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DEVELOPMENT AND EVALUATION OF CYCLODEXTRIN BASED NANOSPONGE LOADED TAZORETENE GEL

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Keywords:

Tazarotene, β-cyclodextrin nanosponges, anti-inflammatory, nanosponge gel, skin permeability

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ABSTRACT: The objective of the present study was to develop cyclodextrin-based nanosponge based topical gel of tazoretene using cross-linker diphenylcarbonate and Carbopol® Ultrez 10 NF polymer. The β-Cyclodextrin nanosponges were prepared using various concentrations of β-Cyclodextrin and diphenyl carbonate and characterized. Based on evaluation parameters the β-Cyclodextrin nanosponges formulations (NS2) displayed narrow particle size, sufficiently high particle size, and maximum solubilization efficiency. Tazarotene was loaded into five β-Cyclodextrin nanosponge formulations by freeze-drying method and evaluated. Owing to the better solubilization and drug loading capacity (45%) TZNS3 was selected for further studies. The particle size of 336 nm, the zeta potential of -23 mV, and maximum drug dissolution of 97%in 12h were displayed by TZNS3 hence formulated into a gel and evaluate. The optimized tazarotene-loaded nanosponge formulation (TZNS3) was incorporated into a model carbopol gel formulation and was evaluated for skin permeation and stability. The flux value for nanosponges based gel formulation (189.342 ± 3.879 µg cm^{-2} h^{-1}) was found to be higher than that for plain tazarotene (106.765 ± 4.123 µg cm^{-2} h^{-1}). Skin permeation studies demonstrated that the cumulative amount of tazarotene penetrating through the skin from gel formulation containing nanosponge encapsulated tazarotene was around 6 times more than that from gel formulation containing free tazarotene at 12 h. The results collectively suggest that because of the controlled drug release, better skin permeation, and good storage stability, cyclodextrin nanosponges-based gel formulation of tazarotene has tremendous potential to serve as a topical delivery system.

INTRODUCTION: Nanoparticles are colloidal systems with particle sizes varying from 10 nm to 1000 nm. These are nano or sub-nano-sized structures which are made of synthetic or semi-synthetic polymers. Nanoparticles of plant medicine are gaining a lot of attention currently.

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Different types of nanoparticles are polymeric nanoparticles, solid lipid nanoparticles, liposomes, proliposomes, niosomes, liquid crystalline systems, quantum dots, etc. But all these nanoparticles have certain disadvantages like drug loading, toxicity, etc. [1-4].

Nanosponges (NS) can conquer these problems. They solubilize poorly water-soluble drugs and provide prolonged release as well as improves drugs' bioavailability. They can load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching.

They can move in the body until they come across the exact target site and attach on the surface and start releasing the drug in a controlled manner [5, 6]. In preparation of NS, cyclodextrins are the preferable polymer because these are having more capacity to increase the solubility of poorly soluble drugs when compared to other polymers.

Cyclodextrins are nanometric biomaterials with a close relationship between molecular status and supramolecular properties. They are a class of cyclic glucopyranose oligomers and are synthesized by enzymatic action on hydrolyzed starch [7-10]. For delivery of NS, topical gels are very attractive drug delivery systems. In-gel preparation usually synthetic and semi-synthetic polymers are used which are expensive and less biocompatible.

Tazarotene, as the third-generation drug for retinoic acid, had been reported to have organizational-reconstruction, normalizing-of-differentiation, and anti-inflammatory functions [11-13]. Preliminary study has shown that tazarotene has the ability to promote angiogenesis and wound healing [14]. The primary pharmacologic effects of tazarotene, including normalization of cellular differentiation, decreased hyperkeratinization, reversal of keratinocyte hyperproliferation, and anti-inflammatory effects, contribute to its therapeutic efficacy in treating acne vulgaris. Overall, tazarotene has a good safety profile and is not associated with carcinogenicity, contact sensitization, mutagenicity, photoallergic reactions, or phototoxicity [15]. Various formulation strategies have been used to prepare tazarotene delivery systems: namely, creams, gels, foams, liposomes, microemulsions, nanosponge and noisome based gels, proniosomal gels, ethosomal gels, electrospun membrane systems, PLGA nanoparticles. A new 0.1 % short-contact tazarotene lotion formulation was designed to increase comfort and convenience, potentially reduce irritation, and improve patient adherence and outcomes [16]. The results of this formulation showed that the lotion was well-tolerated with twice-daily use, suggest its effectiveness. Hence, the loading and release of tazarotene from cyclodextrin nanosponges may be a promising method to enhance skin permeation.

MATERIAL AND METHODS:

Materials: Tazarotene was obtained as a gift sample from Dr. Reddy's Laboratory Ltd., Hyderabad, India. β-Cyclodextrin (Complexol-B) was obtained as a gift sample from Gangwal Chemicals Pvt. Ltd. (Mumbai, India). Diphenyl carbonate was purchased from Sigma Aldrich (Milan, Italy). All other chemicals and reagents used in the study were of analytical grade. Milli Q water (Millipore) was used throughout the studies. Dialysis Bag (Molecular weight cut off 10 kDa) was purchased from Hi-media Pvt. Ltd.,

Preparation of β-Cyclodextrin nanosponges (NS): Cyclodextrin based nanosponges prepared using diphenylcarbonate (DPC) for the crosslinking as reported elsewhere [17]. Five types of nanosponges were prepared using different molar ratios of reactants. The molar ratios and concentrations of both the reactants were used as shown in table 3.1. Briefly, in a 250 ml flask, the required quantity of anhydrous β-Cyclodextrin was dissolved in anhydrous dimethylformamide. Diphenyl carbonate was added to this reaction mixture and refluxed in an oil bath at 90 °C for 6 h under stirring. After completion of the reaction, the obtained product was washed with water and subsequently purified by Soxhlet extraction with ethanol up to 6 h. The white powder thus obtained was dried overnight in an oven at 60 °C and subsequently ground in a mortar. The fine powder obtained was redispersed in water. The colloidal part that remained suspended in water was recovered by lyophilization. The nanosponges were dried under vacuum and stored at 25 °C until further use. The obtained nanosponges were termed NS1, NS2, NS3, NS4, and NS5 based on the molar ratio of reactants. The particle size and practical yield of obtained nanosponges were determined in **Table 1**.

TABLE 1: MOLAR RATIOS AND CONCENTRATIONS OF CYCLODEXTRINS AND THE CROSS-LINKER

S. no.	Type of NS	Molar ratio	Concentration of CD (g)	Concentration of DPC (g)
1	NS1	1:2 (β-CD:DPC)	2.274	0.856
2	NS2	1:4 (β-CD:DPC)	2.274	1.712
3	NS3	1:6 (β-CD:DPC)	2.274	2.568
4	NS4	1:8 (β-CD:DPC)	2.274	3.424
5	NS5	1:10 (β-CD:DPC)	2.274	4.28

Characterization of cyclodextrin Nanosponges:

Particle Size, Polydispersity index and Zeta Potential Determination: The particle size distribution of prepared nanosponges (NS1-NS5) was observed using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK). The zeta potential of the same was determined using a Zetasizer (Malvern Instruments Ltd, Worcestershire, UK) [18].

