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# FORMULATION AND EVALUATION OF NIOSOME GEL ON PSORIASIS

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## ABSTRACT

Psoriasis is an extensive skin disease affecting 2-3 per cent of the world's population. Psoriasis is a skin condition that causes inflammation and is characterized by aberrant proliferation and differentiation. As an excipient cholesterol and non-ionic surfactant are primarily used to generate noisome a non-ionic surfactant-based vesicle. Tacrolimus commonly known as FK-506 or Fujimycin is an immunosuppressive drug used primarily to prevent organ discharge after organ transplantation by lowering the patient's immune system activity that is the possibility of organ discharge. Plaques from psoriasis typically affect the scalp and outer skin surfaces. Less frequently, inverse psoriasis can harm more delicate skin such as that on the neck, genitalia, and intertriginous areas. Although psoriasis cannot be cured there are several drug options that can be used to manage the condition. The aim of this study is to develop and evaluate the tacrolimus niosomal gel and evaluate its pharmacological activity for the topical treatment of psoriasis. To accomplish this niosomal gel preparation was applied to a single product tacrolimus. Nine batches in total were produced by using drug 100 mg and Surfactant: Cholesterol Ratio (0.5: 0.5-1.5) for Niosome Preparation Using Film Hydration method. The drug's compatibility with HPMC, SPAN, Carbapol, sodium alginate, guar gum, and xanthan gum was pre-evaluated. The sizes ranged from 67.8 nm to 121.6 nm which was discovered. The formed niosomal batches were evaluated to determine the best batch and it became clear that as the formulation's hydrophobicity increased the number of vesicles increased due to an increase in cholesterol concentration. Span 20 provided drug content 83.1±1.1 to 91.8±2.6 mg; Span 60 provided 90.3±1.6 to 93.9±1.5mg and Span 80 provided 93.1±1.5 to 85.1±1.0mg.

The evaluation method was repeated throughout the greater drug content and entrapment effectiveness for the NF5 formulation. The NF5 formulation was used to continue forming gel formulations. A total of 10 formulations (F1 to F10) were formed and evaluated. The gel-formed F5 and F6 formulations were discovered to be the clearest, with the best spreadability and uniformity among all the formulations. Out of all the formulation batches tested F6 had the highest viscosity 8166 cPs. It was found that batch F-6 had an optimized formulation which is now being put through stability and pharmacological tests. Animals were separated into 4 groups for 4 in vivo anti-psoriatic trials which were evaluated. Group I is the Negative Control (No Disease),

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Group II is the Positive Control (Disease but No Treatment), Group III is the Treatment (Blank Gel) and Group IV is the Treatment (Best Formulation). Tacrolimus niosomal gel exhibited the lowest PASI score of the therapy group. Additionally, when compared to that of the negative group. It is clear from the research that the Tacrolimus formulation's niosomal gel which contains carbapol 934, 4 mg demonstrated good results for the treatment of psoriasis.

**Keyword:** Drug niosomal dispersion, HPMC, Sodium alginate, Carbapol 934, Tacrolimus niosomal gel etc.

## INTRODUCTION:

### 1. PSORIASIS

Psoriasis is a widespread skin condition affecting 2-3 per cent of the world 's population [1-5]. It can be defined as inflammatory skin condition characterized by abnormal differentiation and proliferation. It is an immune disease in which environmental and genetic factors plays very important role. The name of Psoriasis derives from the Greek term 'psora,' representing 'itch'.[6, 7] As discussed earlier it is inflammatory, dry, non – contagious disease which may covers entire system of person. It can be identified by scaly marinated erythematous plaques develop on skin with symmetrical manner. Psoriasis effects on common sites such as scalp, fingers tips, palms, soles, toes, genitals, under breast, elbows, knees, shins having chances of relapse after some interval.[8]

