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Development Of Cyclodextrin Based Nanosponge Loaded Tazoretene Gel: Characterization & In Vivo Evaluation

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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 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 maximum solubilization efficiency. Tazarotene was loaded into five β-Cyclodextrin nanosponge formulations by freeze drying method and evaluated. The particle size of 336 nm, zeta potential of -23 mV and maximum drug dissolution of 97% in 12h were displayed by TZNS3 hence formulated into gel and evaluated. The optimized tazarotene loaded nanosponge formulation (TZNS3) was incorporated into a model carbopol gel formulation and were evaluated for invivo animal studies, and stability. This reduction may be the result of anti-oxidant and anti-inflammatory activity associated with chitin. Test 2 caused significant reduction in IMQ induced inflammatory process with absence of parakeratosis and decrease in acanthosis. 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.

Keywords: Tazarotene, β-cyclodextrin nanosponges, anti-inflammatory, nanosponge gel, Psoriasis Area and Severity Index, animal studies.

INTRODUCTION

Nanoparticles are the colloidal systems with particle size varying from 10 nm to 1000 nm. Different types of nanoparticles are polymeric nanoparticles, solid lipid nanoparticles, liposomes, proliposomes, niosomes, liquid crystalline systems, quantum dots etc. Nanosponges (NS) conquer the problems of all the nanoparticles and solubilize poorly water-soluble drug and provide prolonged release as well as improves drugs bioavailability (Madhuri et al., 2015). 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 (Ashwini and Pritesh., 2019). For delivery of NS, topical gels are very attractive drug delivery systems. Tazarotene, as the third-generation drug for retinoic acid, had been reported to have organizational-reconstruction, normalizing-of-differentiation, and anti-inflammatory functions (Weinstein et al.,

1997). Overall, tazarotene has a good safety profile and is not associated with carcinogenicity, contact sensitization, mutagenicity, photoallergic reactions, or phototoxicity (Feldman et al., 2013). 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 (Gregoriou et al., 2014). 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 the 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. 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.

Preparation of β-Cyclodextrin nanosponges (NS)

Cyclodextrin based nanosponges prepared using diphenyl carbonate (DPC) for the cross linking as reported elsewhere (Swaminathan et al., 2013). 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 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 nanosponges were dried under vaccum and stored at 25 °C until further use. The obtained nanosponges were termed as NS1, NS2, NS3, NS4 and NS5 based on the molar ratio of reactants. The particle size and practical yield of obtained nanosponges was determined (table 1)

Characterization of cyclodextrin nanosponges

Particle Size, Polydispersity index and Zeta Potential Determination of cyclodextrin 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). (Anandam et al., 2014)

Solubilization Efficiency of nanosponges

An excess quantity of tazarotene (10mg) was suspended with fixed quantity (20 mg) of NS in 20 ml Milli Q water. 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). (Ansari et al., 2011)

Preparation of tazarotene -loaded nanosponges (TZNS)

Drug loaded nanosponges were prepared by freeze drying technique as previously reported. 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 drug loaded NS formulations obtained were named as TZNS1, TZNS2, TZNS3, TZNS4 and TZNS5 depending upon the type of NS. (Swaminathan et al., 2010)

Determination of tazarotene loading in nanosponges

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) analysed as per the procedure adopted for cyclodextrin nanosponges. (Carlotti et al., 2011)

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

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., 2012. 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.

Evaluation of gel formulations

pH determination

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

In-vitro 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.

In Vivo Animal Studies

Imiquimod (IMQ) induced psoriatic animal model and optimization

Activation of Toll receptors expressed on the Dendritic Cells (DC) by IMQ resulted in the production of inflammatory cytokines including interleukins, TNF-α, IFN-δ etc. Secreted cytokines lead to deregulated cell proliferation, with incomplete differentiation of cells leading to psoriatic histological features like acanthosis, parakeratosis, and extended rete pegs. Each day erythema, thickness scaling, is scored and cumulative Psoriatic Area Severity Index (PASI) score is calculated as shown (Fig below). PASI combines the severity of the psoriatic lesions and the area affected in a single score. Ideally to consider an anti-psoriatic therapy effective clinically a 75%reduction in PASI (PASI 75) is needed. (Dan Liu et al., 2013)

