Section A-Research paper
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Nanosponges-based hydrogel formulation containing psoralen to enhance topical delivery

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Abstract

Purpose: The present work aimed to design nanosponge based hydrogel formulation containing psorlen to enhance topical delivery.

Material and Method: The different polymers at various ratios were used to formulate the nanosponges (F1 to F 12). The nanosponges (NS) were studied for entrapment efficiency, particle size, structural properties, size and appearance, and in vitro drug release. The formulation was further evaluated for its anntipsoriatic potential.

Result and Discussion: After primary evaluation formulations F3, F5, F7 and F12 were selected for further studies including drug release and FESM. The nanosponges (F5) made using PVA: EC (1:1) were determined to be superior in the in-vitro release testing and were subsequently selected for antipsoriatic potential investigation.

Conclusion: It was concluded that the formulation F5 showed better antipsoriatic potential was evaluated by topical application of formulation to oxazolone induced psoriasis in mice for 16 days.

Keywords Drug release, Franz diffusion cell, Higuchi diffusion, Nanosponges, Psoralen, Topical delivery.

Introduction

Psoriasis is a chronic, noncommunicable, painful, disfiguring, and disabling illness with no cure that greatly reduces quality of life. Plaque, flexural, guttate, pustular, and erythrodermic these are the types of psoriasis. It is more common among 50–69-year age group [1]. Psoriasis is a global issue with a prevalence of 0.09% [2] to 11.4% [3].

Mild trauma, sunburn, infections, systemic medicines, and stress can induce psoriasis [4]. Comorbidities and skin and nail psoriasis are linked. Skin lesions are frequently symmetrical, sharply delineated, red papules and plaques, and covered in white or silver scales. In addition to hurting, lesions itch and sting. Psoriatic arthritis, which causes joint deformities and impairment, affects 1.3% [5] to 34.7% [6] of people with psoriasis. The percentage of people with psoriasis who experience nail changes ranges from 4.2% to 69% [7–9]. Cardiovascular and other noncommunicable diseases (NCDs) are more likely to develop in people with psoriasis [4,10,11]. Psoriasis causes physical, emotional, and social suffering [12–14]. In general, QoL is frequently seriously compromised [15–22]. Psoriasis causes disfigurement, disability, and significant productivity loss. Treatment of psoriasis is still based on controlling the symptoms. Topical and systemic therapies as well as phototherapy are available. Conventional topical systems such as ointments and creams are less effective for skin permeation due to their poor efficiency and are associated with side effects such as burning, contact dermatitis and stinging sensations owing to uncontrolled release of drug [23,24].

Psoralen, 7H-Furo[3,2-g][1]benzopyran-7-one is a small molecule that belongs to the coumarin family of compounds, is isolated from *Fructus psoraleae*, a common herb used in traditional Chinese medicine. Psoralen and psoralen derivatives are found in plants (Psoralea corylifolia and Ammi majus) and other vegetation such as limes, figs, parsnips, and certain fungi. Psoralen is a photosensitizing drug and was used with sunlight to treat skin diseases in Egypt and India as early as 1200–2000 BC. The ancient Egyptians and Indians applied plant extracts to the skin or ingested the extracts orally and then exposed themselves to sunlight to induce repigmentation in vitiligo.

Topical treatment strategies viz. cream, gels, liposomes, solid lipid nanoparticles, ethosomes, niosomes, lipid-coated microparticles, nanoparticles, magnetic liposomes, flexible membrane vesicles, lipid liposomes are well documented in literature for psoriasis [25]. Nanosponges are encapsulating type of nanoparticles which encapsulates the drugs within its core. Nanosponges possesses various advatages over other systems viz., improve aqueous solubility of lipophilic drugs, protect degradable drug molecules which are unstable in aqueous environment, formulate drug delivery systems for various routes of administration, improve the solubility of the formulation, used to mask unpleasant odours, decrease the side effects and protect the drug from degradation. Predictable release is one of the major advantages of this system compared to other nanoparticle delivery systems under development.

No data on psoralen nanosponges formulation available in literature. Therefore, the current study was aimed to develop a dermatological nanomedicine consisting of psoralen-loaded nanosponges and their evaluation in terms of solubilizing efficiency, encapsulation, particle size, surface charge along with pharmacokinetic evaluation. As the nanosponges offered many advantages of like improved safety, better product stability, and non-irritancy make them suitable approach for development of topical preparations [26]. The formulation also offered the controlled release of drug [27].

Material and method

Material

Psoralen and other materials given in table 1 were purchased from Yucca Enterprises, Mumbai. All the other solvents and reagents were of analytical grade.

Ethical Approval

The experimental study was carried out in both sex Wister albino rats. The rats with weighted between 150- 200 gm were selected trough approval of the committee. The experimental protocol was approved by Institutional Animal Ethics Committee (lAEC) of Rajaram and Tarabai Bandekar college of Pharmacy, Ponda, as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Protocol No. PESRTBCOP/IAEC, 2022R-96).