#### 1.1 Types of Psoriasis

**a. Psoriasis vulgaris (Plaque psoriasis)**

- Among all patients, the most common symptom among psoriasis effects 58 percent (79) to 97 percent (45).[9,10]
- Inflammatory red, strongly demarcated, elevated, dry, plaques of varying sizes, typically covered with white or silvery scales.
- Includes the scalp and the region behind ears, upper arms and shin flexor surfaces (especially elbows and knees), head , neck, hands, soles, and nails.

b. **Intertriginous psoriasis** [11]

- Affects 12 percent (105) to 26 percent (21) of all psoriasis cases.
- Scales are relatively uncommon: Deep-red or white, smooth, sharply demarcated, muddy patches or inscriptions.
- Impacts the axillae, antecubital fossae, inframammary creases, umbilicus, groins, genital region, gluteal cleft, popliteal fossae and other folds of the body mostly predominantly in flexural locations.

c. **Guttate psoriasis** [12, 13] (droplet psoriasis)

- Affects, and generally, between 0.6 percent (45) and 20 percent (8) of people who are diagnosed with psoriasis. This occurs during infancy andadolescence.
- Reddish, drop-like papules and inscriptions, including primarily the trunk, legs and arms.

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- Emergence is correlated with respiratory tract streptococcal infection, and previous skin rash.

d. **Pustular psoriasis**[14, 15]

- Impacts 1.1 percent (45) to 12 percent (105) throughout all psoriasis cases.
- Coalescing pustules, filled with non-infectious pus.
- Either includes specific areas such as palm of hands , fingers, nail and feet soles, or the whole portion of a body just after cause may occur as a single event.

**E. Erythrodermic psoriasis**[16, 17]

- Affects 0.4 percent (45) to 7 percent (105) over all psoriasis cases.
- Fiery redness and exfoliation of most of the body surface.
- A most severe form of rheumatoid arthritis, potentially life-threatening as it can lead to hypothermia, hypoalbuminemia and heart failure at high production.[18, 19]

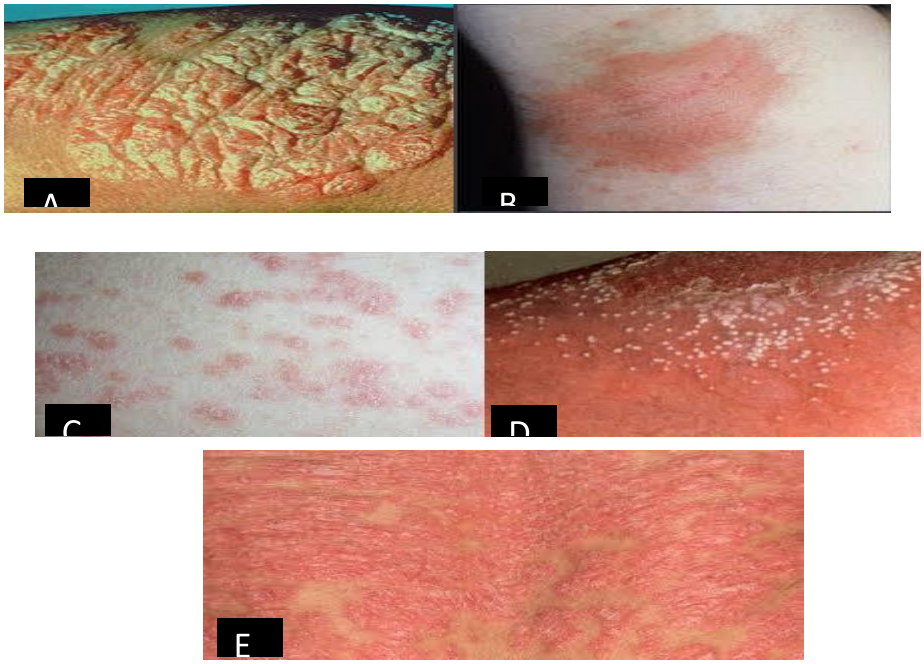


**Fig 1** Types of Psoriatic inflammatory conditions
A: Plaque psoriasis, B: Intertriginous psoriasis, C: Guttate psoriasis D: Pustular psoriasis, E: Erythrodermic psoriasis