Anti-psoriatic activity studies

BALB/c mice of age 8-11 weeks, were treated with commercially available imiquimod cream (Imiquad cream 5%, manufactured by Glenmark pharmaceuticals) to develop psoriatic type skin lesions. Topical application of 62.5 mg of Imiquad cream (3.125mg of imiquimod) on the shaved back of mice for 7 consecutive days resulted in psoriatic type lesions. In order to retain the developed psoriatic lesions, we continued the application of the imiquimod intermittently (for 3 days at an interval of three days) for further two weeks as demonstrated in optimization studies. (Song et al., 2012). The study was approved by institutional animal ethics committee with reference no: 1447/PO/Re/S/11/CPCSEA-24/A.

Scoring of psoriasis

The extend of inflammation on mice back skin was scored by using an objective scoring system; Psoriasis Area and Severity Index (PASI). Markers of inflammation; erythema, thickness and scaling were scored separately from 0 to 4 with none: 0; slight:1; moderate:2; marked:3 and severe:4. PASI scoring was done for every animal on all days.

Photo degradation studies

The degree of photo degradation was measured by comparing the peak areas of tazarotene from the irradiated samples, with those obtained by analysis of an equivalent amount of the non-exposed formulations. (Santo Scalia et al., 2009)

Stability studies

Stability studies were performed on the same formulations utilized for the photodecomposition experiments. 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 for 3months (Bregn et al., 2008)).

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. (Table 2)

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 low polydispersity index. Table 3.
Solubilization efficiency of all the five types of nanosponges for tazarotene was studied and compared with the solubility of free tazarotene in distilled water. Among all NS2 shown more solubilization efficiency (92.232 µg/ml) in comparison with plain tazarotene (1.263 µg/ml). (Figure 1)

Tazarotene loaded nanosponges preparation and evaluation

The drug loading was determined for the estimation of drug association with nanosponges, i.e. Percent drug loading into all the five types of nanosponges (TZNS1-TZNS5) were presented in table 4. 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.
The particle size analysis of TZNS2 and TZNS3 suspensions revealed that the average particle size measured by laser light scattering method is around 292-408 nm with low polydispersity index (table 5).

Characterization of drug loaded nanosponges

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 (Figure 2). Differential scanning calorimetry curves of the free tazarotene, plain nanosponges (NS2 & NS3) and tazarotene nanosponges (TZNS2 & TZNS3) are displayed in figure 3. The tazarotene complex also showed a broad exothermic peak at around 350°C.

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 (figure 4).
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 figure 5.

Drug release study

All formulations showed an increase in dissolution over pure drug, which showed only ≈ 21 % release after 12 h. The In vitro drug release pattern of drug from the optimized formulation was found to be 97.12% after 12h is as shown in figure 6.

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.

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

The cumulative amounts of tazarotene from all formulations (Control1, Test 1, Test 2) at 12 h after dosing were 2.16 ± 0.71 µg/cm2, 6.78 ± 1.43 µg/cm2 and 36.12 ± 1.12 µg/cm2 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 (table 6) (figure 7A and 7B).
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 ($P< 0.05$).

Photo degradation and stability studies

For photo degradation studies free tazarotene, Carbopol gel formulations containing free tazarotene (TZ gel) 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 (table 7).
Around 7.5 % of the drug was degraded within 3 months from free tazarotene and around 4.5 % of drug was degraded from the gel formulation containing non-encapsulated tazarotene (figure 8).

In-vivo animal studies

PASI scoring was done for every animal on all days and is presented in table and skin histology analysis (figure 9) revealed orthokeratotic stratum corneum with intact granular layer and organized thin epidermis in control animals. The histology of Test 1 treated animals showed lining of epidermis with regular acanthosis, focally fused rete pegs, spongiosis, intra epidermal neutrophillic infiltrate forming corneal neutrophillic abscess, overlying dermis with dermal appendages and congested vessels. CNG treated skin histology is very much similar to that of IMQ treated one except that there is slight reduction in IMQ induced acanthosis. Test 2 caused significant reduction in IMQ induced inflammatory process with absence of parakeratosis and decrease in acanthosis (table 8).