Method

Formulation of Nanosponges by Emulsion solvent diffusion method [27]

Various polymers in different ratios were prepared and combined with drug (Table 2). The amount of PVA was dissolved in 100 ml of distilled water to prepare aqueous continuous phase by stirring on hot water bath at 60°C. The dispersed phase was slowly incorporated in the continuous phase using syringe and was magnetically stirred at 1000 rpm for 2 hrs. The prepared dispersion was filtered using 0.45 µm filter paper to separate the solid mass. The product was dried in an oven at 40 °C and was stored in desiccators for 24 hrs to evaporate any residual solvent completely. The final products were packed and stored in air tight containers.

Formulation of nanosponges based Hydrogel

Different conc. of carbopol 934 was added in 80 ml of water and vigorously stir for 24 hrs resulting in uniform mixture. Triethanolamine was then added and stirred for 30 min at room temperature. Dissolved optimized nanosponges equivalent to 100mg in 15 ml of ethanol and add propylene glycol with constant stirring to get solution. The solution was then added into the carbopol-934 polymer solution and mixed well to get the gel for 20 min. Before viscosity studies all the prepared gel samples were allowed to equilibrate for at least 24 hrs.at room temperature. Intermittent checking of the pH of the gel & kept side for 60 min for complete hydration & swelling of gel. Add Methyl paraben and mixing was continued by using a magnetic stirrer till uniform dispersion of the nanopsonges.

Evaluation of psoralen Content in Formulation by Developed and Validated HPLC Method

HPLC Method Development

The determination was done using Agilent Tech. (1100) system. The separation of chromatogram was carried out on column Fortis C18 (100 x 4.6 mm id with 2.5µm particle size) using various solvent systems such as acetonitrile, methanol, water were tried for development of HPLC method for analysis of psoralen. The effect of flow rate was determined by setting flow rates at 0.5 ml/min, 0.7 ml/min, 1.0 ml/min, 1.1 ml/min, 1.2 ml/min and 1.5 ml/min. The solution was scanned between the wavelength range 400-200 nm using the UV spectrophotometer. Quantification of drug was estimated by calculating peak are using CHEMSTATION 10.1 software.

Preparation of mobile phase

Mobile phase was prepared by mixing HPLC grade Acetonitrile: water (0.1% of formic acid) in a ratio 70:30 v/v. The content was sonicated for 15 min and filtered through 0.45 µm membrane filter. Mixed solvents were degassed and used as mobile phase.

Preparation of reference standard and sample solution

Psoralen is used as standard. The standard was prepared by using 10 mg of standard in 10 ml of methanol. The formulation equivalent to 10 mg used to prepared sample solution.

Method validation parameters

The validation of the developed HPLC method was carried out in accordance with ICH guidelines. The linearity was analysed for concentration ranging from 15- 90 µg/ml by using Least-square regression analysis where, peak areas were plotted against the corresponding concentrations. The intra- day and inter- day precision and repeatability was evaluated by triplicates of three different concentrations of each quercetin was spotted and analyzed on same day for intra-day study and two different days for inter-day study with respective chromatographic conditions.

Recovery study method was employed to evaluate accuracy. The samples were spiked with 80, 100 and 120 % of median concentrations of standards.

$$\text{Accuracy} = \frac{\text{spiked concentration} - \text{mean concentration}}{\text{spiked concentration}} \times 100$$

Robustness was carried out by making deliberate changes in the wavelength, flow rate and mobile phase and evaluated their effect on the retention factor. The estimation of LOD and LOQ were done by standard deviation method. Detection limit =3.3σ /S and quantitation limit=10 σ /S (σ is residual standard deviation of a regression line and S is the slope of the calibration curve).

Characterization of Formulation

Physical appearance

The prepared hydrogel formulation was evaluated for appearance and homogeneity by visual observation.

pH determination

The pH of the hydrogel was determined by using a pH meter. For this purpose, the measured quantity (1 %) of the hydrogel was prepared in deionized water and pH measurement was done at 25 °C.

Viscosity

The viscosity of formulated hydrogel was determined using Brookfield viscometer at 100 rpm spindle speed at temperature of 25 °C [16]. The viscosity determination was recorded in triplicate.

Evaluation of Nanosponges

Particle size

The measurement of particle size were done on Zetasizer instrument at 25°C. This technique produces the mean particle diameter and particle size distribution. The analysis was done by the software provided by Malvern Instruments. Before analysis samples were placed in refrigerator maintained at 4°C.

Polydispersity Index

Polydispersity index is indicative of uniformity in the particle size and hence it should be as low as possible. The measurement of PDI was obtained during the analysis of particle size.

Zeta potential

The formulation of nanocochleate was tested for zeta potential using Malvern Zetasizer instrument. Zeta potential was determined by zeta potentiometer. The sample was filled into the cell; an electrode inserted was placed under the microscope and connect them to the zeta meter. The analysis was carried out at 25°C.

Drug loading

HPLC was used to determine drug loading. Using deionized water, one ml of nanosponges formulation was dissolved in 1 ml ethanol, resulting in a volume of 10 ml. After that, the solution was sonicated for 5 minutes. After that, 0.45 m filters are used to filter the solution. The filtrate was then subjected to HPLC analysis.