**Material and Method:**

**2. Preparation of sample stock solution:**

drug equivalent to100 mg was transferred into a 100 ml volumetric flask (1000 µg/ml). From this 10 ml was withdrawn and diluted upto 100 ml with solvent. From this further 1 ml was diluted up to 10 ml and used as stock solution[18,19]**.**

**2.1 Preparation of calibration curve**

From above working std. stock solution of Tacrolimus (100 µg/ml), pippete out from stock solution 0.2 to 1 ml and transferred to series of 10 ml volumetric flasks and final volume

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made up to mark with diluent to form solutions of 2 to 10µg/ml of Tacrolimus. These solutions were then scanned in the range of 1800-400 nm against diluent as blank. The absorbance maxima (λmax) was found to be 291 nm for Tacrolimus and then calibration curve was plotted as absorbance vs. concentration.

## 3. FORMULATION AND DEVELOPMENT OF NIOSOMES

### 3.1 Preparation of niosomes Film hydration method:

Mixture of surfactant (Span 20, 60, 80) and cholesterol (equivalent to 50 mg) were dissolved in 10 ml of chloroform. The solvent was slowly evaporated using a rotary flash evaporator (at 80 rpm, 60°C) under low pressure to produce thin lipid film. In another conical flask, weighed amount of drug (according to dose) was transferred and dissolved in required quantity of Phosphate buffer saline (pH 7.4). The mixture was sonicated for 5 min. by the hand and again resonicated for 5 min. The prepared niosomes were allowed to equilibrate at room temperature. Niosomal dispersion was then kept in refrigerator at 4°C. Total 9 batches of niosomes were prepared according to the variant composition of surfactant Span 20, Span 60 and Span 80 with cholesteraol shown in Table 3.1. The batches were labelled as NF1 to NF9. All the prepared niosomes were subjected to evaluation for the selection of best batch among the others[20-22].

**Table 1**: Formulation code and composition of niosomal batches

| Formulation code | Surfactant | Drug (mg) | Surfactant :cholesterol ratio |
|---|---|---|---|
| NF1 | Span 20 | 100 | 0.5:0.5 |
| NF2 | | 100 | 0.5:1.0 |
| NF3 | | 100 | 0.5:1.5 |
| NF4 | Span 60 | 100 | 0.5:0.5 |
| NF5 | | 100 | 0.5:1.0 |
| NF6 | | 100 | 0.5:1.5 |
| NF7 | Span 80 | 100 | 0.5:0.5 |
| NF8 | | 100 | 0.5:1.0 |
| NF9 | | 100 | 0.5:1.5 |

**EVALUATION OF NIOSOMES**[109-111]

#### 3.1.1 Optical microscopy study:

The particle size of the niosomal suspension was determined by optical microscopy. A drop of niosomal suspension was placed on a glass slide. A cover slip was placed over the niosome suspension and the average vesicle size was measured by an optical microscope (Motic digital microcope) and by using a pre-calibrated ocular eye piece micrometer. The prepared vesicles were studied under 40 X magnification to observe the formation of vesicles.

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3.1.2 **Drug content**

Niosomal suspension equivalent to 10mg taken in a volumetric flask of 100 ml and volume was made up by phosphate buffer pH7.4 after that 1ml of this mixture was diluted to10ml by phosphate buffer pH 7.4 and the percentage dug content was observed at 291 nm using UV spectrophotometer.[23]

3.1.3 **Estimation of entrapment efficiency**

The Entrapment efficiency of niosomes was estimated by ultra-centrifugation method where the niosomal dispersions were centrifuged at 14000 rotations per minute for 90 minutes. The clear supernatant from the resulting solution was diluted appropriately using phosphate buffer pH 7.4 and analyzed for the drug concentration spectrophotometrically. The percentage encapsulation efficiency (EE%) was calculated using following equation.[24]

**Drug Entrapment Efficiency (%) was calculated as follows:**

$$EE\ \% = [(T-C)/T] \times 100$$

Where, T = total amount of drug (calculated both in supernatant and sediment) C = amount of drug found only in the supernatant.