Fourier Transformed Infrared (FTIR) Spectroscopy: The FTIR spectra of β-Cyclodextrin and prepared nanosponges (NS1-NS5) were carried out by the potassium bromide disc method using Tensor 27 FTIR Spectrophotometer (Bruker Optics, Germany) in the region of 4000to 600 cm^{-1}.

Differential Scanning Calorimetry (DSC): β-Cyclodextrin and prepared nanosponges (NS1-NS5) were subjected to differential scanning calorimetry studies using a Perkin Elmer DSC/7 differential scanning calorimeter (Perkin-Elmer, CT-USA) equipped with a TAC 7/DX instrument controller.

X-Ray Powder Diffraction (XRPD): X-ray powder diffraction patterns of β-Cyclodextrin and prepared nanosponges (NS1-NS5) were recorded on X-ray diffractometer (Bruker D8 Advance) at a scan rate of 5 °/min in the 2θ range from 2.5° to 60°.

Transmission Electron Microscopy (TEM): The morphology of the prepared nanosponges (NS1-NS5) was observed under transmission electron microscopy (JEM-2000 EXII; JEOL, Tokyo, Japan). One drop of diluted tazarotene-loaded nanoparticle suspension was deposited on a film-coated copper grid and stained with one drop of 2% (w/v) aqueous solution of phosphotungstic acid, and then allowed to dry for contrast enhancement. The samples were examined at a magnification of 72000× by transmission electron microscopy.

Solubilization Efficiency of Nanosponges: An excess quantity of tazarotene (10 mg) was suspended with a fixed quantity (20 mg) of NS in 20 ml Milli Q water. The volumetric flasks were placed on a mechanical shaker at ambient temperature. After equilibrium for 24 h, the suspensions were then centrifuged for 10 min at 10,000 rpm to separate the free tazarotene, and the colloidal supernatant was collected. 10 ml of methanol was added to the above supernatant to extract the tazarotene encapsulated in nanosponges. The colloidal supernatant solution was then analyzed using a calibration curve for tazarotene concentration by UV spectrophotometer at 246 nm (Systronics -Double Beam Spectrophotometer-2201).

Preparation of Tazarotene-loaded Nanosponges (TZNS): Drug-loaded nanosponges were prepared by freeze-drying technique as previously reported [20]. Accurately weighed quantities of NS were suspended in 50 ml of Milli Q water using a magnetic stirrer, then the excess amount of tazarotene was added, and the mixture was sonicated for 10 min and was kept for 24 h under stirring. The suspensions were centrifuged at 2,000 rpm for 10 min to separate the uncomplexed drug as a residue below the colloidal supernatant. The supernatant was freeze-dried on a lyophilizer (LARK INDIA) at -20 °C temperature and operating pressure 13.33 mbar. The dried powder was stored in a desiccator. The drug-loaded NS formulations obtained were named TZNS1, TZNS2, TZNS3, TZNS4, and TZNS5, depending upon the type of NS.

Determination of Tazarotene Loading in Nanosponges: The weighed amount of tazarotene-loaded nanosponge complexes were dissolved in methanol, sonicated for 10 min to break the complex, diluted suitably, and then analyzed by UV spectrophotometer at 246 nm.

Characterization of Tazarotene-loaded Nanosponges: Particle Size, polydispersity index, zeta potential, FTIR, DSC, PXRD, TEM of tazarotene, plain nanosponges (NS2, NS3), and tazarotene loaded nanosponge complexes (TZNS2, TZNS3) analyzed as per the procedure adopted for cyclodextrin nanosponges [21].

***In-vitro* Release of Tazarotene from Nanosponge Formulations:** The *in-vitro* release study was carried out using multi-compartment (n=6) rotating cells with a dialysis membrane (Sartorius cut off 12,000 Da). The donor phase consisted of formulations containing 5 mg of Tazarotene in 100 ml simulated gastric fluid (pH 6.4). The receptor phase also contains the same medium. The receptor

phase was added with 0.5% w/v sodium lauryl sulfate (1 ml) to maintain proper sink conditions.

The receptor phase was withdrawn completely after fixed time intervals, suitably diluted with distilled water, and was analyzed by using UV spectrophotometer at 246 nm. The experiment was carried out in triplicate.

Kinetic Analysis: To elucidate the mode and mechanism of drug release, the data from the in-vitro release study were fitted into various kinetic models, like zero order, first order, Higuchi's, and KorsemeyerPeppa's model. The release from the tazarotene nanosponge formulation was determined by the curve fitting method. Data obtained from *in-vitro* release studies were fitted to various kinetic equations [22].

Preparation of Tazarotene Gel Formulation: The gel base formulation of tazarotene-loaded nanosponges was prepared by using Carbopol® Ultrez 10 NF Polymer (Lubrizol Corp, Wickliffe, Ohio) as reported by Poonam *et al.* [23]. The polymer was soaked in water for 2 hours and then dispersed in distilled water using a magnetic stirrer so as to obtain a homogeneous gel base of 1% w/w.

To the above gel base 2% w/w of Propylene glycol, 2% w/w of N-methyl-2-pyrolidone, and 1% w/w of Triethanolamine were added with continuous stirring. Finally, tazarotene loaded nanosponges (TZNS) were incorporated into the prepared gel base to get 1% w/w tazarotene in the gel base. Tazarotene reference formulation (control) was prepared by incorporating plain tazarotene into the above gel base.

Evaluation of Gel Formulations:

pH Determination: The pH of both the gel formulations was determined by a digital pH meter.

***In-vitro* and *In-vivo* Skin Permeation Studies:**

Animal Study and Preparation: 6 to 8 weeks old Wistar Albino rats weighing 120 to 150 g were procured for the in vitro permeation studies. The animals were housed in ventilated animal rooms at a temperature of 25 °C with a 12/12 h light/dark cycle. Food and water were provided ad libitum. Animals were allowed 1 week to acclimatize before starting the study. The institutional animal ethics committee with no approved the protocol of animal study:1447/PO/Re/S/11/CPCSEA/17/A [24].

***In-vitro* Skin Permeation and Deposition Studies:** Wister Albino rats were anesthetized with chloral hydrate, and hair on the abdomen was carefully removed with a razor. About 5 cm2 of skin on the left and right sides of the abdomen was excised, and the subcutaneous fat and connective tissue were trimmed. The excised skins were washed and examined to ensure that no obvious defects were present. Diffusion studies were carried out using Franz diffusion cells with an effective diffusion area of 3.14 cm^2 were used to perform the in vitro skin permeation and deposition studies.

The excised skin samples of the dorsal side were clamped between the donor and the receptor chambers of Franz diffusion cells, with the stratum corneum side facing the donor chamber. 0.02 g of the test gel was applied as a thin film to the surface of the stratum corneum with an effective diffusional area of 1 cm^2. The receptor compartment was filled with 20 mL of physiological saline (pH 7.2) containing 1% Tween 80, increasing tazarotene solubility to 155.8 µg/mL and keeping a sink condition for tazarotene penetrated through the skin. The diffusion cells were maintained at 32 ± 1 °C with stirring at 300 rpm throughout the experiment. For each experiment, 0.5 ml of receptor medium was removed at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, or 24 h) and immediately replaced with the same volume of fresh physiological saline solution to maintain sink conditions. The tazarotene concentrations were measured by UV spectrophotometer [25].