Determination of Entrapment efficiency

The nanosponge formulation (10 µg/ml) was centrifuged at 4000 rpm for 18 min at 4°C temperature by using Remi cooling centrifuge. A supernatant contains the nanosponges in suspending stage and oil on the wall of centrifuge tube. The supernatant was again centrifuged at 15000 rpm for 30 min at 4 °C temperature. As a result, a transparent solution of supernatant and nanosponge was attained which was redispersed in mobile phase (100 ml). The solution was further sonicated, the nanosponges were disrupted to discharge the psoralen. The drug was determined for the drug entrapment. The amount of psoralen was estimated by using HPLC system.

$$\text{Percentage Entrapment Efficiency} = \frac{\text{Wc}}{\text{Wt}} \times 100$$

Where amount of drug content (entrapped) in the nanosponge is denoted as Wc and total amount of drug in the dispersion is denoted as Wt.

In- vitro study

In-vitro release study of psoralen from the formulation was carried out in phosphate buffer pH 5.5 acetate buffer by the dialysis membrane method. The amount of formulations equivalent to 10 mg of psoralen was taken in dialysis bags for pH 5.5 medium (by cutoff of 12,000 Da, Sigma). The drug dispersion and formulation containing dialysis bags suspended in a beaker with 100 ml of acetate buffer maintained at pH 5.5 was kept on a magnetic stirrer which is rotated at 100 rpm, with temperature adjusted to 37±0.5°C for a selected time intervals. A 5 ml sample was withdrawn for analysis and replaced with the same quantity of a fresh media. The samples was then filtered through 0.45µm filter. The samples were analyzed for drug release by determining absorbance using HPLC, the rate of psoralen release obtained using the standard curve.

Surface morphology

The particle size and morphology of nanoparticles was examined by transmission electron microscope (TEM) (Tenai G2 20 Twin, FEI Company, Netherland).

Samples of nanosponges such as PVA: HPMC, PVA: EC, Agar: EC, Agar: HPMC were prepared by taking a drop of sample on parafilm. Then, a drop of 2% phosphotungstic acid solution were kept over sample drop and left it for 30 sec. The copper grid was placed on sample. Then air drying of copper grids were performed for 1 h and observed under TEM and photomicrographs were captured.

Animal study

Sensitization and Elicitation (Challenge Application) Procedure

The animals were sensitized by applying 100 µl of 1.5% oxazolone in ethanol to the abdominal region of the animals for six days. Seven days after sensitization, 20 µl of 1% oxazolone in a mixture of acetone and olive oil (4:1) were applied to both sides of the mouse ear on days 7, 10, 13 and 16.

Measurements: During the study, ear thickness was measured with digital Vernier Calipers at various time points. Ear thickness was measured before the sensitization phase (Day 7) and after each elicitation on days 10, 13, 16 and 19 to evaluate swelling ear reactions. Animals were euthanized, and mouse ears were excised, fixed in 10%-buffered formalin solution, embedded in paraffin, cut into 5 µm sections and stained with hematoxylin-eosin, 72 hours after the last application of oxazolone, by standard methods. During the histopathological evaluation, after the microscopic fields were photographed, the epidermal thickness was measured as the distance from the bottom of the stratum corneum to the basement membrane in the interfollicular epidermis. Inhibition of ear swelling (%), ear weight and epidermal thickness were calculated.

Result and discussion

Development and validation of HPLC method

Optimization of RP- HPLC method

The optimization of the RP-HPLC chromatographic parameters were carried out by using different compositions of mobile phase and flow rates. The separation was carried out on Agilent Tech. (1100) system using acetonitrile: water (0.1% of formic acid) in a ratio 70:30 v/v with the flow rate 1.2 ml/min as it gave well resolved peak. Based on peak area quantification was carried out at 247 nm. The Rt for psoralen was found to be 12.035 min given in figure 1.

Method validation parameters

Linearity

The linearity of the method was determined by diluting the standard stock solution to produce the concentration ranges from 15 to 90 µg/ml. The results show excellent correlation existed between peak area and concentration of analyte. By plotting the AUC versus the concentration of analyte, the calibration curve was prepared and analyzed through linear regression (Figure 2).

Accuracy

Good recovery study of the drug was carried out at three different concentrations levels indicating the method was accurate. A known amount of standard drug (80, 100, 120%) was added into pre-analyzed sample and subjected them to the proposed HPLC method. The recoveries were found to be in the range of 100.02- 101.58%. The % recovery was found to be within the limits.

Precision

Data on repeatability and instrumental variation were obtained in triplicate. Method precision was evaluated by repeatedly introducing 60 μg/ml concentration of psoralen. The developed method was found to be precise as % RSD was found to be 0.41. Intraday and interday precision was done in triplicate at 3 distinct concentration levels. The % RSD was found to be 0.97, 1.49 and 0.24 for interday precision and 0.96, 0.29, and 0.24 for intraday precision. RSD < 2%, proved that the method was highly precise.

Robustness

Robustness was done by small changes in the chromatographic conditions like mobile phase, flow rate and wavelength. It was observed that there were no marked changes in the chromatograms. The developed method was found to be robust as the % RSD values were< 2.0 %.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ was found to be 0.30 μg/ml and 3.93 μg/ml respectively.

