation or freeze-drying.

Factors Influencing the Formation of Nanosponge [3, 8]:

Polymer and Cross-linkers: The type of polymer used will affect the formulation and performance of the nanosponge. A high-efficiency efficiency cross-linking transforms the molecular nanocavity into a 3-dimensional nanoporous structure.

Hydrophilic Nanosponges: They are formed by using epichlorohydrin as a cross-linking agent. Hydrophilic nanosponges can change the drug release rate and enhance the absorption of drugs across biological barriers, and can also be used as effective drug carriers even in immediate-release formulations.

The Hydrophobic Nanosponge: They can be synthesized using diphenyl carbonate, pyromellitic anhydride, diisocyanate and carbonyl diimidazole as a cross-linking agent. They are used as sustained-release carriers for water-soluble drugs (including peptide and protein drugs).

Complexation Temperature: The stability constant of the composite depends on temperature changes. The stability constant and temperature rise are negatively correlated. At elevated temperatures, the apparent stability constant decreases due to the decrease of the drug/nanosponge interaction force. Therefore, thorough temperature control should be maintained when preparing nanosponges.

Degree of Substitution: The number, type and position of the substituents on the polymer molecule affect the complexing ability of the nanosponge. The type of substitution is important because β-CD derivatives can be obtained in various forms, which have different functional groups on the surface of cyclodextrin derivatives. When combined with the help of cross-linking, different functional groups will produce different types of composite materials (β-CD nanosponges, CD- carbamate nanosponges, CD-carbonate nanosponges Etc.). The higher the number of substitutions and the degree of cross-linking the more the number of substituents, the greater the possibility of higher cross-linking.

Due to the greater interconnection between the polymers forming the network, a higher cross-linking, will produce highly porous nanosponges. The position of substitution depends on the production conditions. Since the functional groups on the parent compound occupy some different positions, changes in the production process will produce materials with different physical and chemical properties.

Characterization of Nanosponges:

Particle Size and Polydispersity Index: The particle size can be determined by dynamic light scattering using the 90 Plus particle size analyzer or laser diffractometer or Malvern Zeta particle size analyzer equipped with MAS OPTION particle size analysis software. From this, the average diameter and polydispersity index can be determined [9] and value of polydispersity index is given in **Table 1.**

TABLE 1: POLYDISPERSITY INDEX

Polydispersity Index	Type of Dispersion
0-0.05	Monodispersed standard
0.05-0.08	Nearly monodisperse
0.08-0.7	Midrange polydispersity
>0.7	Very polydisperse

Resiliency: Depending on the needs of the final formulation, the resilience (viscoelasticity) of the sponge can be modified to produce softer or stronger beads. Increased cross-linking tends to slow down the release rate. Therefore, by considering the release as a function of cross-linking over time, the elasticity of the sponge will be studied and optimized according to requirements [9].

X-ray Diffraction and Single Crystal X-ray Structure -Analysis: X-ray diffraction can be used to detect inclusion complexes in the solid-state. When the drug molecule is liquid, it does not show its own diffraction, so the diffraction pattern of the newly formed substance is obviously different from that of the uncomplexed nanosponge. The difference in the diffraction patterns indicates the formation of clathrates. When the drug substance is solid in nature, a comparison must be made between the diffractogram of the hypothetical complex and the diffractogram of the mixture of drug and polymer molecules. The formation of the inclusion compound between the drug and the nanosponge changes the diffraction pattern and changes the drug's crystalline nature. The sharpening of existing peaks and the appearance of a few new peaks lead to the formation of clathrates [5, 10].

Microscopy Studies: Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) can be used to study the microscopic aspects of drugs, nanosponges, and products (drug/nanosponge composites). The difference in the crystalline state of the raw materials and products observed under the electron microscope indicates the formation of clathrates (inclusion complexes) [6].

Drug Release Kinetics: In order to study the mechanism of drug release, we analyzed the drug release data using zero-order, first-order, and Higuchi Kosemeyer-Peppas, Hixon Crowell, Kopcha and Makoid-banaker models. The data can be analyzed using graph pad prism software. The software estimates the parameters of non-linear functions and provides a close fit between experimental observations and non-linear functions [6].

Thermoanalytical Methods: The thermal analysis method determines whether the API has undergone some changes before the nanosponge is thermally degraded. The change of the drug substance may be melting, evaporation, decomposition, oxidation or polymorphic transformation. Changes in the drug substance indicate the formation of complexes. The thermogram obtained by differential thermal analysis and differential scanning calorimetry can be observed for the broadening, movement and

appearance of new peaks or the disappearance of some peaks. Changes in weight loss can also provide supporting evidence for the formation of inclusion complexes [11].

