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Formulation And Evaluation of Clotrimazole and Beclomethasone Dipropionate Loaded Nanosponge Based Hydrogel for Improved Topical Delivery

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ABSTRACT

Fungal infections affect over a billion individuals annually and are often inadequately treated with conventional topical therapies due to poor skin permeation, uncontrolled drug release, and associated side effects. This study aimed to formulate and evaluate a nanosponge-based hydrogel (NSH) loaded with clotrimazole (CT) and beclomethasone dipropionate (BD) for enhanced topical delivery. Nanosponges were prepared using the emulsion solvent evaporation method with ethyl cellulose as the rate-retarding polymer and polyvinyl alcohol as the emulsifier. The formulations were characterized for particle size (256–613 nm), polydispersity index, zeta potential (–14.3 to –35.4 mV), entrapment efficiency (up to 89.2% for CT and 86.4% for BD), production yield, and surface morphology via scanning electron microscopy, which revealed porous, spherical particles. FTIR and DSC studies confirmed drug-polymer compatibility and a shift to an amorphous state. The nanosponges were incorporated into a hydrogel using Carbopol 934, along with propylene glycol, methylparaben, propylparaben, and triethanolamine. The resulting hydrogel was evaluated for pH (5.1–5.4), viscosity, spreadability, physical appearance, and drug content. *In vitro* drug release studies demonstrated sustained release from optimized formulations (NS4–NS6), achieving 90.85%, 92.16%, and 94.52% release respectively over 12 hours, following the Higuchi model with Fickian diffusion kinetics. The hydrogel exhibited effective antifungal activity against *Candida albicans* and was confirmed non-irritant via Draize's skin irritation test on rabbits. Stability studies conducted under ICH guidelines at 4 ± 2°C and room temperature for 90 days showed no significant changes in physical properties or drug content.These findings demonstrate that the developed CT and BD-loaded nanosponge hydrogel is a stable, effective, and patient-compliant system for the sustained topical treatment of fungal infections such as candidiasis.

Keywords: *Nanosponge hydrogel, clotrimazole, beclomethasone dipropionate, sustained drug release, Candida albicans, topical delivery, skin irritation.*

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1. INTRODUCTION

Fungal infections, also known as mycoses, are a prevalent global health concern, affecting over one billion people annually [1]. These infections are caused by fungi, which are microorganisms characterized by chitin in their cell walls and can be found in various environments, including soil, plants, household surfaces, and human skin. While some fungi are harmless or even edible, others, such as*Aspergillus*, can be highly dangerous and lead to life-threatening diseases [2,3]. Fungal infections can be contagious, spreading from person to person, and often manifest as skin problems like rashes, bumps, irritation, scaly skin, redness, itching, swelling, and blisters. Common superficial fungal infections include athlete's foot, jock itch, and ringworm, which can occur anywhere on the body [4-6]. More serious systemic fungal infections, such as cryptococcosis and histoplasmosis, can also occur. Treatment for fungal infections typically involves antifungal medicines,

often in the form of creams, or oral/injectable medications, depending on the infection's nature and extent [7]. However, conventional topical systems, like ointments and creams, often prove less effective for skin permeation due to their poor efficiency. These traditional formulations can also lead to side effects such as burning, contact dermatitis, and stinging sensations due to uncontrolled drug release [8,9]. Furthermore, challenges in antifungal therapy include drug resistance, poor bioavailability, drug interactions, and toxicity issues, particularly with widespread use of antifungal agents. Many antifungal agents are lipophilic, leading to low water solubility and formulation difficulties[10].

To overcome the limitations of conventional therapies, there has been a shift towards developing advanced particulate carrier systems, such as microspheres and liposomes, for controlled drug delivery to specific skin regions [11]. These systems aim to regulate drug input rates and minimize systemic absorption, thereby reducing adverse reactions. Nanotechnology has emerged as a significant area of interest, with nanosponges (NS) being a promising novel nano-carrier system for topical delivery. Nanosponges are porous, spongy, spherical polymeric structures designed for controlled and predictable drug release [12-14]. They offer several advantages, including improved safety, enhanced stability, better aesthetic characteristics, and increased formulation flexibility [15]. When incorporated into topical hydrogel drug delivery systems, nanosponges can provide prolonged drug release and retention on the skin, reducing drug toxicity and improving patient compliance by extending dosing intervals. Hydrogels themselves are polymeric materials capable of swelling and retaining significant amounts of water without dissolving, making them promising drug delivery systems for improving bioavailability and therapeutic availability [15,16].

This study focuses on the design and evaluation of a nanosponge-based hydrogel loaded with two key antifungal agents: Clotrimazole and Beclomethasone Dipropionate. Clotrimazole is a synthetic fungistatic agent from the azole group, effective against tinea infections by inhibiting ergosterol synthesis in fungi. Beclomethasone dipropionate is a synthetic halogenated glucocorticoid with anti-inflammatory and vasoconstrictive effects, used for treating steroid-dependent asthma and allergic rhinitis. By combining these active pharmaceutical ingredients within a nanosponge-based hydrogel, this research aims to develop an improved topical delivery system for fungal infections that offers sustained release, reduced side effects, and enhanced patient outcomes.

2. METHOD AND MATERIALS

MATERIALS

Clotrimazole and beclomethasone dipropionate were procured from Bestcare Formulation, Pondicherry. Ethyl cellulose, propylene glycol, triethanolamine, methyl paraben, and propyl paraben were also sourced from the same supplier. Polyvinyl alcohol, dichloromethane, and Carbopol 934 were obtained from Ponmani & Co, Coimbatore. All other reagents used were of analytical grade.

EQUIPMENT

Analytical and formulation studies employed UV-Vis spectrophotometry (Shimadzu UV-1800), FTIR (JASCO 4600), DSC (PerkinElmer), Zetasizer (Malvern Ver. 7.13), SEM (Zeiss Sigma), probe sonicator (V-Tech VTPRO-250), centrifuge (Remi C-30BL), Brookfield viscometer (DV II+ Pro), and general labware from Borosil.

PREFORMULATION STUDIES

Clotrimazole and beclomethasone were characterized for physical appearance, melting point, and solubility in various solvents. UV-Vis spectrophotometric calibration curves were developed at their respective λmax (CT: 260 nm, BD: 236 nm), and FTIR studies confirmed drug-polymer compatibility.

PREPARATION OF NANOSPONGES

Clotrimazole (CT) and beclomethasone dipropionate (BD) were employed as active pharmaceutical ingredients, while ethyl cellulose (EC) served as the polymeric matrix-forming agent, and polyvinyl alcohol (PVA) acted as the stabilizer. The nanosponge formulations (NS1–NS6) were prepared using the **emulsion solvent evaporation technique**, a widely accepted method for nanoscale encapsulation.