Evaluation of formulation

The prepared nanosponges were further studied for different parameters such as particle size, poly dispersibility, zeta potential, % drug loading and entrapment efficiency given in table no. 3, figure 3, 4.

In vitro study F3, F5, F7, f12

The % drug release from nanosponges prepared by using PVA: HPMC (0.5:1), PVA: EC (1:1), Agar: EC (1:0.5) and Agar: HPMC (1:0.5) in first hour is 29.85%, 41.24%, 31.13% and 20.77% respectively. At the end of 8 hrs around 71.85%, 93.02%, 60.92% and 57.05% drug was released from nanosponges prepared using PVA: HPMC (0.5:1), PVA: EC (1:1), Agar: EC (1:0.5) and Agar: HPMC (1:0.5) respectively given in table 4 and figure 5. The nanosponges prepared using PVA: EC (1:1) showed better release of drug from nanosponges.

FESEM

The nanosponges prepared by using PVA: EC (1:1) (F5) combination showed distinct spongy network along with smooth spherical uniform sphere at 4 micron given in figure 6. The other formulations (F3, F7, F12) does not showed smooth spherical uniform sphere.

Animal Study

The effect of formulation F5 was evaluated by topical application of formulation to oxazolone induced psoriasis in mice for 16 days. The animals were sensitized by applying oxazolone to the abdominal region of the mice. The formulation was applied after the challenge. The evaluation was performed by measuring the thickness of ear. After the completion of treatment with nanospoge formulation showed significant decreased in ear thickness, indicating the anti-inflammatory potential of the nanospoge formulation (F5).

The histopathology study revealed that, developed formulation of the active has increased the efficacy with decrease number of inflammatory cells, improved skin surface and reduction in the thickening of the skin when compared with standard Psoralen.

Conclusion

Psoriasis is a chronic, noncommunicable, painful, disfiguring, and disabling illness that reduces the quality of life. Psoralen, 7H-Furo[3,2-g][1]benzopyran-7-one is a small molecule that belongs to the coumarin family of compounds, is isolated from *Fructus psoraleae*, a common herb used in traditional Chinese medicine. The aim of the present work was to prepared psoralen loaded nanosponges and evaluated for its antipsoriatic activity. The different polymers at various ratios were used to formulate the nanosponges (F1 to F 12). The prepared formulations were evaluated for different parameters. After primary evaluation formulations F3, F5, F7 and F12 were selected for further studies including drug release and FESM. The nanosponges (F5) made using PVA: EC (1:1) were determined to be superior in the in-vitro release testing and were subsequently selected for antipsoriatic potential investigation.

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Conflict of interest Authors declared no conflict of interest

Author's contribution statements

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Tables

Table 1: List of chemicals with their uses

Ingredients	**Uses**
Polyvinyl alcohol	Hydrophilic polymer
Hydroxypropyl methylcellulose (HPMC) K15 M	Hydrophilic polymer
Agar	Hydrophilic polymer
Ethyl cellulose	Hydrophobic polymer
Carbopol 940	Gelling agent
Ethanol	Cross linking agent and solvent
Triethanolamine	Used to neutralize Ph
Water	For dispersion

Table 2: Composition of Nanosponges

Ingredients	**Formulations**											
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
Psoralen (mg)	20	20	20	20	20	20	20	20	20	20	20	20
PVA: HPMCK15M (mg)	100: 50	100: 100	50: 100	-	-	-	-	-	-	-	-	-
PVA: EC (mg)	-	-	-	100: 50	100: 100	50: 100	-	-	-	-	-	-
Agar: EC (mg)	-	-	-	-	-	-	100: 50	100: 100	50: 100	-	-	-
Agar: HPMCK15M (mg)	-	-	-	-	-	-	-	-	-	100: 50	100: 100	50: 100
Ethanol (ml)	10	10	10	10	10	10	10	10	10	10	10	10

PVA: Polyvinyl alcohol

HPMC: Hydroxypropyl methyl cellulose

EC: Ethyl cellulose

Table 3: Evaluation of Nanosponges

Sr. No.	Formulation	Particle size (nm)	PDI	Zeta potential (mV)	%Drug loading	% Entrapment efficiency
1.	F1	346.0 ± 0.26	0.618 ± 0.001	-14.1 ± 0.321	68.10 ± 0.87	63.33 ± 0.67
2.	F2	507.3 ± 0.95	0.753 ± 0.001	-24.8 ± 0.322	77.53 ± 1.91	64.17 ± 1.78
3.	F3	282.7 ± 1.13	0.335 ± 0.006	-16.5 ± 0.323	84.78 ± 0.98	65.81 ± 1.61
4.	F4	403.4 ± 1.18	0.525 ± 0.012	-22.8 ± 0.324	88.20 ± 1.59	79.71 ± 1.13
5.	F5	209 ± 0.21	0.198 ± 0.051	-29.85 ± 0.325	93.80 ± 0.80	88.38 ± 1.78
6.	F6	448.3 ± 0.72	0.320 ± 0.003	-25.8 ± 0.326	84.69 ± 0.47	80.26 ± 0.52
7.	F7	594.3 ± 7.39	0.241 ± 0.006	12.6 ± 0.327	81.94 ± 0.96	68.51 ± 1.02
8.	F8	753.4 ± 0.25	0.747 ± 0.014	15.81 ± 0.328	59.27 ± 1.27	53.00 ±1.20
9.	F9	833.3 ± 0.38	0.634 ± 0.019	-26.58 ± 0.329	58.84 ± 1.57	72.18 ± 1.21
10.	F10	629.2 ± 0.40	0.618 ± 0.006	-34.1 ± 0.330	72.64 ± 1.50	62.37 ± 1.42
11.	F11	856.0 ± 0.74	0.586 ± 0.005	-31.5 ± 0.331	79.98 ± 0.20	68.32 ± 0.74
12.	F12	234.7 ± 0.34	0.672 ± 0.001	-50.7 ± 0.332	86.23 ± 0.79	69.84 ± 1.76