Infrared Spectroscopy: The interaction between nanosponges and solid drug molecules can be detected by infrared spectroscopy. After the composite is formed, the nanosponge band changes. If the fraction of guest molecules wrapped in the complex is less than 25% of the band, it can be allocated to the included part of the guest molecule, which is easily concealed by the spectral band of the nanosponge. Infrared spectroscopy is suitable for drugs with characteristic bands such as carbonyl or sulfonyl. This spectroscopic study revealed information about the participation of hydrogen in various functional groups. This usually shifts the absorption band to a lower frequency, increases the strength, and widens the absorption band caused by the stretching vibration of the groups participating in the formation of hydrogen bonds. The hydrogen bond at the hydroxyl group causes the maximum displacement of the stretching vibration band [11].

Thin Layer Chromatography: In thin-layer chromatography, the R_f value of the drug molecule is reduced to a considerable extent, which helps to determine the complex formation between the drug and the nanosponge.

Loading Efficiency and Production Yield: The loading efficiency (%) of the nanosponge can be calculated according to the following formula.

$$\text{Loading efficiency} = \text{Actual drug contents in NS}/\text{Theoretical drug content} \times 100$$

After determining the accurate initial weight of the raw materials and the final weight of the resulting nanosponge, the production yield of the nanosponge can be calculated by the following formula.

$$\text{Production yield} = \text{Practical mass of NS}/\text{Theoretical mass (Polymer + drug)} \times 100$$

Solubility Studies: Higuchi and Connors describe a method for studying inclusion complexation, that is, a phase solubility method for detecting the solubility of drugs in nanosponges.

The phase solubility diagram indicates the degree of complexation. An Erlenmeyer flask is used in this method. Drugs containing different percentages of nanosponge aqueous solution were added to the flask.

The Erlenmeyer flask was stirred on a mechanical shaker at room temperature until a steady state was reached, and the suspension was filtered by centrifugation using a 3000 Dalton molecular filter (MICRON YN 30, Millipore Corporation, Bed ford MA 1730 USA). The solution was analyzed and the drug concentration is determined by high-performance liquid chromatography [11].

Zeta Potential: Zeta potential is used to measure surface charge by using additional electrodes in the particle size measuring device. In this process, take out the sample containing the nanosponge, dilute it with 0.1 mol/L KCl and put it in the electrophoresis cell and apply a 15V/cm electric field. Thus, the average hydrodynamic diameter and polydispersity index are determined after averaging the total measured values [11].

Dissolution Test: The dissolution profile of nanosponges can be studied using the dissolution device USP XXIII, which has a modified basket composed of 5m stainless steel mesh and a rotation speed of 150 rpm. When selecting the dissolution medium, consider the solubility of the active substance to ensure sink conditions. The sample from the dissolution medium can be analyzed by a suitable analytical method.

Applications of Nanosponges:

Solubility Enhancement: Nanosponges can improve the wettability and solubility of molecules with poor solubility in water. The drug can be molecularly dispersed in the nanosponge structure and then released as a molecule, avoiding the dissolution step.

Therefore, the apparent solubility of the drug can be increased. Many formulations and bio-availability problems can be solved by improving the solubility and dissolution of substances and nanosponges can greatly improve the solubility of drugs [9]. **Table 2** provides BCS class II drugs with very low solubility, which are ideal candidates for nanosponges

TABLE 2: BIOPHARMACEUTICAL CLASSIFICATION SYSTEM CLASS II DRUGS

S. no.	Category	Drugs
1	Antihypertensive	Felodipine, Nicardipine, Nifedipine, Nisoldipine
2	Antibiotics	Azithromycin, Ciprofloxacin, Erythromycin, Ofloxacin,
3	Antiarrhythmic agents	Amiodaronehydro chloride
4	Antifungal agents	Econazolenitrate, Griseofulvin, Itraconazole, Ketoconazole
5	Antidiabetic and Antihyperlipidemic	Atorvastatin, Fenofibrate, Glibenclamide, Glipizide, Lovastatin, Troglitazone
6	NSAIDs	Dapsone, Diclofenac, Diflunisal, Etodolac, Etoricoxib, Flurbiprofen, Ibuprofen, Indomethacin, Ketoprofen, Mefenamicacid, Naproxen, Nimesulide, Oxaprozin, Piroxicam
7	Cardiac drugs	Carvedilol, Digoxin, Talinolol
8	Anticoagulant	Warfarin
9	Anticonvulsants	Carbamazepine, Clonazepam, Felbamate, Oxycarbazepine, Primidone.
10	Antipsychotic drugs	Chlorpromazine Hydrochloride Antiretrovirals Indinavir, Nelfinavir, Ritonavir, Saquinavir
11	Antianxiety drugs	Lorazepam
12	Antiepileptic drugs and Steroids	Phenytoin, Danazol, Dexamethasone
13	Immuno suppressants	Cyclosporine, Sirolimus, Tacrolimus
14	Antiulcer drugs	Lansoprazole, Omeprazole
15	Antioxidants	Resveratrol
16	Diuretics	Chlorthalidone, Spironolactone
17	Antineoplastic agents	Camptothecin, Docetaxel, Etoposide, Exemestane, Flutamide, Irinotecan, Paclitaxel, Raloxifene, Tamoxifen, Temozolomide
18	Miscellaneous	Atovaquone, Melarsoprol, Phenazopyridine, Ziprasidone

Nanosponges for Drug Delivery: Nanosponges are solid in nature and can be formulated into oral, parenteral, topical or inhalation dosage forms.