Briefly, accurately weighed quantities of EC (ranging from 50 mg to 300 mg) were dissolved in dichloromethane along with CT and BD under magnetic stirring until a clear organic phase was obtained. This organic phase was then gradually emulsified into a 0.3% (w/v) aqueous solution of PVA. The emulsion process was facilitated using **ultrasonication at 65% amplitude** for 3 minutes in pulsed mode (10 seconds on, 10 seconds off) to ensure uniform droplet formation and nanosponge dispersion.

The resulting emulsion was then subjected to magnetic stirring at ambient conditions for 24 hours to allow for **complete evaporation of the organic solvent (dichloromethane)**. Post-evaporation, the nanosponges were harvested by **centrifugation at 16,900 g for 30 minutes at 4°C**. The obtained pellets were washed multiple times with distilled water to remove any unreacted PVA and residual solvents. Finally, the nanosponges were dried at room temperature, collected, and stored in desiccators for further use [17].

PREPARATION OF NANOSPONGE-LOADED HYDROGEL (NSH)

The dried nanosponges were incorporated into a hydrogel base for topical application. A 1% (w/w) **Carbopol 934** solution was prepared by dispersing the polymer in distilled water with gentle stirring and allowed to hydrate overnight. To this dispersion, the drug-loaded nanosponges were gradually added and homogenized to ensure uniform distribution.

The hydrogel was further formulated by incorporating **propylene glycol** as a permeation enhancer to improve dermal drug absorption. **Methylparaben and propylparaben** were added as antimicrobial preservatives. The gel's pH was adjusted to ~5.5–6.0 (skin-compatible range) using **triethanolamine**, which also served as a gelling and neutralizing agent. A plain drug-loaded gel without nanosponges served as the control formulation [18].

3. CHARACTERIZATION OF NANOSPONGES

Particle size analysis

The particle size analysis of drug loaded NS was performed by using ‘‘Malvern Zetasizer NanoZS. The sample under investigation was diluted with distilled water (1: 200) and filled in disposable polystyrene cuvette. Measurement of particle size, PDI and zeta potential was done based on the dynamic light scattering (DLS) theory. The samples were suitably diluted with distilled water for every measurement[19]. All the samples were tested in triplicate (n = 3) to minimize the error.

Scanning electron microscopy

For the evaluation of the surface morphology of nanosponges, the sample was analyzed in a scanning electron microscope after preparing the sample by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum. The stub containing the coated sample was placed in a scanning electron microscope. The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 20 kV[20]. From the resulting image, average particle size was determined.

Production yield (%)

For calculating production yield, the theoretical mass was calculated initially by taking the mass of solid ingredients added. All the prepared nanosponge formulations were accurately weighed and the weight was recorded [21]. The production yield of the nanosponges was then determined using the following equation:

$$\text{Production yield (\%)} = \frac{\text{Practical mass of nanosponge}}{\text{Theoretical mass (Polymer + Drug)}} \text{ X } 100$$

Drug entrapment efficiency (%)

The drug loaded NS were centrifuges at a high speed of 15,000 rpm for 30 minutes at 4°C and the supernatant liquid was collected, filtered and analysed for non-bound drug in UV Visible spectrophotometer[22]. The drug entrapment efficiency (%) of the nanosponges was calculated according to the following equation:

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

Differential scanning calorimetry (DSC)

Thermal peaks of pure drug BTF, Blank NS and optimized NSs (BNS3) were evaluated for drug encapsulation. The samples (5 mg) were enclosed in aluminium pan and heated at a rate of 10 °C/min in the temperature range of 30–300°C ‘‘(Mettler Toledo: DB V16.10)” [23].

4. CHARACTERIZATION OF NANOSPONGE-BASED HYDROGEL (NSH)

Physical Examination

Gels should have a pleasant appearance with respect to color, consistency etc. The prepared nanosponge loaded hydrogels were inspected visually for their color, homogeneity, and consistency [24].

Drug content (%)

1 g of prepared nanosponge loaded hydrogel formulation containing drug equivalent to 100 mg was dissolved in methanol. The solution was filtered. The absorbance of the resulting solution was measured at isosbestic point using a UV spectrophotometer after suitable dilutions. The drug content of the drug-loaded plain gel was also determined in the same manner[25]. The drug content of the formulation was determined using the following equation:

$$\text{\% Drug Content} = \frac{\text{Actual concentration of drug in the formulation}}{\text{Theoretical concentration of drug}} \text{ X } 100$$

Spreadability studies

Spreadability is a term expressed to denote the extent of the area to which the gel readily spreads on application to the skin. The therapeutic efficacy of a semisolid formulation also depends on its spreading value.1 g of the formulation was placed within a circle of 2 cm diameter pre-marked on a ground glass slide. The gel formulation was sandwiched between this slide and the second slide having the same dimension [26]. The increase in the diameter due to gel spreading was noted. The spreadability was then calculated from the following formula:

$$\text{Spreadability \%} = \frac{D2 - D1}{D1} \text{ X } 100$$

Where; D1 was initial diameter of gel before weight load, and D2 - was final diameter of gel after load.

Determination of pH

The pH of drug loaded NS based topical hydrogel was checked directly by dipping the electrode into the gel and allowed to equilibrate, then the pH was measured by calibrated pH meter maintained at 25°C [27]. The sample was tested in triplicate.

Viscosity

The viscosity of the formulations was determined using Brookfield viscometer with small sample adapter, spindle no. 64. Speed was increased from 10 to 100 rpm and viscosity was noted on cps [28]. Viscosity was measured at 25°C at 100 rpm.

In vitro drug release studies

In vitro release study of Clotrimazole nanosponges, the loaded hydrogel was carried out by using Franz diffusion cell. The formulation was taken in the donor compartment and phosphate buffer saline was taken in the receptor compartment. The cellophane membrane previously soaked overnight in the diffusion medium (PBS 7.4) was placed between the donor and receptor compartment. 1 g of the formulation was spread uniformly on the cellophane membrane, which is in contact with the receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37± 0.5°C. At specific intervals, 1 ml of sample was withdrawn from the receptor compartment and replaced with an equal volume of PBS 7.4. The in vitro drug release of drug loaded nanosponge hydrogel was compared with drug-loaded plain gel. After suitable dilutions, the absorbance of the sample was determined at 261 nm by UV-visible spectrophotometer [29].

Drug release kinetics from porous NS matrix

Drug release data was fitted in four kinetic models, zero-order, first order, Higuchi and Korsmeyer–Peppas kinetics models and regression analysis was performed [30].