Table 4: In-vitro study of nanosponges containing Agar: EC (1:2), PVA: EC (1:1), HPMC: EC (1:2)

Time (hrs)	% Drug Release			
	F3 **PVA: HPMC (0.5:1)**	**F5** **PVA: EC (1:1)**	**F7** **Agar: EC (1:0.5)**	**F12** **Agar: HPMC (1:0.5)**
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	29.85 ± 0.99	41.24 ± 0.99	31.13 ± 0.99	20.77 ± 0.99
2	39.78 ± 1.00	53.71 ± 1.00	32.37 ± 1.00	23.21 ± 1.00
3	51.32 ± 1.01	58.16 ± 1.01	38.69 ± 1.01	28.11 ± 1.01

4	56.80 ± 1.02	68.01 ± 1.02	44.52 ± 1.02	41.51 ± 1.02
5	65.49 ± 1.03	70.29 ± 1.03	46.18 ± 1.03	46.14 ± 1.03
6	67.37 ± 1.04	79.88 ± 1.04	51.92 ± 1.04	50.94 ± 1.04
7	69.99 ± 1.05	82.61 ± 1.05	55.36 ± 1.05	55.52 ± 1.05
8	71.85 ± 1.06	93.02 ± 1.06	60.92 ± 1.06	57.05 ± 1.06