## 4. PREPARATION OF TACROLIMUS NIOSOMAL GEL

The composition of TAC gel formulae is designed in Table 3.3. Tacrolimus (0.1% w/w) was dissolved in a hot mixture containing propylene glycol (25% w/w) and glycerin (10% w/w) as moistening agent. The gel formulations were prepared by dispersing weighed amount of polymers Carbopol 940, HPMC, sodium alginate, guar gum and xanthan gum in water with constant stirring using magnetic stirrer at a moderate speed. Then, mixture containing drug was added. The pH of gel were adjusted using TEA. Finally, preservatives methyl and propylparaben were added slowly with continuous stirring. The prepared gels were packed in wide mouth glass containers covered with screw-capped plastic lid. The containers covered with an aluminum foil and were kept in dark and cool place [25, 26].

Table 2: **PREPARATION OF TACROLIMUS NIOSOMAL GEL**

| **Ingredients** | **Formulations** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** | **F10** |
| **Drug niosomal dispersion** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **HPMC** | 2 | 4 | - | - | - | - | - | - | - | - |
| **Sodium alginate** | - | - | 2 | 4 | - | - | - | - | - | - |
| **Carbapol 934** | - | - | - | - | 2 | 4 | - | - | - | - |
| **Xanthan gum** | - | - | - | - | - | - | 2 | 4 | - | - |
| **Guar gum** | - | - | - | - | - | - | - | - | 2 | 4 |
| **Glycerin** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Methyl Paraben** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Propyl Paraben** | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| **Propylene glycol** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Water up to** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

## 5. **EVALUATION OF TACROLIMUS NIOSOMAL GEL** [28-30]

### 5.1 **Visual examinations**

All prepared gel formulations were inspected for their color, syneresis, and presence of lumps by visual inspection after the gels have been kept in the containers.

#### 5.1.1 **Homogeneity**

All prepared gels were tested for homogeneity after the gels have been set in the container. They were tested for their appearance and presence of any aggregates and results for the same were noted.

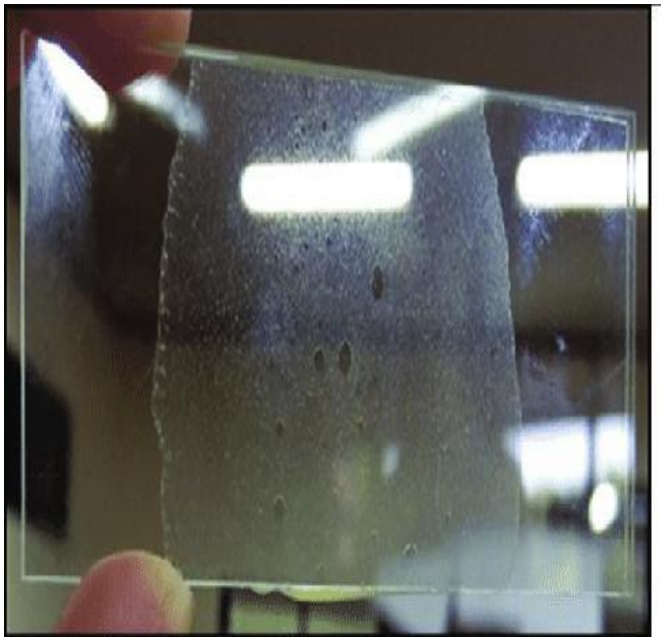

**Fig. 2:** TAC loaded Niosomal Gel Homogenity

#### 5.1.2 Grittiness

The formulations were evaluated microscopically for the presence of particles if any. No appreciable particulate matter was seen under light microscope.