Skin irritancy study

Acute dermal irritation studies were performed using Draize's method on healthy Newzealand white rabbit . Visual observations for erythema and edema were made at 24, 48, and 72 hours post-application. Newzealand white rabbit were obtained from Biogen laboratory animal facility, Bangalore Laboratory Animal Facility, Bangalore, Reg. No: KMCRET/ReRc/MPharm/50/2022). Skin irritation test was performed for the nanosponge hydrogel formulation NS on rabbit to find out any irritation problems which could make it unsuitable for topical use. About 1 g of formulation was applied to the sensitive part of the skin. The site of application was inspected for irritancy, erythema, and edema using draize's method. Skin reaction at the site of application was subjectively assessed and scored once daily at 1, 24, 48 and 72 hours [31]. The reaction at the site of application was assessed.

Evaluation Of Antifungal Activity

Antifungal efficacy of the NSH was assessed against *Candida albicans* using the agar well diffusion method. Each Petri dish is divided into 4 parts, in each part sample discs such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (100µg) disc (discs are soaked overnight in sample solution) and Std Fluconazole 10µg, is placed in the plate with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4º C or at room temperature for 1 hour for diffusion. Incubate at 28 º C for 48hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition [33].

Stability Studies

Stability testing was conducted as per ICH guidelines (Q1A-R2) over a period of 90 days. NSH formulations were stored at 4 ± 2°C and room temperature (25 ± 2°C). Samples were periodically assessed for changes in appearance, pH, drug content, and viscosity to ensure stability under varying environmental conditions [34].

5. RESULTS AND DISCUSSION

PRE-FORMULATION STUDIES

Pre-formulation studies were conducted to characterize the raw materials, Clotrimazole (CT) and Beclomethasone

Dipropionate (BD), before their incorporation into the nanosponge hydrogel system.

Physical Appearance

Both Clotrimazole and Beclomethasone Dipropionate were analyzed and found to be white, odorless, and tasteless crystalline powders.

Melting Point

The melting points of the pure drugs were determined using a melting point apparatus. Clotrimazole had a melting point of 141°C, while Beclomethasone Dipropionate melted at 209°C .

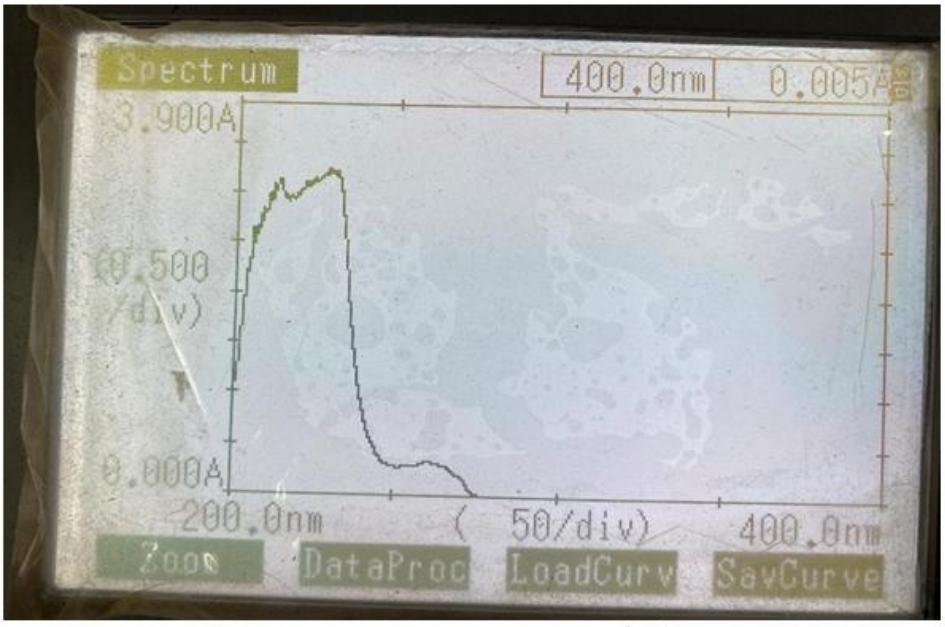
Solubility

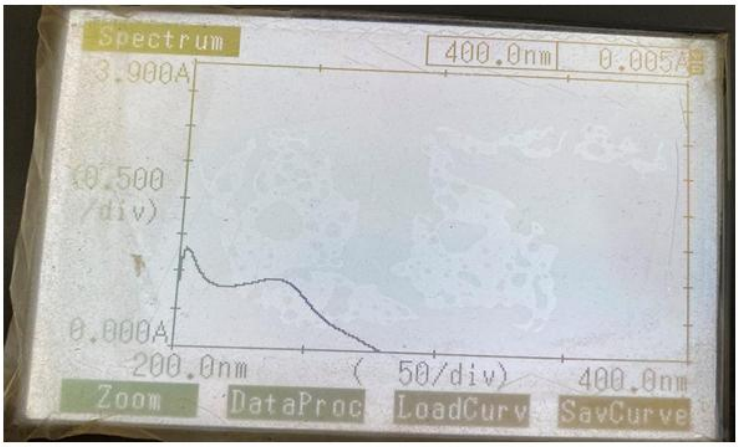
Solubility tests were performed for CT and BD in various solvents. Clotrimazole was found to be soluble in methanol, dimethyl sulfoxide (DMSO), and chloroform, sparingly soluble in ethanol, and practically insoluble in water. Beclomethasone Dipropionate was soluble in methanol, dichloromethane, and chloroform, sparingly soluble in ethanol, and practically insoluble in water.

S.no	Solvent	Soluble		Sparingly soluble		Insoluble	
		CT	BD	CT	BD	CT	BD
1.	Methanol	✓	✓	-	-	-	-
2.	Ethanol	✓	-	-	✓	-	-
3.	Dichloromethane	✓	✓	-	-	-	-
4.	DMSO	✓	-	-	-	-	✓
5.	Chloroform	✓	✓	-	-	-	-