Figures

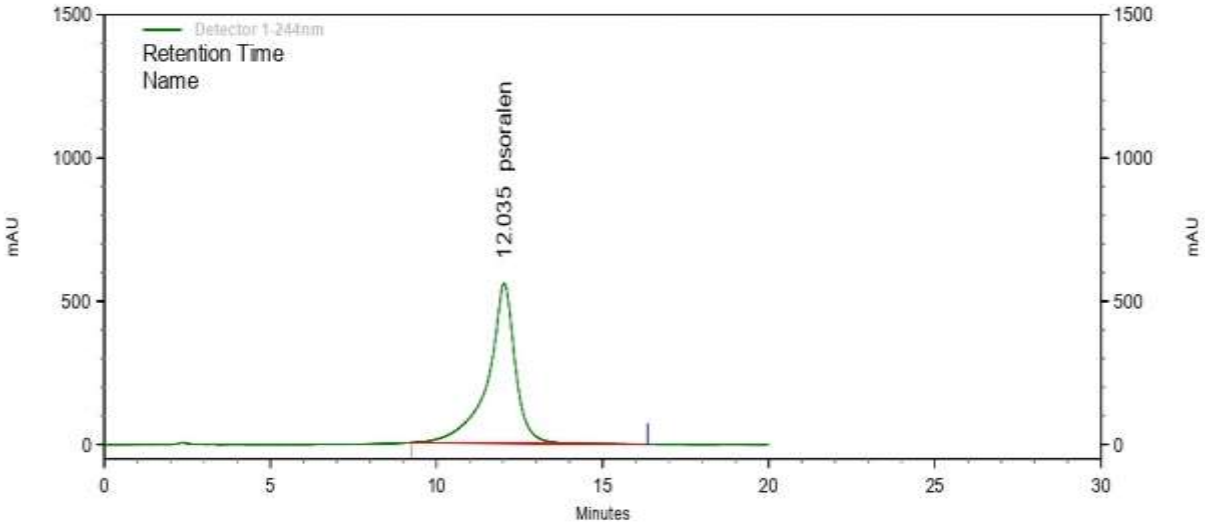

Figure 1: Chromatogram of Psoralen standard

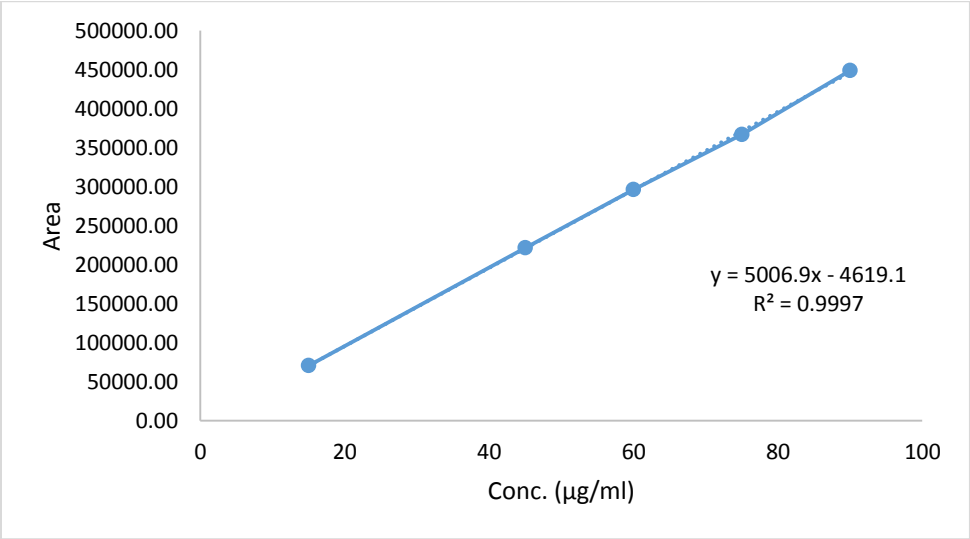

Figure 2: Linearity curve of psoralen

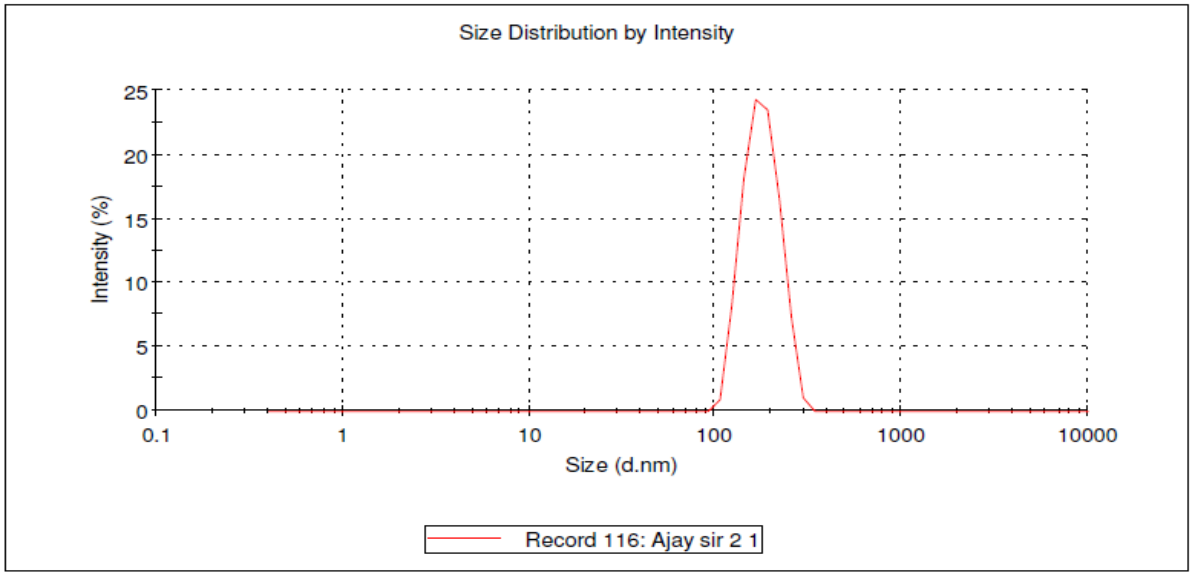

Figure 3: Particle size distribution of formulation F5