For oral administration, the complex can be dispersed in a matrix suitable for the preparation of capsules or tablets with excipients, diluents, lubricants, and anti-caking agents. For parenteral administration, the complex can simply be carried in sterile water, saline or other aqueous solutions. For topical administration, they can be effectively incorporated into topical hydrogels [12].

Topical Agents: The nanosponge delivery system is a unique technology used to control the release of topical drugs that extend drug release and retention of drug form on the skin.

Local anesthetics, antifungals, and antibiotics belong to the category of drugs that can be easily formulated into topical nanosponges. When the active ingredient penetrates into the skin, a rash or more serious side effects may occur.

In contrast, this technology allows for a uniform and sustained release rate, reducing irritation while maintaining efficiency. A variety of substances can be incorporated into the formulated product, such as gels, lotions, creams, ointments, liquids, or powders [10].

Nanosponges Serve as the Carrier of Biocatalysts and the Delivery and Release of Enzymes, Proteins, Vaccines and Antibodies: Many systems for carrying enzymes and proteins have been developed, such as nanoparticles and microparticles, liposomes and hydrogels. The carrier in a specific system can protect proteins from decomposition, change their pharmacokinetics and improve their *in-vivo* stability. Now, it has been found that nanosponges based on cyclodextrin are particularly suitable for adsorbing proteins, enzymes, antibodies and macromolecules.

Especially when enzymes are used, they can maintain their activity, efficiency, extend their operation and extend the active pH and temperature range and allow continuous flow processes. In addition, proteins and other macromolecules can be carried by adsorbing or encapsulating them in cyclodextrin nanosponges [13].

Nanosponges as a Carrier for Delivery of Gases: Gas plays an important role in medicine, whether it is used for diagnostic or therapeutic purposes. The lack of an adequate supply of oxygen, called hypoxia, is associated with various pathologies, from inflammation to cancer. In clinical practice, it is sometimes difficult to deliver oxygen in an

appropriate form and dose. Cavalli *et al.* develop nanosponge formula as an oxygen delivery system for topical application, with the ability to store and release oxygen slowly over time [11].

Nanosponges AS Protective Agent against Photo Degradation: Sapino *et al.* reported that γ-Oryzanol (a mixture of ferulic acid esters) is an antioxidant, usually used to stabilize food and pharmaceutical raw materials and also used as a sunscreen in the cosmetics industry. Due to its high instability and photodegradability, its application is limited. Nanosponges are prepared by encapsulating γ-Oryzanol and show good protection from photodegradation. The nanosponges loaded with gamma oryzanol can be formulated as gels and O/W emulsions [2].

Removal of Organic Pollutants from Water: β-cyclodextrin nanosponges are completely insoluble in water and have the characteristics of encapsulating organic pollutants in water. Ceramic porous filters can be impregnated with these nanosponges to form organic/inorganic hybrid filter modules. These hybrid filter modules have been tested to purify water, employing a variety of water pollutants effectively. It has been determined that polycyclic aromatic hydrocarbons (PAH) can be removed very effectively (over 95%). It can also remove representatives of pollutant groups such as trihalomethanes (THM), monoaromatic hydro-carbons (BTX) and pesticides (simazine). **Table 3** enlist various research studies done on the formulation of nanosponges of various drugs for the desirable characteristics.