Selection of Wavelength of Maximum Absorbance

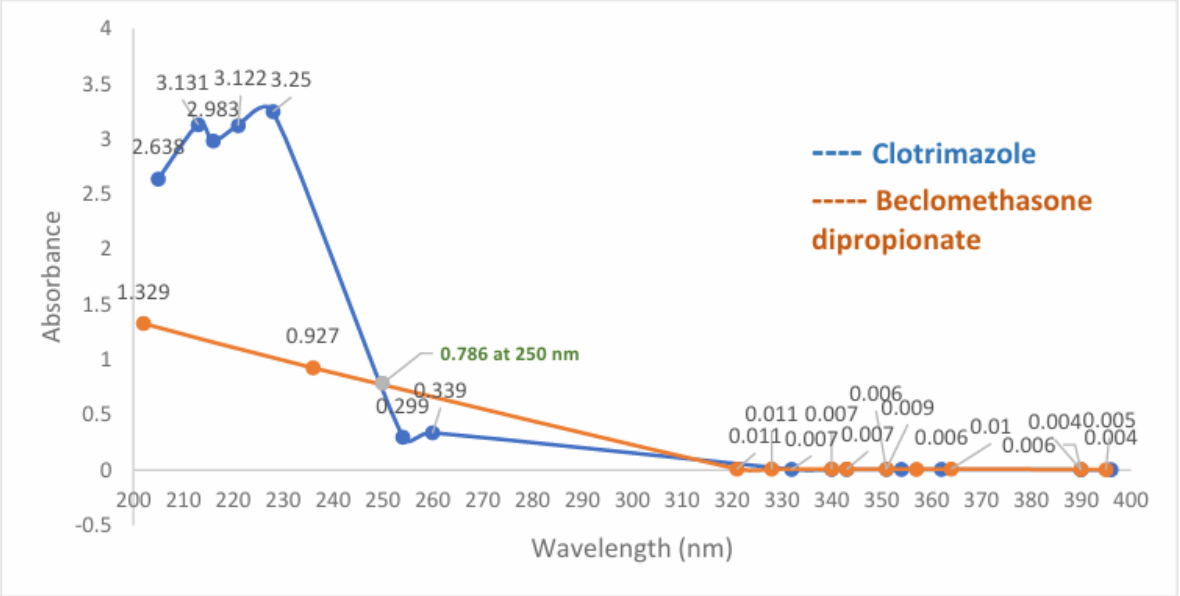
The Q-absorption ratio method was used to determine the maximum absorbance wavelength for simultaneous estimation of CT and BD using a UV-Visible spectrophotometer. The maximum absorbance for a 200 µg/ml solution of CT was observed at 260 nm, and for a 30 µg/ml solution of BD, it was 236 nm .





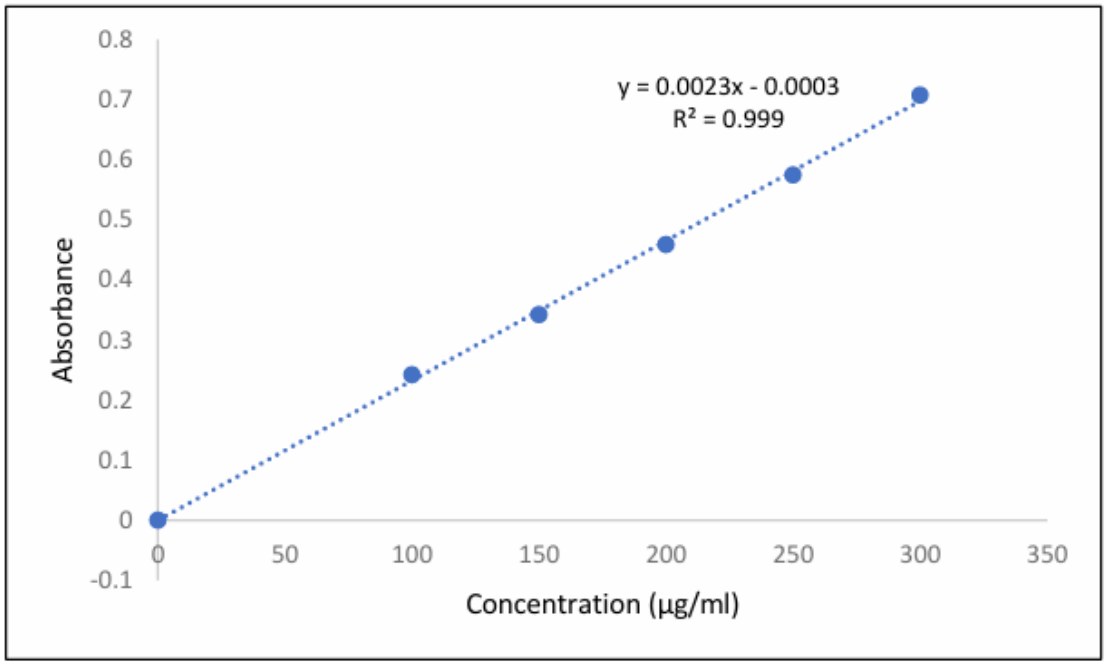
Determination of Iso-absorptive Point

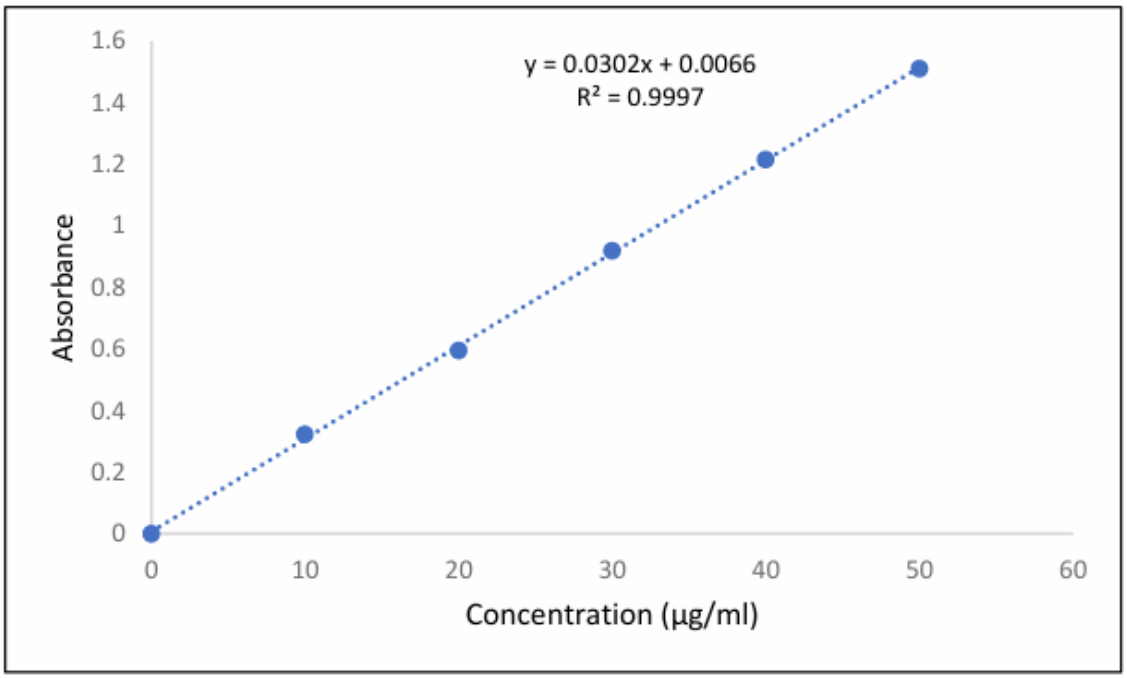
An iso-absorptive point was determined by preparing 200 µg/ml solutions of CT and 30 µg/ml of BD and scanning them in the UV-visible spectrophotometer. The overlain spectrum of CT and BD revealed an iso-absorptive point at 250 nm, which was selected for further analysis .