After 12 h, the skin surfaces were thoroughly washed with distilled water to remove excess formulation. The epidermis layer was then stripped using cellophane adhesive tape [37] to extract the tazarotene deposited on the stratum corneum. The skin was cut into small pieces and homogenized. The tazarotene attached to the tape and remaining on the homogenized skin was extracted with methanol. The solution was then centrifuged at 4000 rpm for 10 min, and the concentration of tazarotene in the supernatant was determined by a UV spectrophotometer

***In-vivo* Permeation Study in Mice:** Twenty-four hours prior to the experiment, the dorsal hair was removed with a razor, and the bare skins were washed with physiological solution. 0.02 g of the test gel was applied as a thin film on the dorsal surface (3.14 cm^2). At hours 3, 6, 9 and 12 thereafter, the mice were killed by cervical dislocation, and the treated skin area was dissected. Further steps for skin sample treatment and extraction of tazarotene from skin tissues were performed using the same methods as described above. The amount of tazarotene in the epidermis and dermis was analyzed by UV spectrophotometer [26].

Skin-irritation Testing (Draize patch test): The irritation potential of the nanosponges-based tazarotene gel in comparison with marketed tazarotene gel was evaluated by carrying out the Draize patch test on rabbits. Animal care and handling throughout the experimental procedure were performed in accordance with the CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethical Committee with No: 1447/PO/Re/S/11/CPCSEA/ 15/A. White New Zealand rabbits weighing 2.5–3 kg were acclimatized before the beginning of the study. Animals were divided into four groups (n = 3) as follows:

Group 1: No application (control).

Group 2: Plain tazarotene gel formulation (tazarotene gel containing 0.05% w/w).

Group 3: Gel formulation without tazarotene (placebo gel).

Group 4: Nanosponge-based gel containing tazarotene (0.05%, w/w).

The back of the rabbits was clipped free of hair 24 h prior to the application of the formulations. 0.5 g formulations were applied on the hair-free skin of rabbits by uniform spreading within the area of 4 cm^2. The skin was observed for any visible change such as erythema (redness) at 24, 48, and 72 h after the application of various formulations. The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4.

Photodegradation Studies: Photodegradation experiments were performed using a UVA lamp with a 320-400 nm wavelength range. The systems under study were carbopol gel formulation of nanosponge complex of encapsulated tazarotene (1%, w/w) and gel formulation of an equivalent amount of the plain tazarotene as control. An aliquot (40 mg) of test gels was evenly spread through a syringe onto the bottom of a beaker and then irradiated for 2 h. After the exposure interval, the beaker was removed, its content quantitatively transferred into a 20-ml calibrated flask, and subjected to sonication for 15min. The resulting sample was adjusted to volume (20 ml), filtered (0.45 μm membrane filters), and analyzed by UV spectrophotometer. The degree of photodegradation was measured by comparing the peak areas of tazarotene from the irradiated samples with those obtained by analyzing an equivalent amount of the non-exposed formulations. Each sample was prepared and analyzed in triplicate [27].

Long-term Stability Studies: Stability studies were performed on the same formulations utilized for the photodecomposition experiments. The formulations were stored in stoppered containers for 3months, at room temperature and in the dark. At appropriate time intervals, aliquots (40–50 mg) were withdrawn from the emulsions and transferred into calibrated flasks (20 ml). The samples were extracted with ethanol under sonication, diluted to volume, filtered (0.45 μm membrane filters), and analyzed by UV spectrophotometer for the assay of the remaining tazarotene content. All measurements were performed in triplicate [28].

RESULTS AND DISCUSSION:

Characterization of Prepared Nanosponges: The cyclodextrin-based nanosponges were synthesized in five different molar concentrations (1:2, 1:4, 1:6, 1:8 & 1:10) from β-Cyclodextrin and diphenyl carbonate and characterized prior to use to have uniform batches. The practical yield of nanosponges was found to be very less for lower molar concentrations as shown in **Table 2**. The practical yield was proportional to the concentration of the cross linking agent.

TABLE 2: THE PRACTICAL YIELD OF CYCLODEXTRIN NANOSPONGES

S. no.	Type of NS	Molar ratio	Concentration of CD (g)	Concentration of DPC (g)	Practical yield (g)
1	NS1	1:2 (β-CD:DPC)	2.274	0.856	1.425
2	NS2	1:4 (β-CD:DPC)	2.274	1.712	2.215
3	NS3	1:6 (β-CD:DPC)	2.274	2.568	3.635
4	NS4	1:8 (β-CD:DPC)	2.274	3.424	3.810
5	NS5	1:10 (β-CD:DPC)	2.274	4.28	3.825

The particle size analysis of prepared nanosponges revealed that the average particle size measured by laser light scattering method is around 276-322 nm with a low polydispersity index. The particle size distribution is unimodal and having a narrow range, as seen in **Table 3**. A sufficiently high zeta potential indicates that the complexes would be stable, and the tendency to agglomerate would be miniscule. A narrow PI means that the colloidal suspensions are homogenous in nature.

TABLE 3: PARTICLE SIZE, POLYDISPERSITY INDEX AND ZETA POTENTIAL OF PREPARED NANOSPONGES

Sample	Mean hydrodynamic diameter ± SD (nm)	Polydispersity Index	Zeta potential (mV)
NS1	284 ± 21	0.17±0.005	-16.34±1.2
NS2	322 ± 32	0.22±0.005	-19.65±1.3
NS3	292 ± 17	0.20±0.005	-22.55±2.1
NS4	276 ± 37	0.25±0.005	-24.46± 1.5
NS5	304 ± 12	0.19±0.005	-17.23± 2.5

(All determinations were performed in triplicate and values were expressed as mean±S.D., n=3)

Cyclodextrin-based nanosponges have attracted the attention of many formulation experts due to their nature of improving the solubility of poorly water-soluble drugs. These nanosponges can form both inclusion and noninclusion complexes with the drugs and can accommodate a wide variety of drugs. In addition to improving the solubility, these nanosponges can protect the labile groups from the gastric environment and enhance the stability of the drug.

FTIR spectra of nanosponges showed a characteristic peak of carbonate bond at around 1720–1750 cm^{-1} which confirms the formation of cyclodextrin-based nanosponges. In addition, the other characteristics peak of nanosponges were found at 2918 cm^{-1} due to the C– H stretching vibration, 1418 cm^{-1} due to C–H bending vibration and 1026 cm^{-1} due to C–O stretching vibration of primary alcohol **Fig. 1** [33-34]. The DSC thermogram of β- cyclodextrin exhibited an endothermic peak at 289 °C corresponding to its melting transition point **Fig. 2**. The DSC analysis of nanosponges showed no peak before 350 °C, meaning that this material has high thermal stability. The plain β-Cyclodextrin (not cross-linked) showed a crystalline structure at XRPD, as shown in **Fig. 3**. Whereas, XRPD pattern of nanosponges shows the crystallinity degree with a weak long-range order characterized by some broad reflections that appear as narrow peaks in the XRPD of β-Cyclodextrin.