TABLE 3: VARIOUS EXAMPLES OF NANOSPONGES

Drug	Nanosponge Vehicle	Category of drug	Study
Itraconazole	Beta cyclodextrin and copolyvidonum	Antifungal	Solubility
Voriconazole	Ethyl cellulose, Poly methyl methacrylate (PMMA), PluronicF-68.	Antifungal	Drug release
Miconazole Nitrate	Beta cyclodextrin, Di-phenylcarbonate	Antifungal	Drug release
Celecoxib	Beta cyclodextrin, N,N methylene diacylamine	NSAID	Solubility
Erlotinib	Beta cyclodextrin	Tyrosine kinase inhibitor (Anticancer)	Solubility, bioavailability and *In-vitro* cytotoxicity
Econazole Nitrate	Ethyl cellulose, PVA	Antifungal	Irritation study, Adsorption
Isoniazid	Ethyl cellulose, PVA	Anti-tubercular	Drug release
Cephalexin	Ethyl cellulose, PVA	Antibiotic	Drug release and stability
Norfloxacin	Beta cyclodextrin and Diphenyl carbonate	Antibiotic	Bioavailability
L-Dopa	Beta cyclodextrin	Parkinson's Disease	Drug release
Fenofibrate	Maize starch, SDS	Fibrate	Solubility and Bioavailability
Nifedipine	Beta cyclodextrin	Calcium-channel blocker	Solubility
Glipizide	Beta cyclodextrin	Sulfonylurea	Drug release
Ibuprofen	Ethylcellulose and PVA	NSAID	Drug release
Resveratrol	Cyclodextrin	Antioxidant	Stability, cytotoxicity and permeation
Paclitaxel	Beta cyclodextrin	Antineoplastic	Bioavailability
Camptothecin	Beta cyclodextrin	Antineoplastic	Stability and solubility
Tamoxifen	Beta cyclodextrin	Antiestrogen	Solubility
Temozolomide	Poly(valerolactineallylvalerolactone) and poly (Valerolactoneallylvalerolactoneoxepanedione)	Antitumor	Drug release
Dexamethasone	Beta cyclodextrin	Antitumor	Drug release
γ-Oryzanol	Beta cyclodextrin	Anti-oxidant	Stability
Telmisartan	Carbonated cross-linkers	Antihypertensive	Dissolution rate
Lysozyme	Cyclodextrin-based poly (amidoamine)	Enzyme	Solubility and drug release
Nelfinavir Mesylate	Beta cyclodextrin	Antiviral	Solubility and drug release

Formulation of Luliconazole Loaded Nano-sponges: Topical drug administration refers to the application of a drug-containing preparation to the skin or mucosal surface to directly treat skin diseases or the skin manifestations of systemic diseases, with the purpose of limiting the pharmacological or other effects of the drug to the surface of the skin or within the skin [14]. The combination of active ingredients and base provides opportunities for a wide range of topical formulations, such as gels, creams, foams, ointments, lotions, *etc.*, which are suitable for various types of drug delivery and therapeutic terms, and are used to perform topical formulations on the base.

The classification is based on their physical properties or their intended use or their composition, in which therapeutically active ingredients are incorporated. The outcome of topical dermatological drug treatment is significantly affected by choice of carrier or delivery system. Examples of the most common dosage forms for topical dosage forms include solutions, suspensions, emulsions (such as lotions), semi-solids (ointments, creams, pastes, gels), solids such as powders and aerosols, sprays [4, 15]. Porous polymer delivery systems, in which small spherical particles with large porous surfaces are called nanosponges, are used to passively target cosmetics to the skin to avoid systemic absorption. Nanosponges can encapsulate a variety of substances.

They have the ability to solubilize poorly soluble drugs and extend the release of drugs by increasing their bioavailability. Due to the internal hydrophobic cavity and external hydrophilic branches, both hydrophilic and lipophilic drug molecules can be loaded into the nanosponge [16]. Now, targeted drug delivery has always been a long-term problem for medical researchers. How to deliver the drug to the body at the right place at the right time, how to control the release of the drug, its effect on the body, its therapeutic effect, safety, how to control the release to prevent overdose effects. The use of nanotechnology has solved these problems with the aid of nanosponges. This nanosponge can easily target specifically targeted cells or tissues [17]. For feasible and useful treatments, different methods have been used to improve the entry of sedative atoms encapsulated by ineffective solvents. One of the fascinating elements is the possibility of nanosponges. For this reason, the frame must be connected to the commonly used skin carrier. For example, the gel keeps in mind that the ultimate goal is to have proper semi-solid consistency. Therefore, this method was chosen to combine the nanosponge technology with the principle of transdermal drug delivery to improve the systemic and local administration of luliconazole, thereby effectively transferring the drug to the skin [17].

Material: The following materials are used with AR/LR grades or the best grades available, provided by the manufacturer without further purification or investigation. The drug luliconazole used was a gift sample from Mankind Pharma Ltd in Delhi. Ethylcellulose, DMSO Carbopol 940, HMPC, polyvinyl alcohol, sodium alginate, gum arabic, methylparaben, and propylparaben were purchased from Scientific Chemicals, Latur. All reagents and solvents used in the research are of pharmacopeial grade and analytical grade.

Preparation of Ethyl Cellulose Nanosponges: The ethyl cellulose-based nanosponge is prepared by the emulsion solvent diffusion method using polymer and a crosslinking agent. For each ratio, the dispersed phase with polymer, cross-linking agent (PVA) is accurately weighed and dissolved in the solvent DMSO. Finally, the homogenized ethyl cellulose and DMSO are placed in an Erlenmeyer flask. The mixture was added to the water phase and then continuously stirred on a magnetic stirrer at 1000 rpm for three hours. The reaction mixture was cooled, and the required nanosponges were collected through a filtration process, and the nanosponges were purified by acetone and kept in an oven for drying at 40°C. for 12 h. After purification, store the nanosponges at 250°C for further use.