CONCLUSION

The present study demonstrated the preparation of tazarotene loaded nanosponges by freeze drying technique. 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. The nanosponge formulations were incorporated into a model carbopol gel formulation and were evaluated for in-vivo animal studies and stability. The IMQ-induced inflammatory process was significantly reduced in Test 2, with no parakeratosis and a decrease in acanthosis. Moreover, the chemical instability of tazarotene, during 3-month storage of the formulations at room temperature and in the dark, was almost completely suppressed by the nanosponge based gel formulation. 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.

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TABLES

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

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

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)

Table 4: Percent drug loading in nanosponges

S.NO	Name of the formulation	Drug loading (%)
1	TZNS1	21.32 ± 1.31
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)

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)

Table 6. In-vitro skin penetration of tazarotene solution in propylene glycol and the carbopol gel formulations containing encapsulated or nonencapsulated 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 ($p < 0.01$)

Table 7. Photo degradation 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 ($p < 0.05$)

Table.8: PASI score of animals in anti-psoriatic activity studies of various treatment.

Treatment	Average PASI		Reduction in PASI (%)
	On Psoriasis induction	At the end of therapy	
Control	7.5	6.1	21.43±5.21
Test 1	7.66	0.83	89.22±5.48
Test 2	7.33	0.5	93.28±5.12