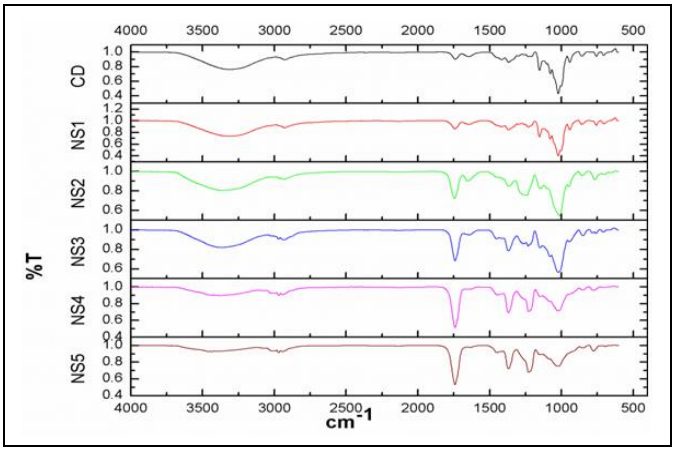


FIG. 1: FTIR SPECTRA OF B-CYCLODEXTRIN AND PREPARED NANOSPONGES (NS1-NS5)

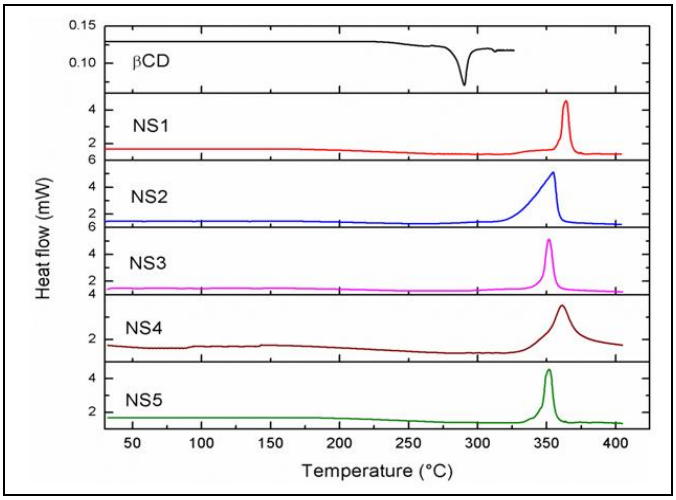


FIG. 2: DSC THERMOGRAMS OF B-CYCLODEXTRIN AND PREPARED NANOSPONGES (NS1-NS5)

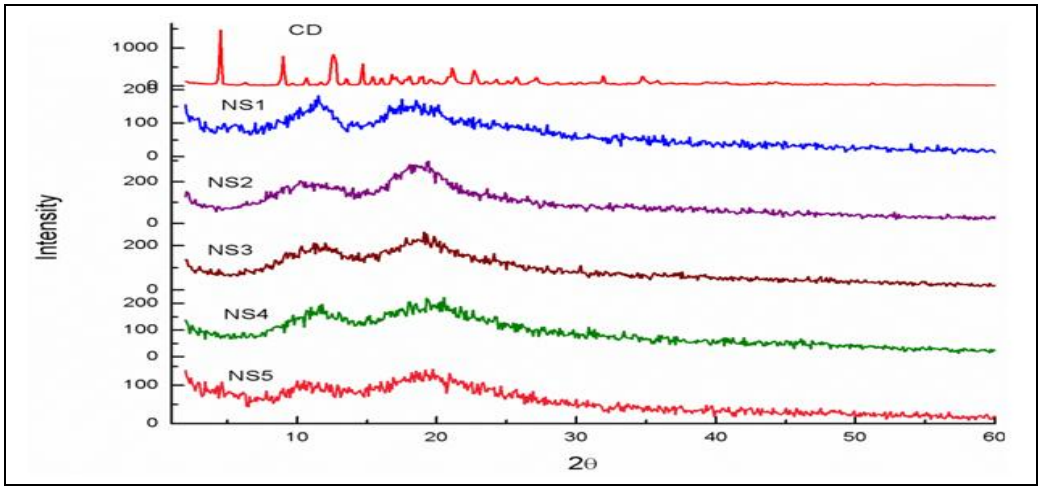
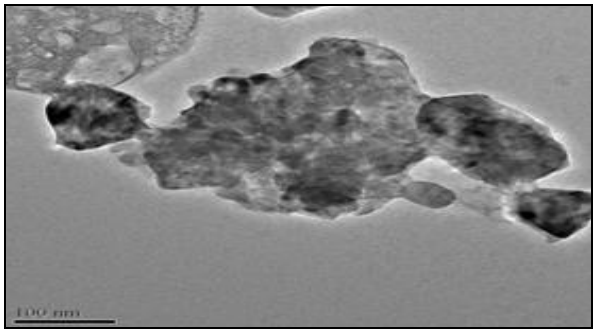
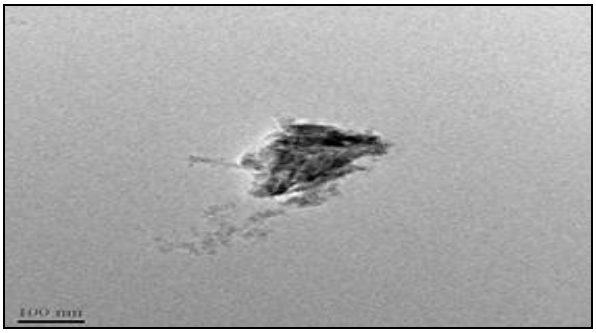


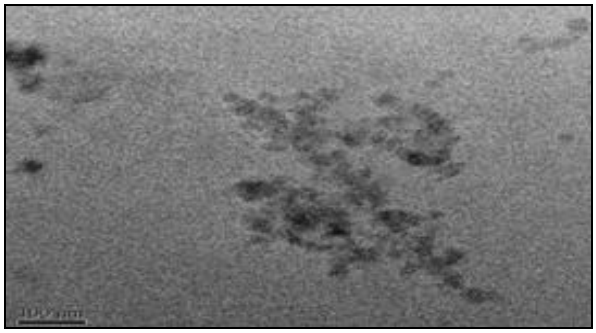
FIG. 3: XRD SPECTRA OF B-CYCLODEXTRIN AND PREPARED NANOSPONGES (NS1-NS5)



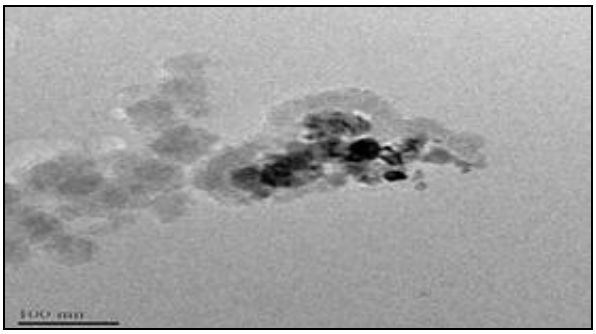
NS1



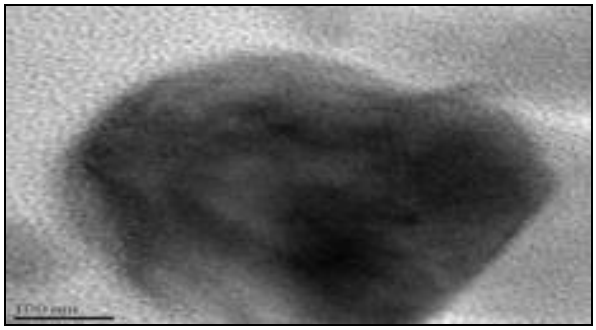
NS2



NS3



NS3



NS4

Fig. 4: TEM images of prepared nanosponges (NS1-NS5)

The solubilization efficiency of all the five types of nanosponges for tazarotene was studied and compared with the solubility of free tazarotene in distilled water. All the nanosponges (NS1-NS5) significantly enhanced the solubility, as shown in **Fig. 5**. Among all NS2 shown more solubilization efficiency (92.232 µg/ml) in comparison with plain tazarotene (1.263 µg/ml).