Preparation of Luliconazole-loaded Nanosponges: Luliconazole was loaded into ethyl cellulose nanosponges by solvent evaporation technology. The solvents used are acetone and ethanol. Mix the ethylcellulose nanosponge with a suitable solvent (organic phase) in 100ml. Preferably, the mixture is added to the excess cross-linking agent in a ratio of 4:16. *i.e* Dissolve

4000 mg luliconazole drug in 100 ml solvent. The reaction proceeds at 100 ℃ until the solvent is refluxed for 1-48 h. The solution was cooled at room temperature, and distilled water was added. Filter the mixture. The product was recovered by filtration and dried in an oven at 40 ℃.

Formulation of Topical Gel Containing Luliconazole Loaded Nanosponges: The dried drug-encapsulated nanosponges were collected and transfer the required amount of drug equivalent to the nanosponges, that is, 0.2 gm, into a 250 ml volumetric flask containing 100 ml of ethanol to remove free unencapsulated drugs by dissolving in ethanol. The drug-encapsulated nanosponge is separated from the free drug by membrane filtration. Collect the remaining drug-loaded nanosponges and disperse them in distilled water by ultrasonic treatment to form a nano-suspension. Disperse 250 mg of gelling agent in 5 ml of distilled water and allow for swelling overnight. Use different gelling agents, such as Carbopol940, gum arabic, HPMC, sodium alginate. Stir continuously in a magnetic stirrer for 1-2 h and add the weighed other excipients to the previously soaked Carbopo l940. Triethanolamine was used to adjust the pH. Transfer the gel to a graduated cylinder and makeup to 20 ml with distilled water.

TABLE 4: FORMULA FOR NANOSPONGES

Ingredients	NS1	NS2	NS3	NS4
Ratio	**1:1**	**1:2**	**1:3**	**1:4**
Ethyl cellulose (mg)	60	120	180	240
DMSO (ml)	20	20	20	20
%PVA(in 100 ml)	0.5	0.5	0.5	0.5

TABLE 5: COMPOSITION OF LULICONAZOLE NANOSPONGES GELS CONTAINING VARIOUS POLYMERS

Ingredients	NS1	NS2	NS3	NS4	NS5	NS6
Nanosponges(gm)	0.2	0.2	0.2	0.2	0.2	0.2
Sod.alginate(gm)	1	-	-	-	0.5	-
HPMC K-15(gm)	-	1	-	-	0.5	0.5
Acacia(gm)	-	-	1	-	-	0.5
Carbopol934(gm)	-	-	-	1	-	-
MethylParaben(mg)	0.1	0.1	0.1	0.1	0.1	0.1
PropylParaben(mg)	0.05	0.05	0.05	0.05	0.05	0.05
DMSO(ml)	10	10	10	10	10	10
Water(ml)	20	20	20	20	20	20

Evaluation of Nanosponges:

Physical Appearance: The physical appearance of the prepared nanosponge was observed with naked eyes. A white spongy powder was observed. The spherical appearance of the nanosponge depends on the viscosity of the ethylcellulose solution.

Production Yield: The production yield was determined by the following formula.

Production yield = Practicle mass of nanosponges / Theoretical mass (drug + polymer) × 100

Drug Entrapment Efficiency: Accurately weigh 10 mg of nanosponge and suspend it in 100 ml of phosphate pH 6.8 buffer solution. After that, the solution was filtered through filter paper, an appropriate dilution was made from the filtrate, and the absorbance was measured at 296 nm using a (Shimadzu UV1800) dual-beam UV spectro-photometer. The entrapment efficiency is determined by the following formula,

Entrapment efficiency = (Total drug-free drug)/(Total drug) × 100

Actual Drug Content: An accurately weighed equal amount (10 mg) of the nanosponge containing the drug was continuously stirred for one hour in 100 ml of phosphate buffer pH 7.4 solution. Use an ultraviolet spectrophotometer to analyze further the filtered sample at 296 nm next to the blank. Estimation of drug content for all batches was done using the following expressions:

Actual Drug content (%) = Nact/Nms × 100

Where Nact = actual luliconazole content in the weighed quantity of nanosponges, Nms = weighed quantity of nanosponges and 'N' is the theoretical Luliconazole content in nanosponges.

Swelling and Water uptake: The prepared nanosponge was immersed in an aqueous solvent to determine the swelling and water absorption rate. Swelling index and water uptake were calculated using the equation.

Swelling index = (Mass of Hydrogel after 72 h)/(Initial mass of polymer) × 100

Water uptake = (Mass of Hydrogel after 72 h)/(Initial mass of polymer) × 100

Infrared Spectroscopy: The FTIR spectrum of luliconazole nanosponge formulation batches was recorded in the wavelength range of 4000 to 400 cm^{-1}. The characteristics of IR absorption peaks of luliconazole were studied.

Particle Size: The average particle size analysis was performed with the help of a zeta sizer (Malvern) to evaluate the effect of polymer concentration on particle size.