FIGURES

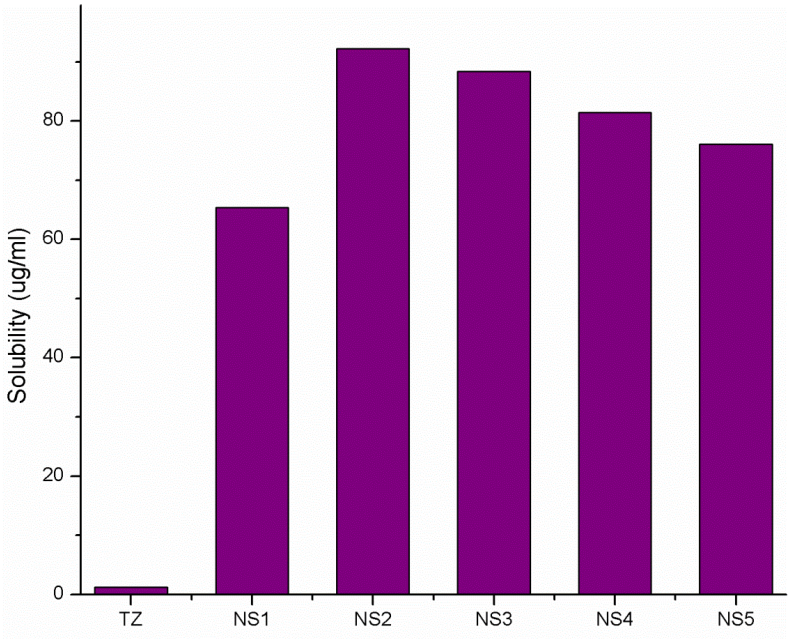


Figure 1: Solubilization efficiency of nanosponges

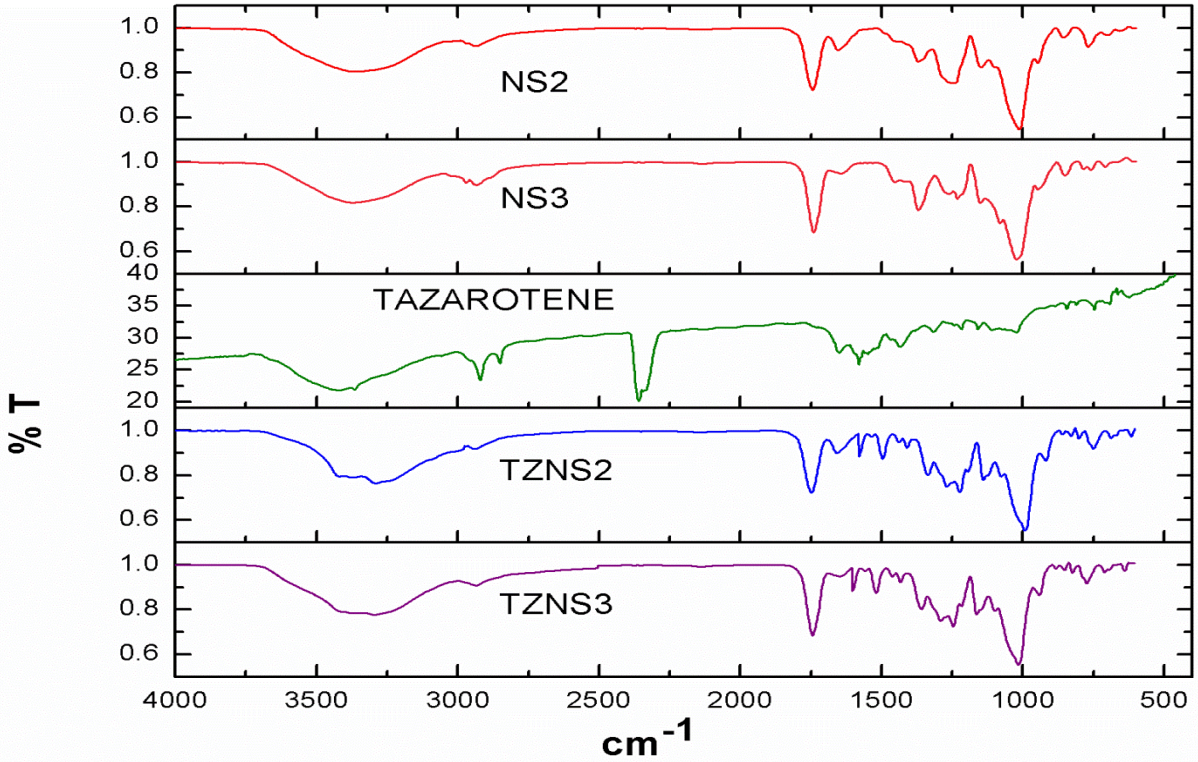


Figure 2: FTIR spectra of Plain nanosponges (NS2 & NS3), tazarotene and complexes (TZNS2 & TZNS3)

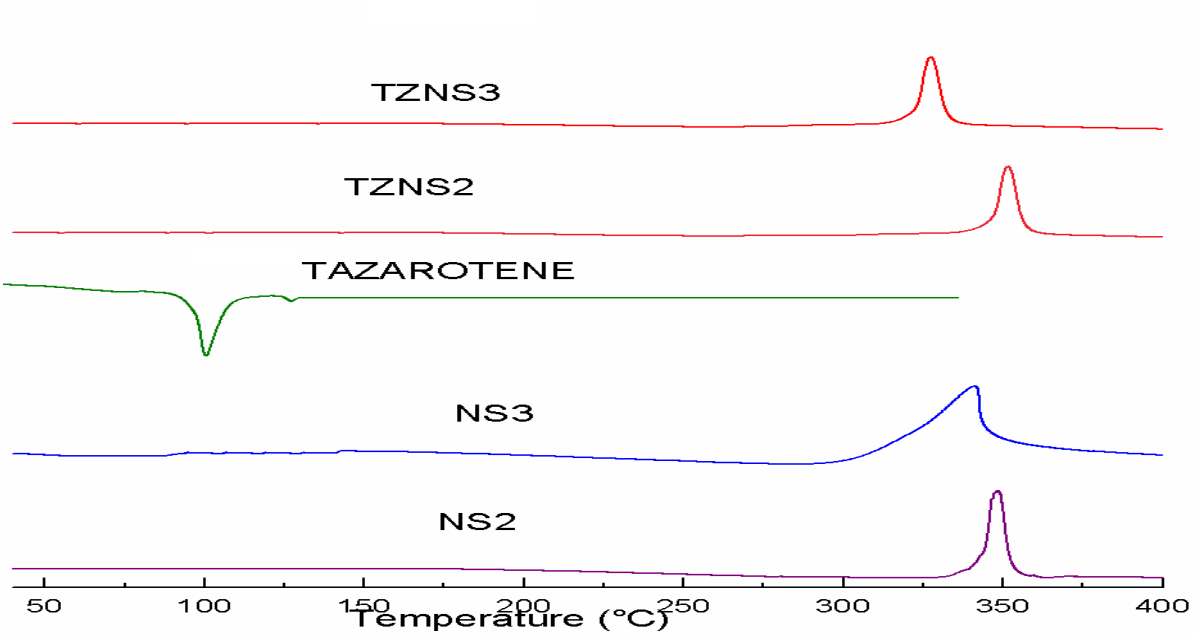


Figure 3: DSC thermogram of Plain nanosponges (NS2 & NS3), tazarotene and complexes (TZNS2 & TZNS3)