Construction of Calibration Curves

Calibration curves were prepared by plotting the absorbance against concentration for both CT and BD. The standard concentration of CT (100-300 µg/ml) at 260 nm showed linearity with an R^2 value of 0.999, indicating it obeys Beer-Lambert's law . Similarly, the standard concentration of BD (10-50 µg/ml) at 236 nm showed linearity with an R^2 value of 0.9997, also confirming adherence to Beer-Lambert's law.





Compatibility Studies using FT-IR Spectroscopy

FTIR spectra of the pure drugs, excipients, and their mixtures were analyzed to check for potential chemical interactions. It was observed that there was no deviation in the characteristic peaks of the drugs in the physical mixture, suggesting no interaction between the drug and excipients .

FTIR Interpretation of Clotrimazole

The FTIR spectrum of Clotrimazole showed characteristic peaks: C=N stretching at 1674.21 cm^{-1}, aromatic ring C-H stretching between 748.38-671.23 cm^{-1}, C=C bending at 1566.20 cm^{-1}, and a peak at 563.21 cm^{-1} .

FTIR Interpretation of Beclomethasone Dipropionate

The FTIR spectrum of Beclomethasone Dipropionate displayed peaks for C-H stretching at 2993.52 cm^{-1}, C=O at 1728.22 cm^{-1}, β-lactam at 1658.78 cm^{-1}, C=C at 1612.49 cm^{-1}, C-H bending of the methyl group at 1450.47 cm^{-1}, C-O at 1296.16 cm^{-1}, and Cl at 547.78 cm^{-1} .

FTIR Interpretation of Ethyl Cellulose

Ethyl Cellulose showed C-H stretching at 2978.09 cm^{-1}, C=O stretching at 1728.22 cm^{-1}, and C-O stretching between 1300-1000 cm^{-1} .

FTIR Interpretation of Polyvinyl Alcohol

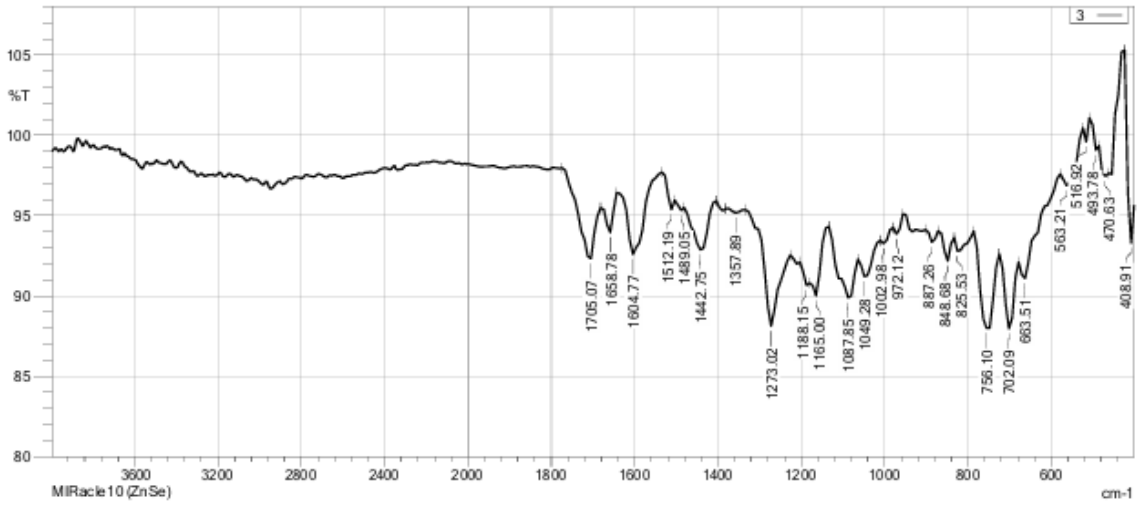
Polyvinyl Alcohol exhibited peaks for COOH at 1728.22 cm^{-1}, alkanes bending at 1419.61 cm^{-1}, C-O stretching between 1000-1300 cm^{-1}, and C-H rocking between 600-800 cm^{-1} .

FTIR Interpretation of Carbopol 934

Carbopol 934 showed C=O stretching at 1697.36 cm^{-1}, O-H bending at 1411.89 cm^{-1}, C-O stretching at 1219.01 cm^{-1}, and C=C bending at 972.12 and 894.97 cm^{-1} .

FTIR Interpretation of Mixture Sample

The FTIR spectrum of the mixture sample showed peaks for β-lactam (BD drug) at 1658.78 cm^{-1}, Cl (CT & BD) at 563.21 cm^{-1}, C=N stretching (CT drug) at 1658.78 cm^{-1}, and aromatic ring C-H stretching at 756.10 cm^{-1} . The absence of new peaks and retention of existing peaks confirmed compatibility between the drug, polymer, and other excipients.



6. CHARACTERIZATION OF PREPARED NANOSPONGES

Particle Size and Zeta Potential Analysis

The particle size, polydispersity index (PDI), and zeta potential of the nanosponge formulations (NS1-NS6) were analyzed. All formulations fell within the nanometer size range (256 nm to 613 nm). The mean diameter of the formulations varied with the concentration of ethyl cellulose (EC) . The PDI values ranged from 0.163 ± 0.03 to 0.390 ± 0.05, indicating a homogeneous and heterogeneous size distribution . The zeta potential values ranged from -14.3 ± 0.09 mV to -35.4 ± 0.16 mV, suggesting that the formulated nanosponges are stable . An increase in polymer (EC) concentration led to an increase in the charge of the NS formulation .