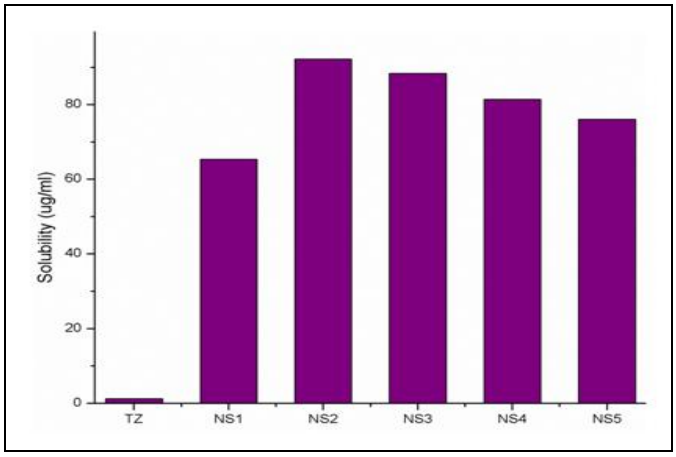


FIG. 5: SOLUBILIZATION EFFICIENCY OF NANOSPONGES

Tazarotene Loaded Nanosponges Preparation and Evaluation: Tazarotene was loaded into all five types of nanosponges by the freeze-drying method (TZNS1-TZNS5). The drug loading was determined for the estimation of drug association with nanosponges, *i.e.*, the amount of drug encapsulated into nanosponges, calculated by analyzing a fixed amount of formulation. The percent drug association is calculated by a formula as mentioned previously. Percent drug loading into all the five types of nanosponges (TZNS1-TZNS5) was presented in **Table 4**.

TABLE 4: PERCENT DRUG LOADING IN NANOSPONGES

S. no.	Name of the formulation	Drug loading (%)
1	TZNS1	21.32 ± 1.3
2	TZNS2	44.35 ± 2.42
3	TZNS3	45.86 ± 3.38
4	TZNS4	29.76 ± 2.12
5	TZNS5	24.56 ± 3.12

(All determinations were performed in triplicate and values were expressed as mean ± S.D., n=3)

Among the five types of nanosponges (NS1-NS5), the loading efficiency was found to be higher in NS3 (1:6 β-CD: DPC) as much as 45.86 % w/w, while lowest 21.32 % w/w in NS1. Owing to the better solubilization and loading capacities, TZNS3 was selected for further studies. The NS4 and NS5, the higher amount of cross-linker might have provided a hyper cross-linking of β-CD, and the interaction of tazarotene with β-CD cavities might have been hindered. It is evident from this; the degree of cross-linking will be a major factor affecting the extent to which the nanosponge forms inclusion complexation with the drug.

The particle size analysis of TZNS2 and TZNS3 suspensions revealed that the average particle size measured by the laser light scattering method is around 292-408 nm with a low polydispersity index. The particle size distribution is unimodal and having a narrow range, as seen in **Table 5**.

A sufficiently high zeta potential indicates that the complexes would be stable, and the tendency to agglomerate would be miniscule. A narrow PI means that the colloidal suspensions are homogenous in nature.

TABLE 5: PARTICLE SIZE, POLYDISPERSITY INDEX, AND ZETA POTENTIAL OF NANOSPONGES AND TAZAROTENE COMPLEXES

Sample	Mean hydrodynamic diameter ± SD (nm)	Polydispersity Index	Zeta potential (mV)
NS2	322 ± 32	0.22±0.005	-19.65±1.3
NS3	292 ± 17	0.20±0.005	-22.55±2.1
TZNS2	408 ± 27	0.35±0.005	-21.55±2.2
TZNS3	336 ± 47	0.25±0.005	-23.12±2.7

(All determinations were performed in triplicate and values were expressed as mean±S.D., n=3)

Characterization of Drug Loaded Nanosponges: Fig. 6 shows a comparison of FTIR spectra of NS2, NS3, tazarotene, TZNS2, and TZNS3 complexes. FTIR studies showed that there are weak interactions between NS and tazarotene that were evident from broadenings and disappearance of the drug peaks in case of complexes. The Comparison of FTIR spectra of tazarotene and complex showed that there is a major change in the fingerprint region *i.e.* 900 to 1,400 cm^{-1}. These characteristic peaks were broadened or shifted in the formulations suggesting definite interactions between tazarotene and nanosponges. Differential scanning calorimetry curves of the free tazarotene, plain nanosponges (NS2 & NS3), and tazarotene nanosponges (TZNS2 & TZNS3) are displayed in **Fig. 7**.

The DSC spectra of tazarotene showed a sharp endothermic peak at 98 °C correspondings to its melting point. The DSC spectra of nanosponges showed exothermic peaks at around 350°C. The tazarotene complex also showed a broad exothermic peak at around 350 °C. The complete disappearance of the drug endothermal peak was observed for the formulations obtained by freeze-drying. This phenomenon can be assumed as proof

of interactions between the components of the formulation. This can be considered as indicative of drug amorphization and/or inclusion complex formation. To study the physical nature of tazarotene with in the cyclodextrin nanosponges, XRD pattern of pure tazarotene, plain nanosponges (NS2 & NS3) and tazarotene loaded nanosponges (TZNS2 &TZNS3) were investigated. Some of the characteristic peaks of tazarotene demonstrated the high crystalline structure as shown in **Fig. 8**. However, there were no characteristics peaks of pure tazarotene were observed in nanosponge complexes. The absence of such crystalline peaks of tazarotene in complex clearly indicates the amorphization of drug within the porous structure of nanosponges.