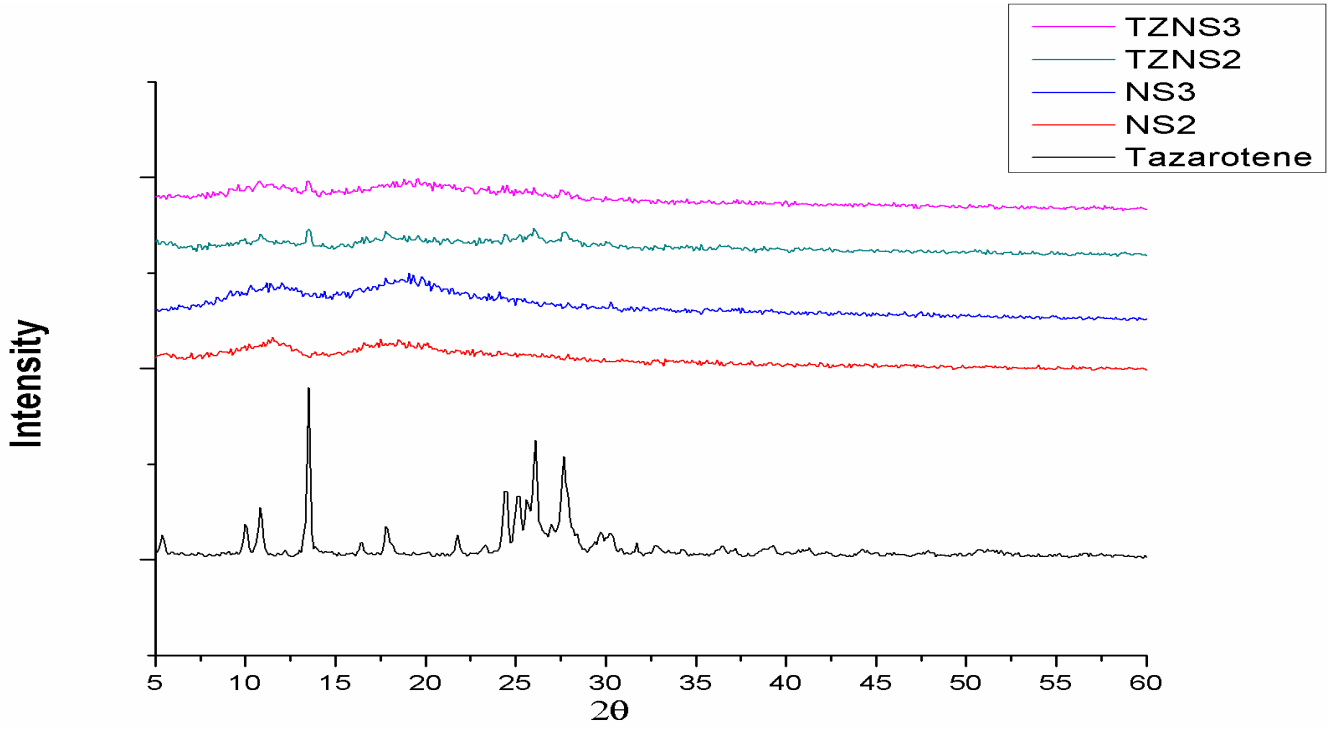


Figure 4. XRD spectra of Plain nanosponges (NS2 & NS3), tazarotene and complexes (TZNS2 & TZNS3)