Evaluation of Topical Nanosponges Loaded Gel: Evaluation parameter for gel is pH, viscosity, drug content, skin irritation test, *in-vitro* dissolution test spreadability.

Physical Evaluation: Homogeneity and clarity are observed. A digital pH meter was used to measure the pH of the formulation. First, adjust the pH of the water to 7 pH and then measure the pH of the prepared gel.

Viscosity: Viscosity of prepared gel was measured using Brook field viscometer (HBDVE) at different RPM and noted.

Drug Content: Dissolve 1gm of gel which was quantity equivalent to dose, that is, 2% of the drug in 100 ml of phosphate buffer $_{P}$H 7.4 and measure the absorbance at λ_{max} 296 nm.

Calculate the drug content based on the slope and intercept obtained from the linear equation, that is, y = mx + C for the pure drug.

Spreadability Test: Weigh 1 gm of gel and place it on a spreadable device with a glass slide on the lower side and another glass slide on the upper side, and the weight is tied to the upper glass slide. After placing the gel on the download slide, place the upper slide on the download slide and calculate the time required to slide the two slides from the gel. And use the formula to calculate the spreadability, (Length of slide = 7.5 cm): S = M*L/TS = spreadability, M = weight tied to upper slide, L = length of glass slide. T=Time taken to separate two slides (sec).

***In-vitro* Drug Release Study:** The *in-vitro* study of the gel was performed on a dialysis membrane. The receptor compartment is filled with phosphate-buffered saline (PBS) of pH 7.4. The cut-out dialysis membrane was used for the study. The entire device was placed on a thermostatic magnetic stirrer, and the temperature was maintained at 37 ℃ during the entire study. The study lasted for 6 h. regular. The sample was taken out and analyzed by spectrophotometry at 296 nm.

Skin Irritation Test: In this study, all experiments were performed with the permission of the Animal Ethics Committee (CPCSEA), and all animal care and handling guidelines were followed. The permission of the ethics committee has been obtained. Perform the skin irritation test according to the procedure. Skin irritation studies on 10 healthy rats. With the help of sharp surgical scissors and chemical depilatory, the hair on the back were physically removed. Then the skin was washed properly one day prior to use. The animals were divided into 2 experimental groups with 5 animals in each group.

Group 1: Control and 0.8% formalin solution, Group 2: Control and medicated gel (with drug). The latter observed for any sensitivity after 24hrs, 48 h, 72 h and the reaction if any graded as: Score Erythema scale: 0 No reaction, 1 Slight, patchy erythema, 2 Slight but confluent or moderate but patchy erythema, 3 Moderate erythema, 4 Severe erythema with or without edema.

Antifungal Activity (Zone of Inhibition): Weigh 16.25 g of Sabouraud's dextrose agar and transfer it to a 500 ml Erlenmeyer flask and 250 ml of purified water, and heat to completely dissolve it. Sterilize in an autoclave at 121 ℃ and 15 pounds of pressure for 15 min about 20 min. Then cool at room temperature, disperse the fungal strain (Candida albicans) in the culture medium, then pour the culture medium into three petri dishes, let it cool for a while at room temperature, until it forms solidification at room temperature and then each petri dish is drilled with a 6 mm sterile steel hole, and the calculated concentration of standard drug (luliconazole), gel preparation (NS1) and placebo gel are placed in the hole and incubated in the petri dish at 37 ℃ for 72 h. Then observe the zone of inhibition and calculate the radius of the zone of inhibition.

Infrared Spectroscopy: The FTIR spectrum of luliconazole nanosponge Formulation batch NS4 was recorded in the wavelength range of 4000 to 400 cm^{-1}. The characteristics of IR absorption peaks of luliconazole were studied.

Stability Study: A stability study of the formulation giving the maximum dissolution rate was conducted to indicate that the optimized batch's visual physical or chemical stability was assessed at 40 ± 20 ºC / 75 ± 5% RH according to ICH guidelines. The optimized batch of nanosponges loaded with luliconazole was packaged in aluminum strips and stored for three months. After 90 days, the physical appearance and drug encapsulation efficiency of the samples were analyzed.

RESULT AND DISCUSSION:

Evaluation of Nanosponges of Luliconazole: The physical appearance of the prepared nanosponge was observed with naked eyes. All the batches observed are spongy white powder. It was observed that the % yield of batches NS1 to NS4 was in a wide range of 67.35 to 75.65%. It can be seen that the NS1 and NS4 batches have good production yields (72.42% and 75.65%). The conclusion is that the ethyl cellulose concentration and cross-linking time affect the yield of nanosponges. Due to changes in polymer concentration, yields may vary. The % Entrapment efficiency of batches NS1 to NS4 is in the range of 64.83 to 79.16%. The highest % entrapment efficiencies shown in batches NS1 and NS4 were 72.40% and 79.16%, respectively. The conclusion is that the concentration of ethyl cellulose increases and then the % entrapment efficiency increases. The prepared nanosponge were immersed in an aqueous solvent to determine the swelling and water absorption rate. Swelling index and water uptake were calculated using the equation. An accurately weighed equal amount (10 mg) of the nanosponge containing the drug was continuously stirred for one hour in 100 ml of phosphate buffer pH 7.4 solution. A (UV1800) UV dual-beam spectrophotometer was used to further analyze the filtered sample at 296 nm next to the blank.