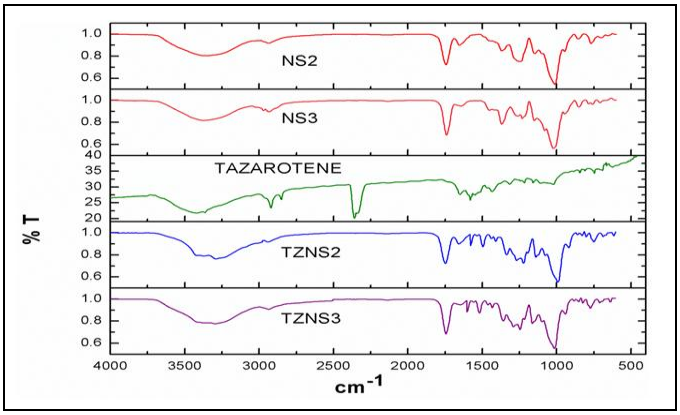


FIG. 6: FTIR SPECTRA OF PLAIN NANOSPONGES (NS2 & NS3), TAZAROTENE AND COMPLEXES (TZNS2 & TZNS3)

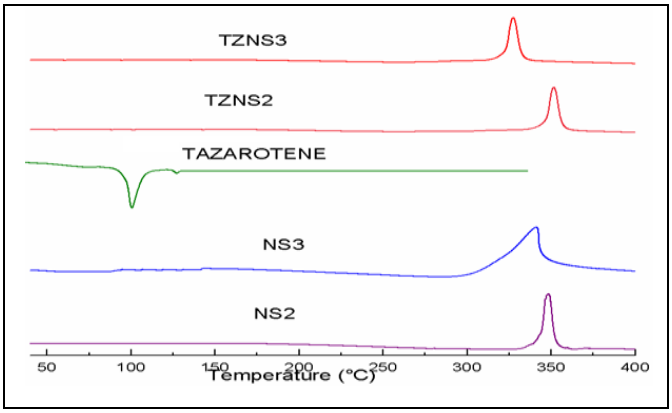


FIG. 7: DSC THERMOGRAM OF PLAIN NANO-SPONGES (NS2 & NS3), TAZAROTENE AND COMPLEXES (TZNS2 & TZNS3)

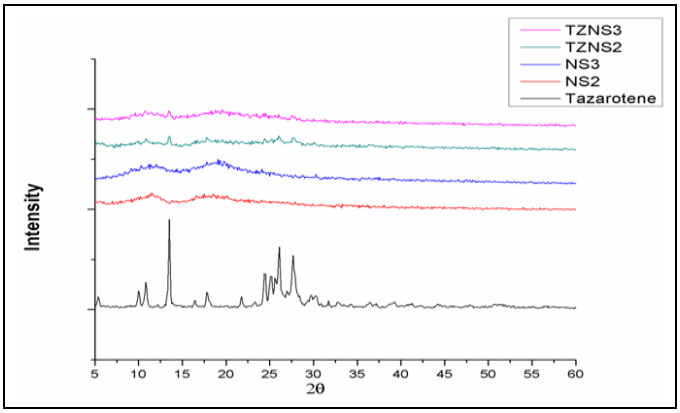
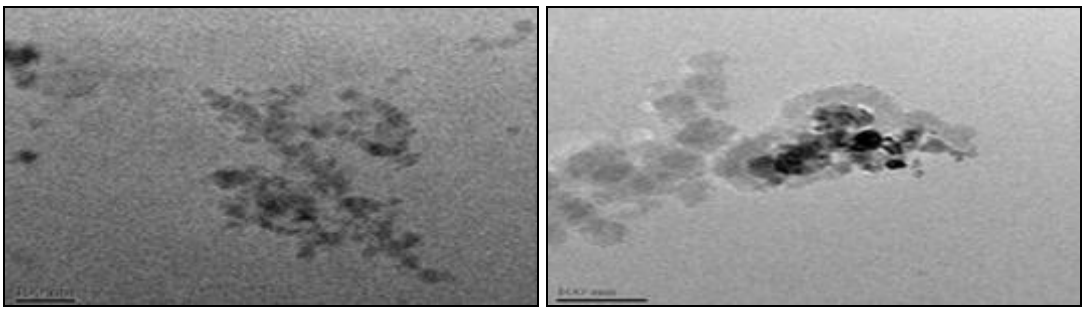


FIG. 8: XRD SPECTRA OF PLAIN NANOSPONGES (NS2 & NS3), TAZAROTENE AND COMPLEXES (TZNS2 & TZNS3)



TEM OF SN2 **TEM OF SN3**

TEM OF TZSN2 **TEM OF TZSN3**

FIG. 9: TEM IMAGES OF PLAIN NANOSPONGES AND DRUG LOADED NANOSPONGES

Transmission electron microscopy (TEM) studies showed that the regular spherical shape of both the nanosponges that are unaffected even after drug encapsulation as shown in **Fig. 9**.

Drug Release Study: Dissolution rates of tazarotene pure drug suspension and nanosponge complexes were evaluated. All formulations showed an increase in dissolution over the pure drug, which showed only ≈ 21 % release after 12 h. The Relatively higher dissolution enhancement could be credited to more intimate drug-carrier interaction during the formulation of nanoparticles. The Invitro drug release pattern of the drug from the optimized batches is as shown in **Fig. 10**.

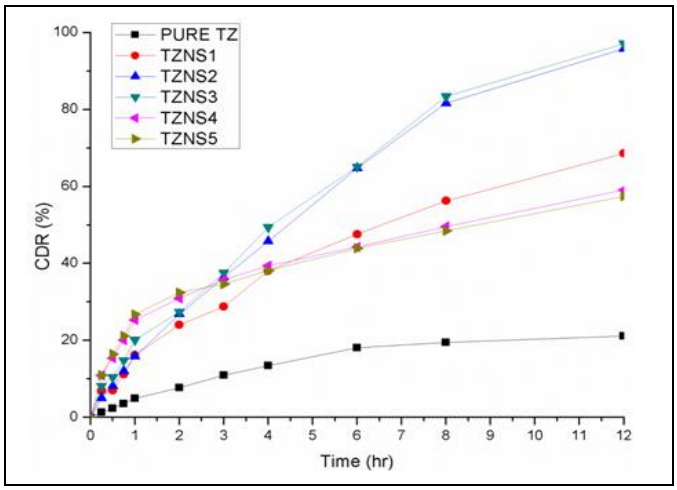


FIG. 10: DISSOLUTION PROFILE OF PURE TAZAROTENE AND TAZAROTENE LOADED NANOSPONGE FORMULATIONS

The drug release data indicate high linearity when plotted by both zero-order and first-order equations. Further, the translation of the data from the dissolution studies suggested the possibility of understanding the mechanism of drug release by configuring the data in various mathematical modeling such as Higuchi and Korsmeyer-Peppas plots. In the Korsemeyer-Peppas model, the value of n characterizes the release mechanism of the drug. If the n value is less than 0.45 ($0.45 \leq n$) indicates Fickian diffusion mechanism, $0.45 < n < 0.89$ indicates non-Fickian transport, n= 0.89 indicates case II (relaxational) transport, and $n > 0.89$ indicates super case II transport.

The n value obtained from the Korsmeyer-Peppas plots *i.e.* 0.679 indicating non-Fickian diffusion mechanism transport thus it projected that delivered its active ingredient in a controlled manner **Fig. 11**.