Formulation	Particle size (nm) ± S.D*	PDI ± S.D*	Zeta potential (mv) ± S.D*
NS1	256 ± 0.15	0.163 ± 0.03	-14.3 ± 0.09
NS2	376 ± 0.12	0.312 ± 0.04	-25.4 ± 0.14
NS3	438 ± 0.18	0.280 ± 0.03	-24.6 ± 0.13
NS4	512 ± 0.11	0.318 ± 0.02	-31.4 ± 0.10
NS5	572 ± 0.16	0.321 ± 0.03	-35.2 ± 0.15
NS6	613 ± 0.20	0.390 ± 0.05	-34.4 ± 0.16

*Values are expressed as mean ± SD (n=3)

Production Yield (%)

The percentage yield obtained after the whole process was less than 100%. The yield of the formulated nanosponges ranged from 44.5 ± 0.08% to 85.2 ± 0.06% .

Formulation	NS yield (%) ± S.D*
NS 1	44.5 ± 0.08
NS 2	48.4 ± 0.07
NS 3	56.7 ± 0.08
NS 4	82.9 ± 0.10
NS 5	84.8 ± 0.07
NS 6	85.2 ± 0.06

*Values are expressed as mean ± SD (n=3)

Drug Entrapment Efficiency (EE%)

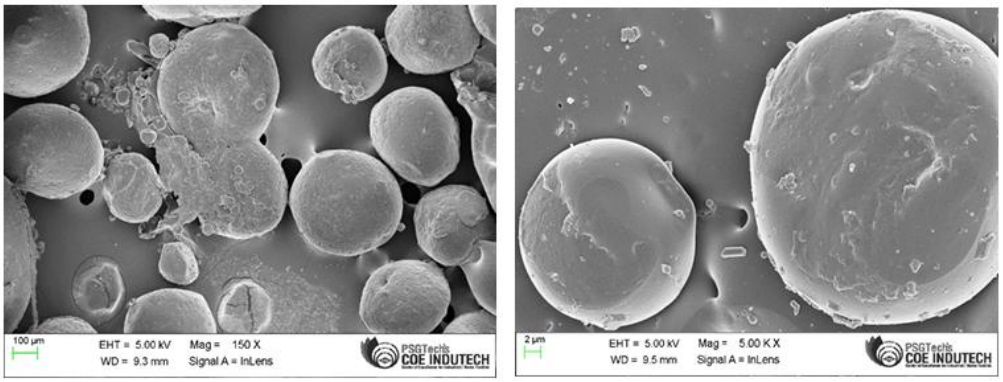
Drug encapsulation efficiency depends on the polymer used; a higher concentration of polymer (EC) generally leads to maximum entrapment . The EE% for Clotrimazole (CT) ranged from 62.8 ± 0.17% (NS2) to 89.2 ± 0.19% (NS6), and for Beclomethasone Dipropionate (BD) from 59.6 ± 0.16% (NS1) to 86.4 ± 0.14% (NS6) .

Formulation	EE (%) ± S.D*	
	CT	BD
NS 1	64.3 ± 0.19	59.6 ± 0.16
NS 2	62.8 ± 0.17	60.4 ± 0.15
NS 3	78.4 ± 0.21	74.3 ± 0.18
NS 4	87.5 ± 0.23	85.6 ± 0.20
NS 5	86.7 ± 0.18	83.3 ± 0.17
NS 6	89.2 ± 0.19	86.4 ± 0.14

*Values are expressed as mean ± SD (n=3)

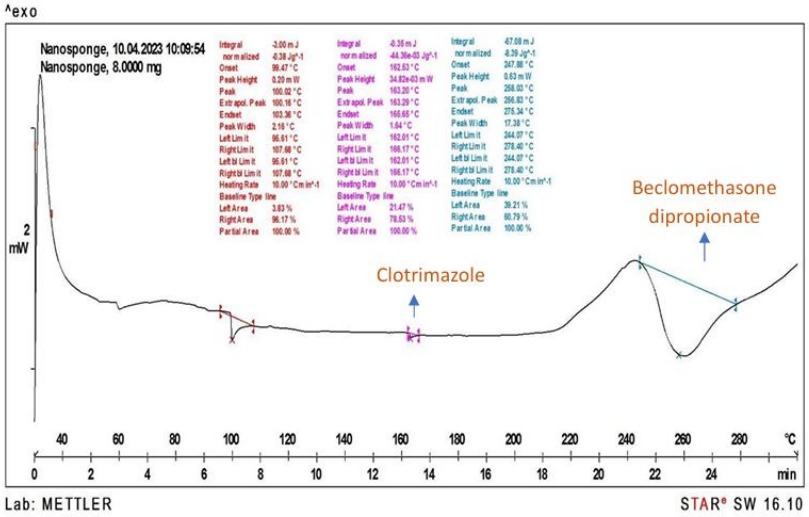
Scanning Electron Microscopy (SEM)

SEM images of the optimized NS4 formulation revealed that the nanosponges were spherical and porous with a smooth surface morphology. This porous and spongy feature is attributed to the inward diffusion of dichloromethane into the EC polymeric surface during fabrication .



DSC Studies

Differential Scanning Calorimetry (DSC) was performed on pure drugs (CT and BD) and the formulated NS. The endothermic DSC curves of the pure drugs showed melting points at 145°C (CT) and 210°C (BD). The DSC curve of the nanosponge formulation (NS4) showed a broad peak and an increased melting point at 162°C and 247°C, resulting from the coalescence of EC and the drugs. The disappearance of sharp peaks and an upward shift in the baseline indicated the fusion of the drugs with the polymer, confirming the crystalline nature of CT and BD was transformed to an amorphous nature due to the fabrication of NS, leading to stable drug-loaded nanosponges .



7. CHARACTERIZATION OF PREPARED NANOSPONGE LOADED GEL

Physical Examination

All prepared nanosponge hydrogel formulations containing 1% Carbopol 934 were visually evaluated for homogeneity. They were found to be clear and transparent .

Drug Content (%)

The drug content of the formulated NS hydrogel was analyzed using the Q-analysis method. The percentage of CT and BD drug present was satisfactory and uniformly distributed across all formulations. For CT, drug content ranged from 73.21 ± 0.02% (NS1) to 98.52 ± 0.01% (NS6), and for BD, it ranged from 70.32 ± 0.05% (NS1) to 96.89 ± 0.014% (NS6) .

Formulation	% Drug content ± S.D*	
	CT	BD
NS 1	73.21 ± 0.02	70.32 ± 0.05
NS 2	82.31 ± 0.004	79.41 ± 0.03
NS 3	88.52 ± 0.002	82.45 ± 0.005
NS 4	97.89± 0.015	95.54 ± 0.013
NS 5	96.29 ± 0.011	94.32 ± 0.012
NS 6	98.52 ± 0.01	96.89 ± 0.014

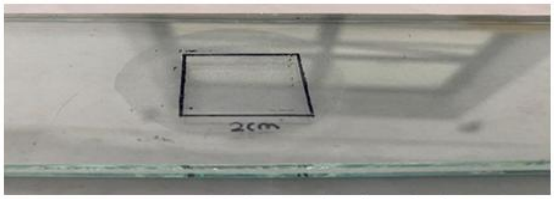
*Values are expressed as mean ± SD (n=3)

Spreadability Studies

The spreadability of the formulated NS hydrogel formulations ranged from 2.12 ± 0.01 cm (NS6) to 2.21 ± 0.04 cm (NS5). These values indicate that the formulated NSH is suitable for topical application.