TABLE 6: ACTUAL DRUG CONTENT, PRODUCTION YIELD, ENTRAPMENT EFFICIENCY, WELLING INDEX AND WATER UPTAKE FLUCONAZOLE NANOSPONGES

Formulation Code	Production Yield	Entrapment efficiency	Actual drug content	% Swelling index	Water uptake
NS1	72.42	72.40	17.43	75	96
NS2	67.35	64.83	28.67	80	108.69
NS3	70.99	69.92	48.654	74.35	129.26
NS4	75.65	79.16	35.112	73.91	114.28

Evaluation of Gel: Physical appearance *i.e.*, clarity and homogeneity, was observed visually. Viscosity was measured by HBDVE Brook field viscometer.

TABLE 7: VISCOSITY OF DIFFERENT FORMULATIONS

Formulation Code	Torque	RPM	Viscosity
	97.50	60	22014
NS1	99.2	100	12154
	94	150	10052
	96	200	11026
	94	60	19672
	95	100	17653
NS2	95	150	15638
	88	200	12656
	94	60	11754
	91	100	13620
NS3	91	150	14854
	88	200	12058
	98	60	18254
	94	100	16185
NS4	91	150	14210
	88	200	12052
	97	60	11495

	96	100	13072
NS5	92	150	13668
	88	200	12114
	96	60	14192
	92	100	15415
NS6	91	150	14543
	88	200	12252

TABLE 8: CLARITY AND HOMOGENICITY OF THE FORMULATIONS

Formulation code	Clarity	Homogenicity
NS1	+	Good
NS2	++	Good
NS3	++	Good
NS4	+++	Good
NS5	+++	Good
NS6	++	Good

+turbid, ++clear, +++veryclear

TABLE 9: ACTUAL DRUG CONTENT, SPREADABILITY AND PH

Formulation code	Actual drug content	Spreadability	pH
NS1	88.32	5.922	7.0
NS2	89.26	6.128	7.1
NS3	74.43	6.266	7.3
NS4	94.58	6.368	7.2
NS5	90.65	6.185	7.0
NS6	91.46	6.230	6.9

A diffusion rate study was performed to evaluate the diffusion characteristics of luliconazole from the prepared nanosponge hydrogel. **Table 10** shows the release profiles of all batches. Diffusion studies of all formulations showed that the drug release percentage was found to be between 57.21 and 85.65% in a given time period. According to the data, NS4 shows the highest drug release compared to any other batches and NS1 batch shows the lowest drug release, so NS4 is considered to be the best formulation based on its kinetic release characteristics because the polymer uses ethylcellulose. During the diffusion experiment, maintaining the temperature and stirring speed is important for consistent and accurate measurement of the diffusion rate. This can be maintained throughout the diffusion study, and the solubility of the drug will not be a deciding factor leading to the delay of drug release in the formulation study.

TABLE 10: % DRUG RELEASE OF VARIOUS FORMULATIONS

Time (h)	Formulation code					
	NS1	NS2	NS3	NS4	NS5	NS6
1	4.87±0.01	4.62±0.02	4.72±0.01	7.43±0.021	5.76±0.02	5.94±0.01
2	5.26±0.03	6.43±0.01	6.88±0.02	11.24±0.04	7.85±0.03	8.90±0.02
3	15.48±0.01	11.02±0.02	14.68±0.02	22.96±0.01	16.84±0.01	15.43±0.03
4	18.42±0.03	17.68±0.01	25.44±0.02	32.14±0.04	28.65±0.01	28.42±0.02
5	24.54±0.02	22.88±0.02	28.68±0.03	38.24±0.02	36.06±0.02	32.32±0.01
6	32.11±0.01	29.18±0.03	34.36±0.02	51.02±0.01	40.39±0.01	44.17±0.03
7	41.12±0.03	34.84±0.02	42.12±0.03	59.42±0.02	51.02±0.01	55.12±0.01
8	44.20±0.01	41.21±0.01	44.42±0.02	66.42±0.04	54.66±0.03	57.94±0.01
9	51.34±0.02	48.44±0.04	51.27±0.01	69.76±0.01	61.54±0.03	62.92±0.01
10	52.12±0.01	54.14±0.03	53.16±0.03	77.21±0.02	65.63±0.01	67.1±0.01
11	55.24±0.01	56.64±0.03	58.94±0.01	82.75±0.01	67.90±0.04	71.33±0.02
12	57.21±0.02	61.62±0.01	62.07±0.02	85.65±0.02	68.96±0.01	75.76±0.03