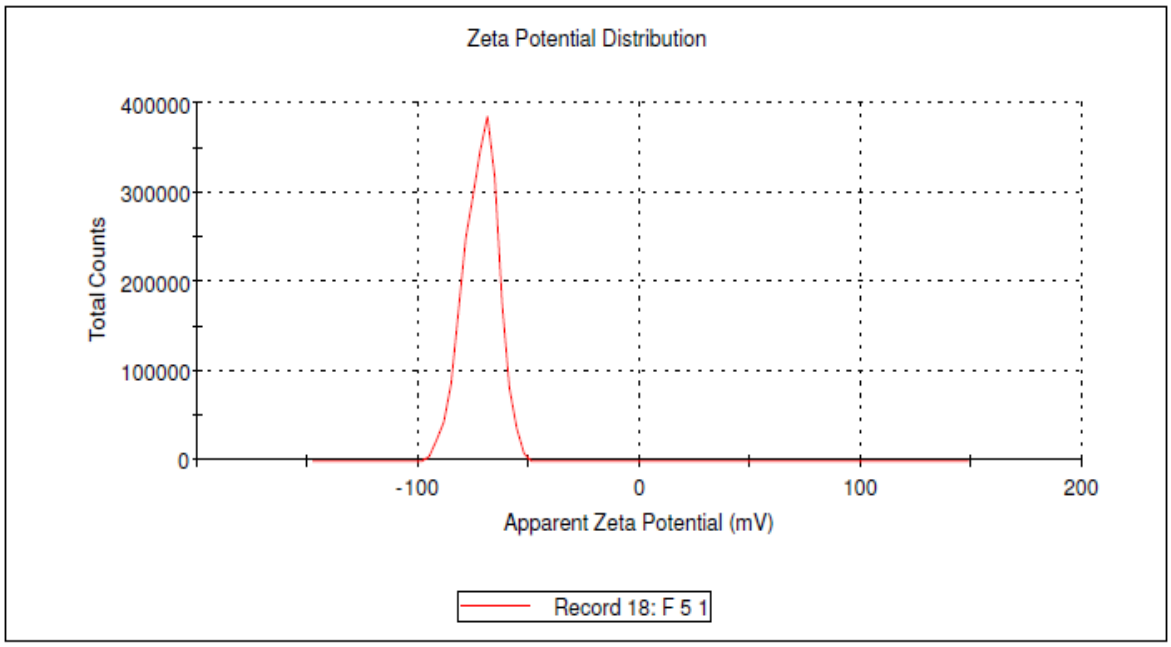

Figure 4: Zeta potential of formulation F5

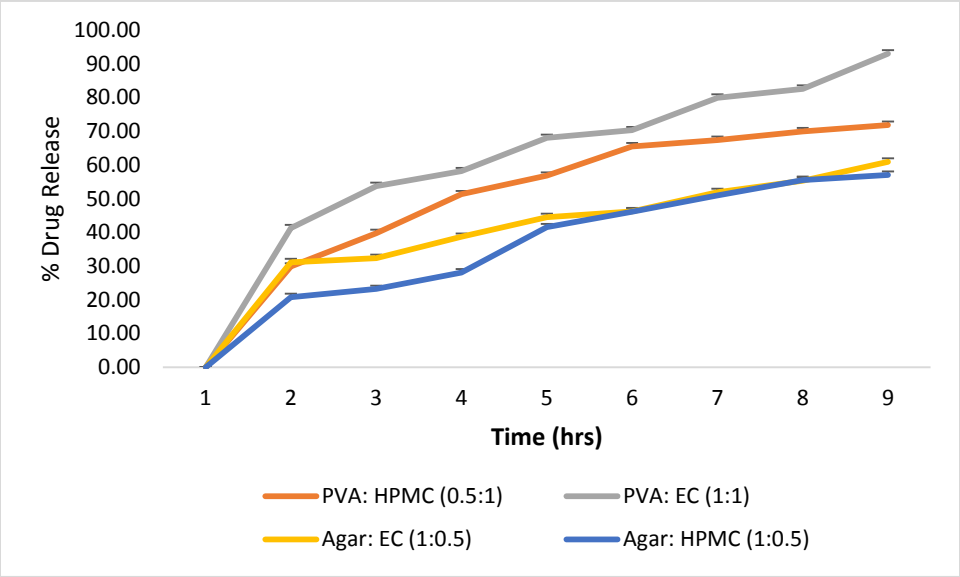

Figure 5: In-vitro study of nanosponges containing Agar: EC (1:2), PVA: EC (1:1), HPMC: EC (1:2)

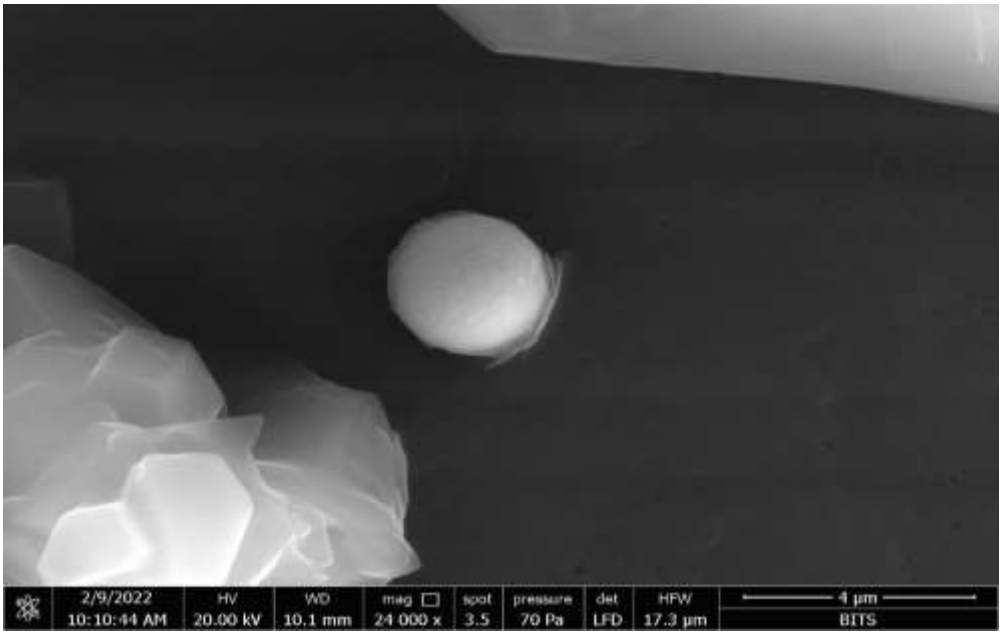

Figure 6: FESEM evaluation of nanosponges (F5)