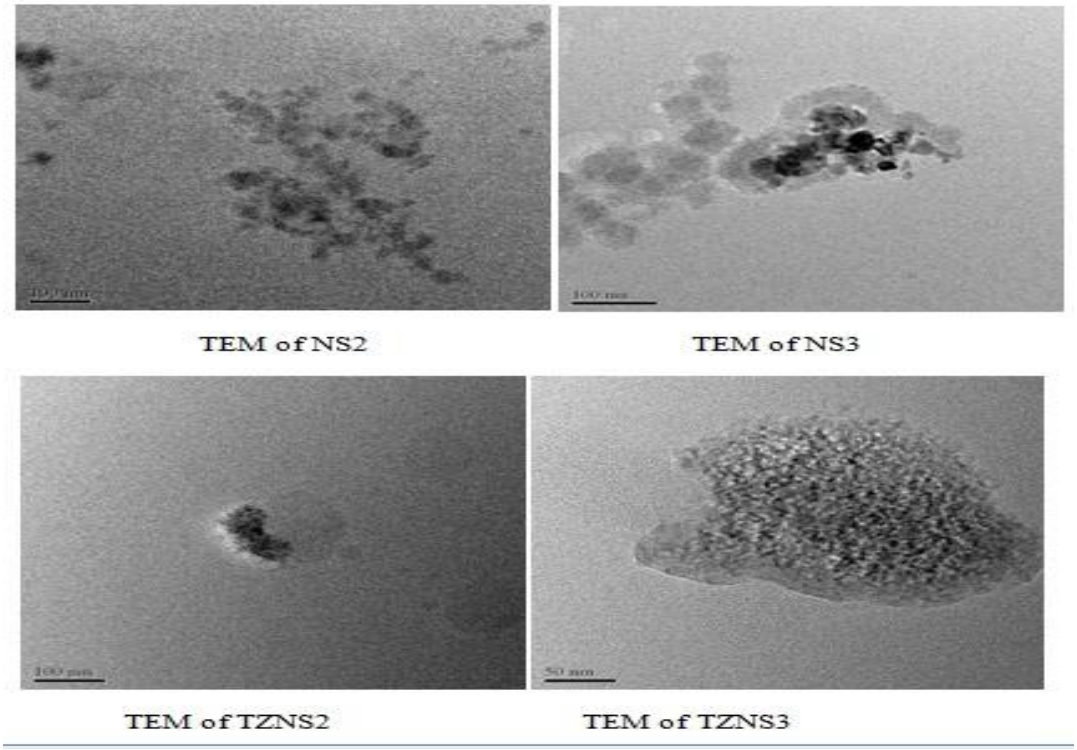


Figure 5: TEM images of plain nanosponges and drug loaded nanosponges

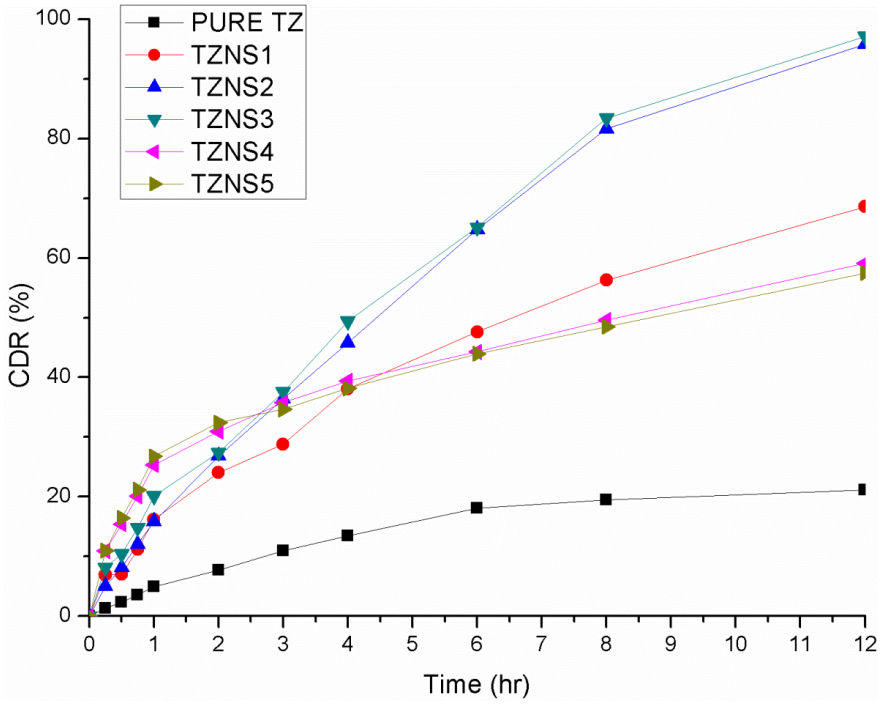


Figure 6: Dissolution profile of pure tazarotene and tazarotene loaded nanosponge formulations