TABLE 11: SKINIRRITATIO NTEST

Formulation	Presence of edema 24 h.	Presence of edema 48 h.	Presence of edema 72 h
Control	0	0	0
Aqueous formalin solution	2	3	3
Medicated Gel	0	0	0

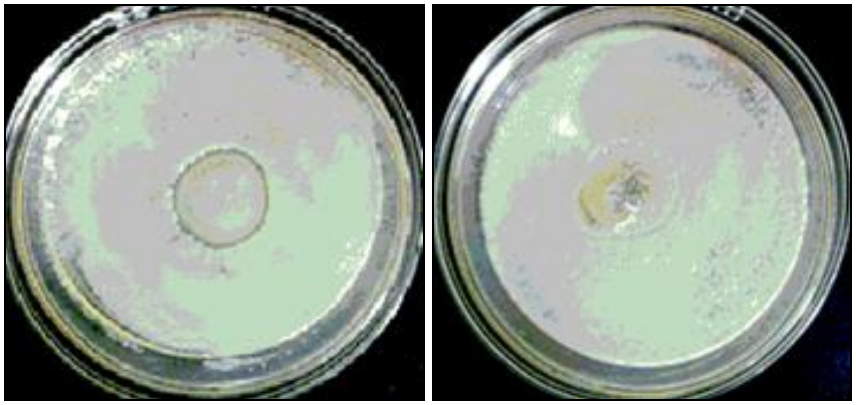
Skin Irritation Test: It was performed by using *in-vitro* skin irritation test method. The formulation did not show any signs of skin irritation, such as redness or any change in the skin. Therefore, it can be concluded that the formulation has no possibility of skin irritation and is safe for topical application.

Zone of Inhibition: In this study, the fungus used was *Candida albicans*. The optimized batch NS4 was studied.

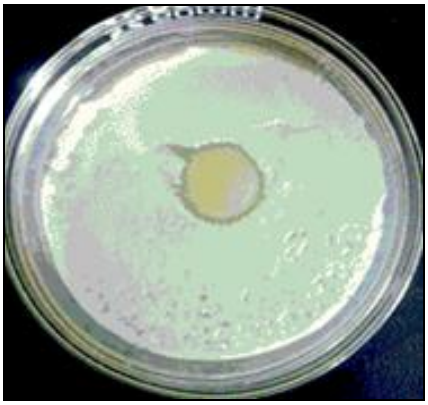
It was observed that the inhibition zone of NS4 was 7.2 mm 2, the placebo gel was 0 mm^2 and the pure drug luliconazole was 6.8 mm^2. This indicates that the luliconazole gel is effective against the fungus *Candida albicans* and luliconazole nanosponges proved as a better scaffold for topical application.

TABLE 12: ZONE OF INHIBITION

S. no	Formulation	Zone of inhibition (mm^2)
1	Placebo gel	0
2	Pure drug (luliconazole)	6.8
3	Formulation batch NS4	7.2



ZONE OF IN HIBITION NS4 BATCH ZONE OF INHIBITION PLACEBO GEL



ZONE OF INHIBITION FORMULATION OF PURE DRUG

FIG. 2: ZONE OF INHIBITION

Stability Studies: The results of stability studies showed that no significant changes in physical appearance, entrapment efficiency and *in-vitro* drug release studies.

Stability studies also showed that there was no change in the drug release profile. This indicates that the selected batch NS4 is stable and reproducible.

TABLE 13: STABILITY TEST STUDY OF NANOSPONGES GEL

Parameters	Before stability testing	After stability testing
Color	White Translucent	White Translucent
Visual appearance	Clear and homogenous	Clear and homogenous
% Drug release	84.60%%	84.45%

CONCLUSION: From all the above observations and results, it can be concluded that all formulations exhibit satisfactory organoleptic properties. The nanosponge loaded with luliconazole was prepared by a hyper-crosslinking method and the entrapment efficiency, physical properties, yield, swelling index and water uptake, drug content and particle size of the nanosponge were tested. The results obtained from the research confirmed that the maximum entrapment efficiency was found to be 79.16% in the formulation code NS4, and the formula was optimized. Therefore, particle size and morphology studies were performed on the optimized formulation. It was found that the average diameter of the optimized formula was 175.3 nm.

The prepared nanogels were evaluated for its viscosity, *in-vitro* drug diffusion study and *in-vivo* animal study of nanosponge gel and the results were compared with the commercially available conventional gels. In this study, we have developed topical gel formulation containing nanosponges loaded with antifungal drug luliconazole, which proved as novel approach for topical delivery and most suitable than conventional gels. Thus, it could provide better scaffold for topical application in near future to treat fungal infections. Though the current state of the art on such nanoscale sponges is still at its infancy, as the activities and competencies of this type of nanomaterial to interact with biological matter is growing, many new possibilities are anticipated in the near future.

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