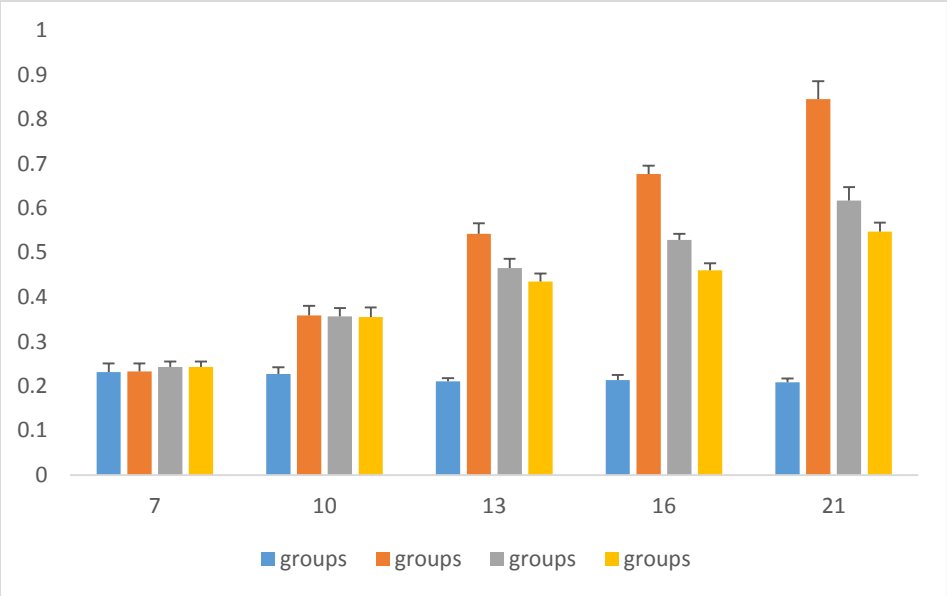

Figure 7: Determination of anti- psoriatic action of Nanosponge by measurement of ear thickness

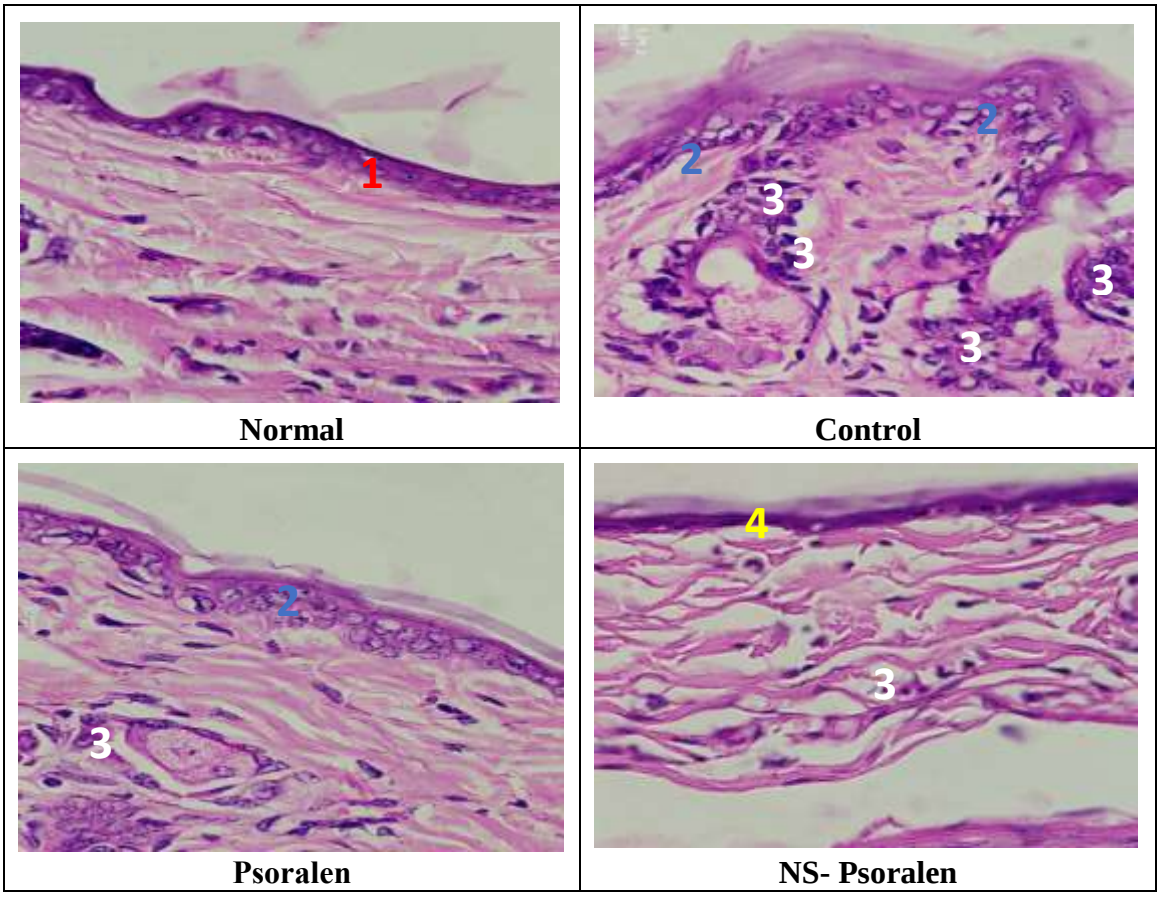

Figure 8: Histopathological study of skin in mice with oxazolone-induced psoriasis

* 1: normal epidermis,

2: thickened epidermis with psoriatic cells,

3: inflammatory cells,

4: keratinized layer