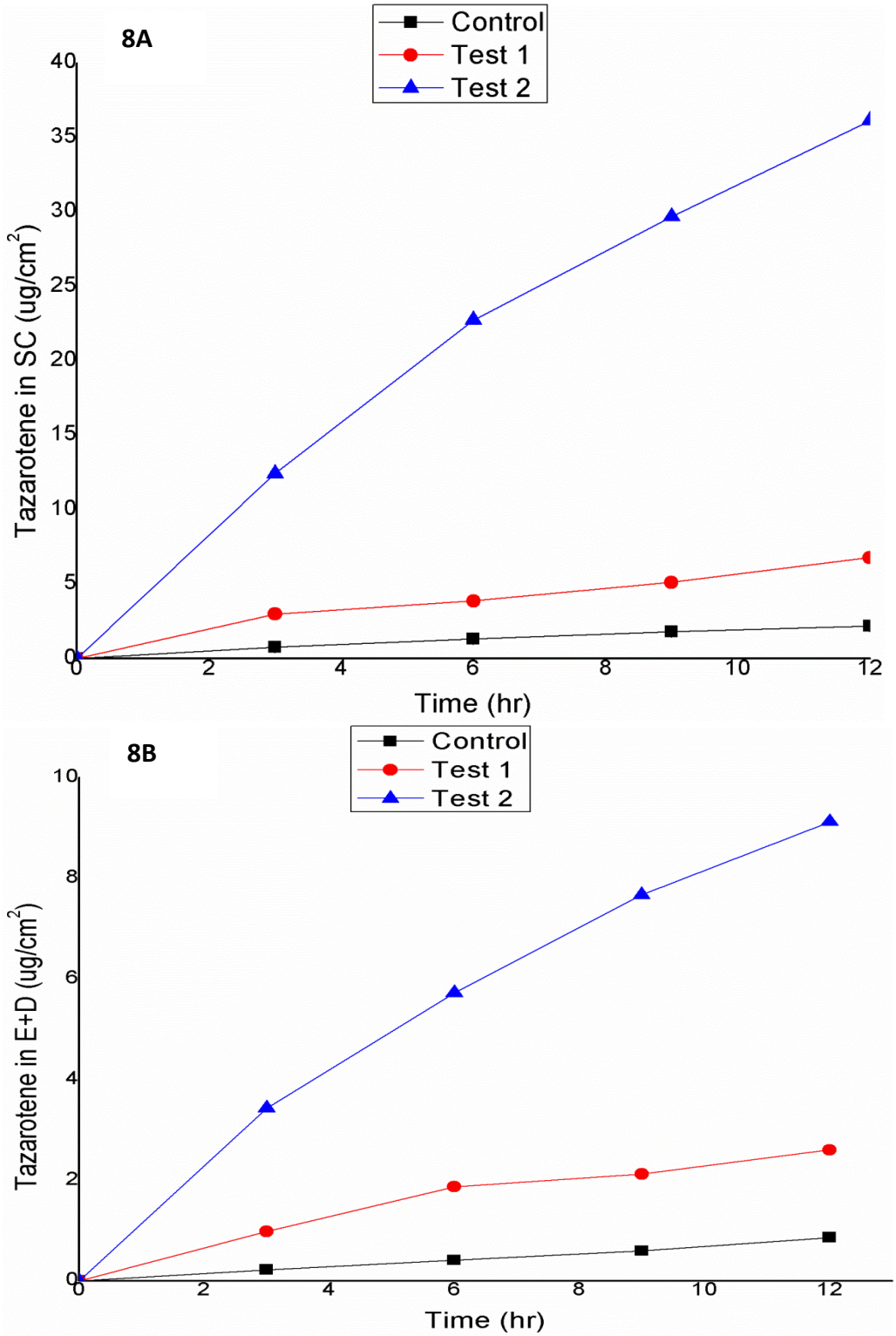


Figure 7. The time course of in-vitro skin permeation of tazarotene incorporated into gel formulation or control formulation in A. Stratum corneum B. Epidermis and dermis