Formulation	spreadability ± S.D*
NS 1	2.15 ± 0.02
NS 2	2.13 ± 0.01
NS 3	2.15 ± 0.03
NS 4	2.14 ± 0.01
NS 5	2.21 ± 0.04
NS 6	2.12 ± 0.01

*Values are expressed as mean ± SD (n=3)



Determination of pH

The pH values of the NSH formulations were found to be in the range of 5.1 ± 0.02 (NS6) to 5.4 ± 0.02 (NS3), which falls within the acceptable pH range for skin application, minimizing the risk of irritation.

Formulation	pH ± S.D*
NS 1	5.2 ± 0.01
NS 2	5.3 ± 0.03
NS 3	5.4 ± 0.02
NS 4	5.3 ± 0.01
NS 5	5.2 ± 0.02
NS 6	5.3 ± 0.04

*Values are expressed as mean ± SD (n=3)

Viscosity

The viscosity of the NSH formulations ranged from 11685 ± 2.8 cP (NS1) to 11800 ± 1.2 cP (NS6). The viscosity of the gel increased with increasing polymeric ratio.

Formulation	Viscosity ± S.D*
NS 1	11685 ± 2.8
NS 2	11695 ± 2.3
NS 3	11716 ± 1.9
NS 4	11743 ± 1.8
NS 5	11720 ± 1.5
NS 6	11800 ± 1.2

***Values are expressed as mean ± SD (n=3)**

In vitro Drug Release Studies

In vitro drug release studies were conducted using a Franz diffusion cell for 10 hours. The maximum cumulative drug release for the NS4 hydrogel formulation was 97.5 ± 1.2% . The fabricated drug-loaded nanosponges showed sustained and progressive drug release due to the porous matrix formed by ethyl cellulose .

Time (Hr)	% Cumulative drug release ± S.D*					
	NS1	NS2	NS3	NS 4	NS5	NS6
0	0	0	0	0	0	0
1	10.90 ± 1.2	11.44 ±0.2	10.32 ±1.3	13.44 ± 1.2	11.93± 1.4	10.32±1.3
2	19.62 ± 0.3	16.48 ±2.1	18.35 ±0.2	16.48 ±0.2	20.26± 0.4	17.32±0.2
3	25.76 ±2.1	22.39 ±1.2	22.14±1 .2	22.39 ±0.5	24.89± 0.3	20.23±1.2
4	29.00 ±0.4	27.18±1 .5	27.04±1 .5	27.18 ±1.4	30.01± 0.5	22.32±0.5
5	35.23 ±0.6	31.4 ±2.4	30.05 ±0.5	31.4± 0.3	37.37± 1.2	28.82 ± 1.7
6	38.94 ±1.4	36.16 ±0.4	34.24 ±1.4	36.16 ±1.4	42.73± 0.2	35.32±1.2
7	48.47 ±0.5	41.64 ±0.6	39.08 ±0.7	41.64 ±0.2	47.03± 1.4	40.21 ± 1.3
8	55.18 ±2.1	45.19 ±1.3	43.61 ±1.2	55.19 ±1.5	59.96± 0.2	59.43 ± 1.2
9	59.04 ±1.3	51.4 ±2.1	52.35 ±1.1	61.4± 0.5	62.74± 0.3	68.54 ± 0.4
10	66.14 ±2.4	54.16 ±1.5	63.67 ±1.3	74.16 ±1.3	76.16± 0.4	79.62 ± 1.1
11	71.17 ±0.3	68.41 ±0.3	72.53 ±0.2	82.41 ±1.5	85.73± 1.3	86.73 ± 1.3
12	79.23 ±0.2	79.42 ±0.1	80.23 ±1.2	90.85 ±1.3	92.16± 0.3	94.52 ±1.2

***Values are expressed as mean ± SD (n=3)**

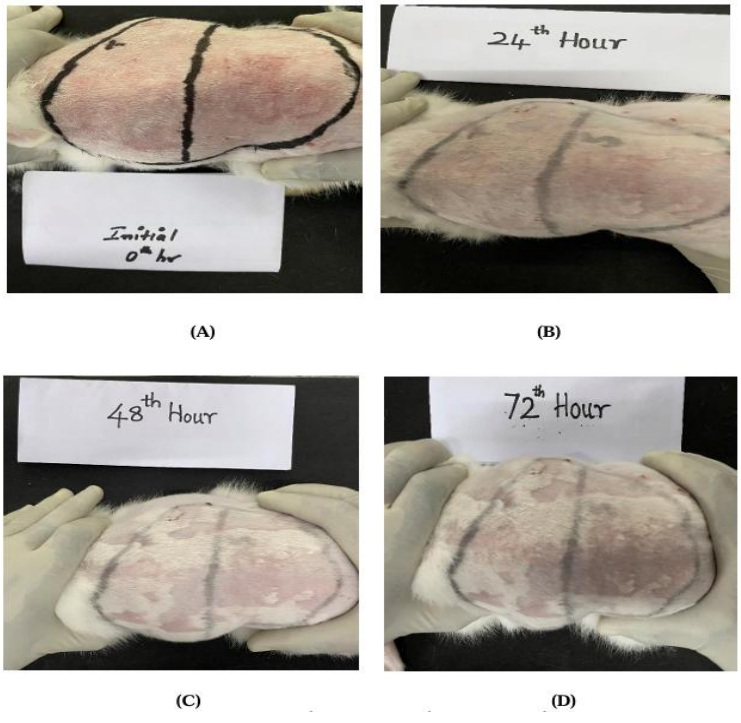
Drug Release Kinetics from Porous NS Matrix

The drug release data were fitted to four kinetic models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The Higuchi model was selected as the best-fitted model, as indicated by the highest R^2 values (ranging from 0.9839 to 0.996). This confirms that the formulation NS hydrogel followed Fick's law of diffusion.