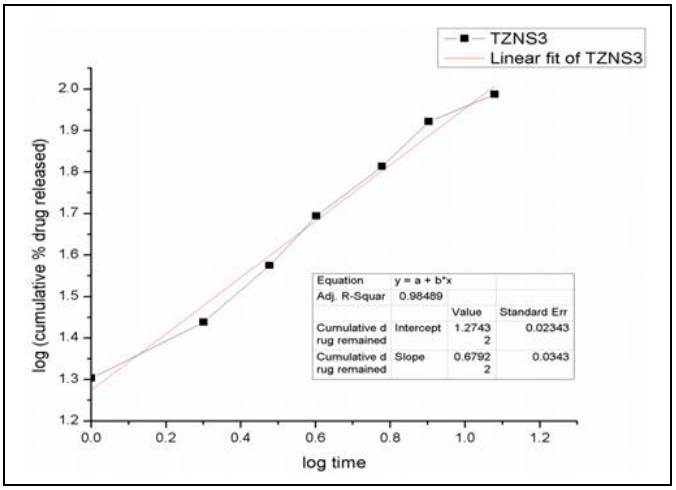


FIG. 11: PLOT OF KORSMEYER- PEPPAS RELEASE KINETICS OF THE NANOFORMULATION

Preparation and characterization of Gel Formulation: Based on reported literature, Carbopol 934 polymer was used to prepare the gel base formulation of tazarotene-loaded nanosponges [40]. The nanosponges containing tazarotene have been formulated with Carbopol 934 as hydrogel using 2% w/w of Propylene glycol and 2% w/w of N-methyl-2-pyrolidone as permeation enhancers. 1% w/w of Triethanolamine was used for neutralization of the gel. In a previous study, carbopol hydrogel with the same composition resulted in the enhanced stability and permeation of econazole nitrate [41].

pH of Gel Formulations: The pH of both the formulations was found between 4.35 and 5.12, thus lying in the normal pH range of skin, 3.0–9.0; hence the preparation will be non-irritating.

***In-vitro* and *In-vivo* Skin Permeation Studies:** In order to evaluate the influence of nanosponge encapsulation on tazarotene permeation and deposition in the skin, in vitro skin permeation studies across hairless mice skin were conducted using vertical Franz diffusion cells at 37 °C. Skin permeation studies were carried out with gel formulation of tazarotene-loaded nanosponges and plain tazarotene gel formulation, using tazarotene solution as the control. Hereafter the formulations will be mentioned as Control (plain tazarotene solution), Test 1 (gel formulation of plain tazarotene), Test 2 (gel formulation of TZNS3). In our study, sampling was carried out from the receptor media at 0.5, 1, 2, 4, 6, 8, 10, and 12 h to determine if there was any transdermal delivery of

tazarotene. However, no tazarotene was detected in the receptor phase after 12 h of both control and Test formulations, indicating the lack of transdermal delivery. At the end of the 12 h permeation study, the amount of tazarotene deposited in the stratum corneum and epidermis/dermis of rat skin was determined. The results of the skin permeation parameters are listed in **Table 6**.

TABLE 6: *IN-VITRO* SKIN PENETRATION OF TAZAROTENE SOLUTION IN PROPYLENE GLYCOL AND THE CARBOPOL GEL FORMULATIONS CONTAINING ENCAPSULATED OR NON-ENCAPSULATED TAZAROTENE, 12 H AFTER TOPICAL APPLICATION

Sample	Tazarotene (µg/cm^2)	
	Stratum corneum (SC) (µg/cm^2)	(Epidermis + Dermis) (µg/cm^2)
Control	2.16 ± 0.71	0.86 ± 0.22
Test1	6.78 ± 1.43*	2.6 ± 0.74*
Test 2	36.12 ± 1.12**	9.12 ± 1.03**

Results are represented by mean ± S.D. (n = 3). *Significant statistical difference compared to control ($p < 0.05$). **Significant statistical difference compared to control ($p < 0.01$)

Compared to control, both the test formulations significantly enhanced the penetration of tazarotene 12 h after application. The cumulative amounts of tazarotene from all formulations (Control1, Test 1, Test 2) at 12 h after dosing were 2.16 ± 0.71 µg/cm^2, 6.78 ± 1.43 µg/cm2 and 36.12 ± 1.12 µg/cm^2 respectively. In other words, the cumulative amount of tazarotene penetrating through the skin from gel formulation containing nanosponge encapsulated tazarotene was around 6 times more than that from gel formulation containing free tazarotene at 12 h. The distribution of tazarotene in the excised rat skin is as shown in **Fig. 3** [20]. The time-course of the *in-vitro* skin penetration showed that when tazarotene was incorporated in nanosponges, its concentration in SC was significantly enhanced at 3 h, 6 h, 9 h, and 12 h ($P< 0.05$) post-application. Similarly, tazarotene concentration in the [E + D] also was significantly enhanced after 3 h, 6 h, 9 h, and 12 h ($P< 0.05$). The higher permeation and deposition of tazarotene might be due to the combination of nanosponge encapsulation and the penetration-enhancing effect of Carbopol gel formulation. The release rate (flux) of tazarotene across the membrane and excised skin differs significantly. **Table 7**, which indicates the barrier properties of the skin. Interaction between the skin and nanoformulation components may justify these differences. Association and fusion of nanoparticles to the skin surface resulted in higher flux due to the direct transfer of drugs from the vesicles. The flux value for nanosponges based gel formulation (189.342 ± 3.879 µg cm^{-2} h^{-1}) was found to be higher than that for plain tazarotene (106.765 ± 4.123 µg cm^{-2} h^{-1}). Same observations were also made while considering the permeability coefficient (Kp). The difference in the flux and Kp values of plain tazarotene gel and nanosponges-based gel was also significant ($p <0.05$). The higher flux and Kp values of nanosponges-based gel suggest that it might be able to enter the skin easily compared with plain tazarotene gel with an advantage of low interfacial tension of the emulsifier film ensures an excellent contact to the skin.

TABLE 7: FLUX OF TAZAROTENE FROM NANO-SPONGES BASED GEL FORMULATION

Formulation	Flux (µg cm^{-2} h^{-1})	
	Egg membrane	Rat skin
TZNS gel	59.542 ± 2.12	189.342 ± 3.879
Tazarotene gel	34.356 ± 5.13	106.765 ± 4.123
Control	22.834 ± 2.89	36.782 ± 3.88

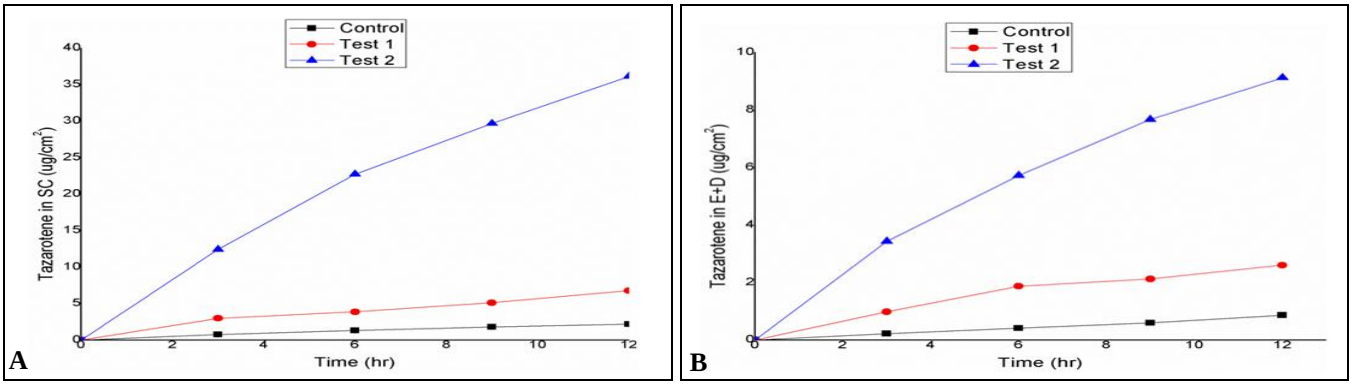


FIG. 12: THE TIME COURSE OF IN-VITRO SKIN PERMEATION OF TAZAROTENE INCORPORATED INTO GEL FORMULATION OR CONTROL FORMULATION IN A. STRATUM CORNEUM B. EPIDERMIS AND DERMIS

Draize PATCH TEST: One of the major disadvantages associated with tazarotene therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. Ideally, the delivery system of tazarotene should be able to diminish or abolish these erythematic episodes. However, most of the currently marketed conventional dosage forms such as creams, lotions, and gels cannot reduce the irritation caused by topical application of tazarotene. It was hypothesized that encapsulation of tazarotene in the cyclodextrin nanosponges would reduce skin irritation. The results obtained from the primary skin irritation studies are listed in **Table 8**.