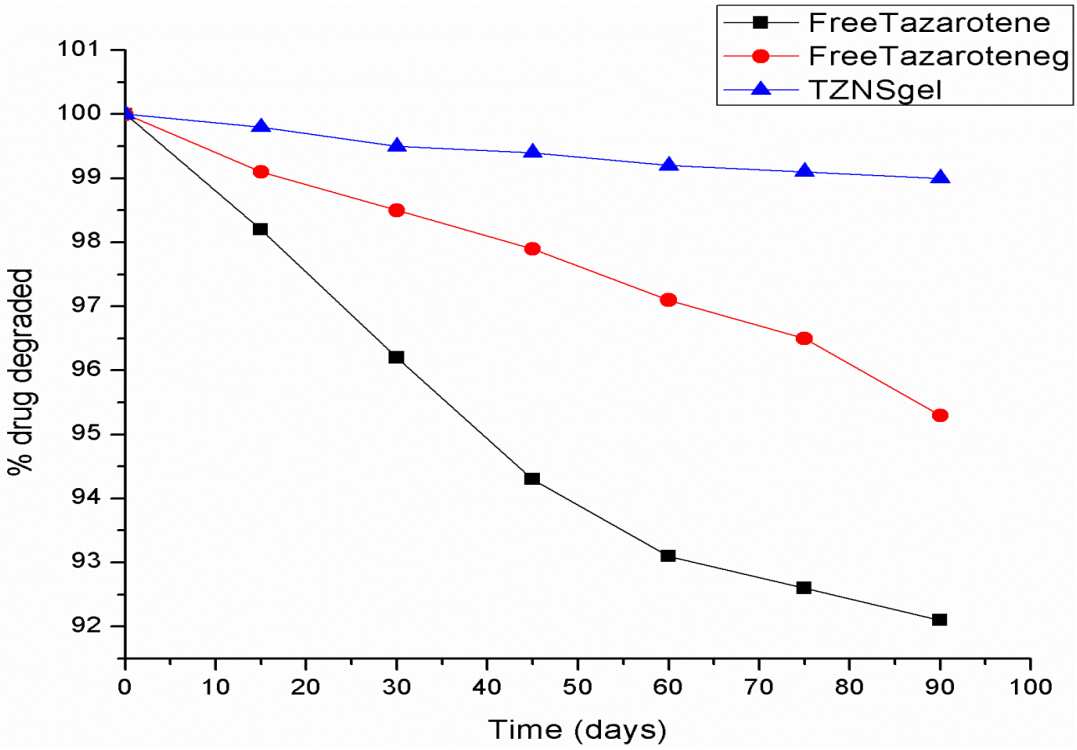


Figure 8. Stability of free and encapsulated tazarotene for 3 months

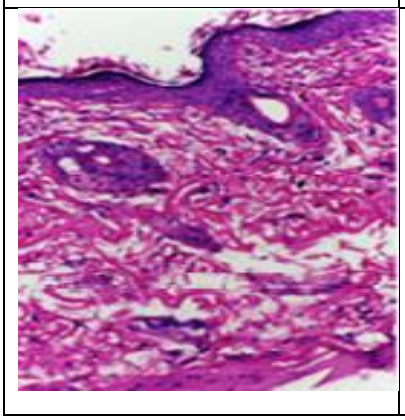
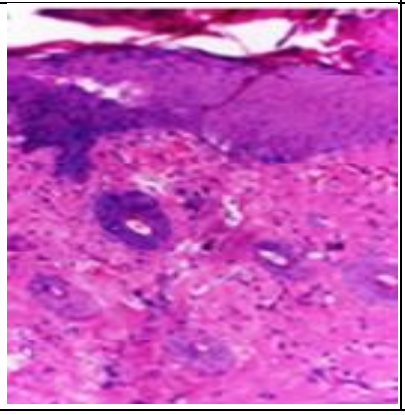
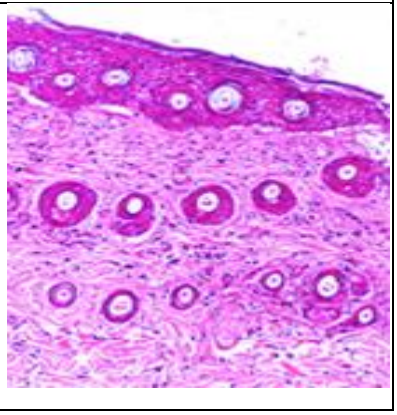
Control	Test 1	Test 2
		

Fig 9. Skin histology of animals in anti-psoriatic activity