Formula	Zero order	First order	Higuchi plot	Mechanism	Korsmeyer-Peppas Model		
	r^2				r^2	n	Mechanism
F1	0.9933	0.8878	0.996	Diffusion	0.9509	0.157	Fickian
F2	0.9747	0.8198	0.9839	Diffusion	0.9794	0.1564	Fickian
F3	0.9724	0.7964	0.9906	Diffusion	0.9877	0.1639	Fickian
F4	0.975	0.7797	0.9953	Diffusion	0.9844	0.1741	Fickian
F5	0.9881	0.8347	0.9946	Diffusion	0.9723	0.1696	Fickian
F6	0.9656	0.768	0.986	Diffusion	0.9616	0.1971	Fickian

Skin Irritancy Study

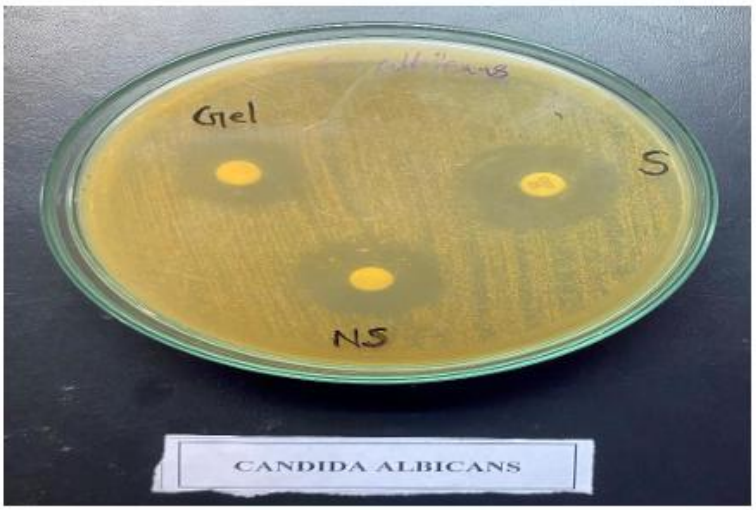
The NS4 hydrogel sample was tested for skin irritancy on New Zealand white rabbits using Draize's method and was found to be 'non-irritant'. The results showed a score index of 0.0, indicating no erythema or edema at 0, 24, 48, and 72 hours.



(A) (B) (C) (D)

8. EVALUATION OF ANTIFUNGAL ACTIVITY

The in vitro antifungal activity of the NS4 hydrogel was studied against *Candida albicans* using the disc diffusion method. The NS4 hydrogel showed a zone of inhibition of 20 ± 0.4 mm, while the standard fluconazole showed 26 ± 0.9 mm. According to the classification of antimicrobial activity, a zone of inhibition greater than 20 mm indicates strong activity. Therefore, the developed NS hydrogel formulation was considered to have high fungicidal activity against *Candida albicans*.



9. STABILITY STUDIES

Stability studies were performed on the optimized NS4 hydrogel for 90 days at two different temperatures: 4 ± 2°C and room temperature (28 ± 1°C), following ICH guidelines. The physical appearance, pH, and drug content were evaluated at 30, 60, and 90 days.

Physical Appearance

No significant change was observed in the physical appearance of the NS5 hydrogel over 90 days at both room and accelerated temperatures .

S.no	Day of sample withdrawing	Physical appearance
1.	1st day	No changes
2.	30th day	No changes
3.	60th day	No changes
4.	90th day	No changes

***Values are expressed as mean± SD (n=3)**

pH Values

A mild change in pH was observed over the storage period, but the values remained within the permitted limits .

S.no	Day of sample withdrawing	pH ± S.D*	
		At 4 ± 2°C	At R.T
1.	1st day	5.3 ± 0.02	5.3 ± 0.02
2.	30th day	5.3 ± 0.1	5.3 ± 0.1
3.	60th day	5.2 ± 0.2	5.3 ± 0.01
4.	90th day	5.1 ± 0.2	5.3 ± 0.02

***Values are expressed as mean± SD (n=3)**

Drug Content

The drug content of the NS hydrogel remained stable over the 90-day period at both temperatures. For CT, content ranged from 97.80 ± 0.004% (1st day at 4 ± 2°C) to 95.39 ± 0.5% (90th day at 4 ± 2°C) and 97.89 ± 0.003% (1st day at R.T) to 96.09 ± 0.02% (90th day at R.T). For BD, content ranged from 95.44 ± 0.003% (1st day at 4 ± 2°C) to 93.24 ± 0.6% (90th

day at 4 ± 2°C) and 95.54 ± 0.09% (1st day at R.T) to 94.02 ± 0.03% (90th day at R.T).

S.no	Day of sample withdrawing	Drug content (%) ± S.D*			
		At 4 ± 2°C		At R. T	
		CT	BD	CT	BD
1.	1st day	97.80± 0.004	95.44 ± 0.003	97.89± 0.003	95.54 ± 0.09
2.	30th day	97.19± 0.001	94.34 ± 0.03	97.48± 0.012	95.12 ± 0.07
3.	60th day	97.09± 0.05	94.04 ± 0.3	96.12± 0.05	95.04 ± 0.013
4.	90th day	95.39± 0.5	93.24 ± 0.6	96.09± 0.02	94.02 ± 0.03

***Values are expressed as mean± SD (n=3)**

10. CONCLUSION

This study successfully developed and evaluated a nanosponge-based hydrogel formulation containing both Clotrimazole (CT) and Beclomethasone Dipropionate (BD) for enhanced topical treatment of fungal infections. The formulation process, utilizing the emulsion solvent evaporation method with ethyl cellulose as the polymer, proved to be both simple and cost-effective. Characterization of the nanosponges revealed desirable properties, including appropriate nanometer-scale particle sizes (256-613 nm) and good stability as indicated by zeta potential values. High entrapment efficiency was achieved, particularly with increased polymer concentration, and scanning electron microscopy confirmed the porous, spherical morphology essential for sustained drug release. Differential Scanning Calorimetry further supported the successful conversion of the crystalline drugs into an amorphous form within the nanosponges, contributing to formulation stability. The resulting hydrogel formulations exhibited excellent physical characteristics, including homogeneity, clarity, and transparency. They demonstrated satisfactory drug content uniformity, good spreadability for topical application, and skin-compatible pH values. In vitro drug release studies showed a sustained release profile over 12 hours, with formulations NS4, NS5, and NS6 achieving optimal release rates, and the release kinetics best fit the Higuchi model, indicating a diffusion-controlled mechanism. Crucially, the hydrogel was found to be non-irritant in skin irritancy tests on rabbits, and it exhibited significant antifungal activity against *Candida albicans*. Furthermore, stability studies over 90 days confirmed the formulation's integrity in terms of physical appearance, pH, and drug content. While the study successfully demonstrated the potential of this nanosponge-based hydrogel for sustained and effective topical delivery, a notable limitation is the absence of in vivo efficacy studies in human subjects, which would be essential to fully validate its clinical applicability and long-term benefits.

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