A Draize patch test is a reliable method, and the results obtained from this study can be linked to that obtained in humans. The skin-irritation studies indicated that nanosponges-based gel containing tazarotene did not show any sign of skin irritation as compared to moderate erythema shown by plain tazarotene gel formulation after 72 h of application **Table 8**. Thus, nanosponges-based gel formulation demonstrated an advantage over-marketed formulation in improving the skin tolerability of tazarotene indicating their potential in improving patient acceptance and topical delivery of tazarotene.

TABLE 8: MEAN ERYTHEMAL SCORES FOR VARIOUS TAZAROTENE FORMULATIONS OBTAINED AT THE END OF 24, 48, AND 72H

Formulations	Erythmeal score (n = 3)		
	24 h	48 h	72 h
Control (Group 1)	0	0	0
Plain tazarotene gel (Group 2)	0	1	1
Nanosponges based gel without tazarotene (Group 3)	0	0	0
Nanosponges based gel containing tazarotene (Group 4)	0	0	0

Photodegradation and Longterm Stability Studies: For photodegradation studies, free tazarotene, Carbopol gel formulations containing free tazarotene (TZgel), and nanosponge encapsulated tazarotene (TZNS3gel) were exposed to UVA lamp with a 320-400 nm wavelength range and the extent of degradation was measured by HPLC. 22.52 % of the drug was degraded when the gel formulation containing plain tazarotene exposed to UVA lamp. A marked reduction in the extent of tazarotene photodegradation was attained in the formulations containing the nanosponge encapsulated tazarotene, which demonstrated the protective effect of the nanosponges. The results were as presented in **Table 9**. In order to evaluate whether the gel formulation achieved the enhancement of tazarotene photostability, photostability experiments were performed with free tazarotene. Around 28.34% of drug was degraded when it is exposed as such to UVA lamp. This result is indicating that the carbopol gel formulation is contributing to some protection against photodegradation. In order to evaluate whether the enhancement of tazarotene photostability achieved by the nanosponges varied with time, additional photolysis experiments were performed after 3-month storage of the same formulations at room temperature and in the dark. The percentage loss of the tazarotene upon irradiation of the examined formulations was presented in **Table 9**. The results indicated the phytostabilization properties of the nanosponges in gel formulation were retained after 3-month storage.

TABLE 9: PHOTODEGRADATION VALUES FOR FREE AND NANOSPONGE ENCAPSULATED TAZAROTENE IN GEL FORMULATIONS, IMMEDIATELY AFTER PREPARATION AND AFTER 3-MONTH STORAGE

Sample	% tazarotene degraded	
	Immediately after preparation	After 3-month storage
Free tazarotene (Control 1)	28.34 ± 2.1	29.14 ± 3.1
Tazarotene in gel formulation (Control 2)	22.52 ± 2.4*	24.13 ± 2.4*
TZNS3 in gel formulation (Test 1)	6.53 ± 1.1**	7.68 ± 1.1**

Each value is the mean ± S.D. of three determinations. *Significant statistical difference compared to control 1 ($p < 0.05$). ** Significant statistical difference compared to control 2 ($p < 0.05$)

To examine the effect of nanosponges on the chemical stability of tazarotene, an aging study was performed on the same formulations used in photodegradation studies. The formulations were analyzed for tazarotene over 3 months at room temperature and in the dark, and the generated results are illustrated in figure 13. Around 7.5 % of the drug was degraded within 3 months from free tazarotene, and around 4.5 % of the drug was

degraded from the gel formulation containing non-encapsulated tazarotene. On the other hand, no decrease of the tazarotene was detected, after the same time interval, in the nanosponge encapsulated formulations. These results signify the protective capabilities of cyclodextrin nanosponges.

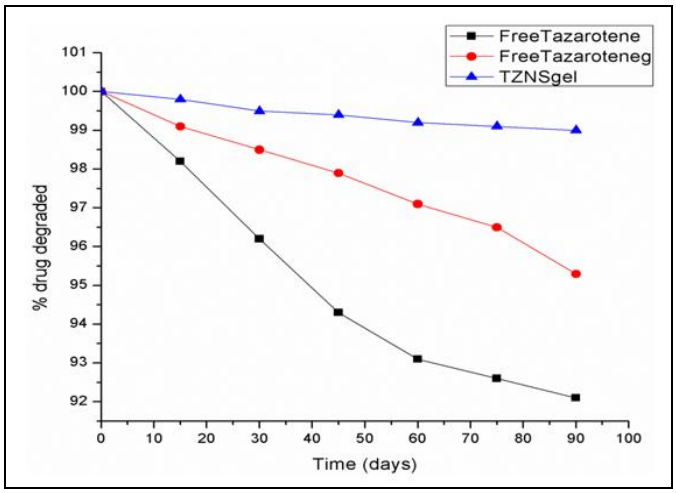


FIG. 13: STABILITY OF FREE AND ENCAPSULATED TAZAROTENE FOR 3 MONTHS

CONCLUSION: The present study demonstrated the preparation of tazarotene-loaded nanosponges by freeze-drying technique. FTIR, DSC and XRD studies confirmed the formation of inclusion complex of tazarotene with nanosponges. The dissolution of the tazarotene nanosponges was significantly higher compared with the pure drug due to the reduction of drug particle size, the formation of a high-energy amorphous state, and the intermolecular hydrogen bonding. The release kinetics may be prolonged or accelerated according to the type of nanosponges. The relative bioavailability of tazarotene nanosponges with respect to plain tazarotene was obtained as 850 %. The nanosponge formulations were incorporated into a model carbopol gel formulation and were evaluated for skin permeation, antifungal activity, and stability. Skin permeation studies demonstrated that nanosponge encapsulated tazarotene was able to exhibit controlled release of tazarotene for 12 hours across rat skin, and percent drug diffused from nanosponge formulation was nearly 8 fold higher than free tazarotene gel formulation.The flux value for nanosponges based gel formulation (189.342 ± 3.879 µg cm^{-2} h^{-1}) was found to be higher than that for plain tazarotene (106.765 ± 4.123 µg cm^{-2} h^{-1}), indicating easier penetration. Moreover, during 3-month storage of the formulations at room temperature and in the dark, the chemical instability of tazarotene was almost completely suppressed by the nanosponge-based gel formulation. The results collectively suggest that because of the controlled drug release, better skin permation and good storage stability, cyclodextrin nanosponges-based gel formulation of tazarotene has tremendous potential to serve as a topical delivery system.

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CONFLICTS OF INTEREST: Nil

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