#### 5.1.3 Spreadability test

A sample of 0.5 g of each formulation was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 min where no more spreading was expected. Diameters of spreaded circles formed due to press were measured in cm and were taken as comparative values for

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spreadability.

**5.1.4 pH determination**

The pH of the formulated Tacrolimus gels was determined using digital pH meter (Systonic). Readings noted.

**5.1.5 Viscosity studies**

The measurement of viscosity of the prepared gels was done using Brookfield viscometer. The gel was evaluated using spindle no. 64.

**5.1.6 Extrudability**

The prepared gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The amount of gel extruded was calculated (>90% extrudability: Excellent, >80% extrudability: Good, and >70% extrudability: Fair).

**5.1.7 Skin irritation studies Acute dermal irritation:**

The acute dermal irritation study was performed in accordance with the OECD Guidelines 404 –Acute dermal irritation/corrosion‖. A positive control group received 0.8% w/v aqueous solution of formaldehyde as a standard irritant; a control group received placebo patch and a treated group received Tacrolimus Niosomal Gel. Tacrolimus Niosomal Gel was mixed in a minimum amount of olive oil to create paste preparation for dermal application (2 mg/kg). Around 5 cm × 5 cm of rabbit's trunk was unclipped for experimental use. The test article was then applied under a 2.5 cm × 2.5 cm gauze patch to one intact site per rabbit and wrapped with an occlusive dressing. The animals were fitted with Elizabethan collars during the application. The test articles were removed from the test site by gently washing with soaked in lukewarm water at the end of the exposure period, prior to scoring for dermal reactions. No dermal reactions were observed at 3 min, 1 h and 4 h after gel removal. The test was repeated with two additional rabbits to confirm the initial findings, since the rabbits in the initial test did not exhibit any dermal reaction.

**5.1.8 *a. In vitro* Release of Tacrolimus Niosome Gel**

*In vitro* release study was performed using Modified-Franz diffusion cell. Tacrolimus Niosomal gel formulation (0.045% w/w) was used in the study. Phosphate buffered saline (0.01 M, pH 7.4) was used as a release medium (acceptor compartment). Gel sample (1 g) was placed on a cellulose nitrate membrane (0.1 mm pore diameter), which acted as a diffusion barrier (donor compartment). The assembly was water jacketed to maintain 32±0.5 $^{0}$C. Aliquots of samples were withdrawn at different time intervals during a time period of 48 h and were analyzed using the validated HPLC procedure[31-32].

**6. Stability studies**

The purpose of stability testing is to provide evidence on how the quality of an active substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. In any, rationale design and evaluation of

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dosage forms for drugs, the stability of the active component is the major criteria in determining their acceptance or rejection. During the stability studies the product is exposed to normal conditions of temperature and humidity. However, the studies take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. As per ICH requirements, stability testing of the optimized gel formulation was carried out. Gel was packed in clean, lacquered, collapsible aluminum tubes and different replicates were held in a humidity chamber at 25 ± 2 ° C and 60 ± 5% RH. Gel was evaluated at an interval of 0 - 6 months for alteration of appearance, pH, and drug content and in vitro release profile.

**Results and Discussion:**

**7. PREPARATION OF TACROLIMUS NIOSOMES:**

Three batches from each surfactants Span 20, 60 and 80 were prepared taking various compositions of surfactant: Cholesterol composition. Formulations were labelled as NF1 to NF9. Three ratio of surfactant: Cholesterol ratio i.e. 0.5:0.5, 0.5:1.0, 0.5:1.5 were used for the formulation. Concentration of drug was kept constant as 100 mg.

**7.1 EVALUATION OF NIOSOMES**

**7.1.1 Vesicle size and SEM:**

Details of Tacrolimus particle sizes are described in (Table 4.2), which shows that Vesicle shaped with Span 20 is smaller than Span 80 and Span 60 shaped vesicle. When diffusion was agitated vesicle size was that. The explanation for this is the energy exerted in agitation that results in larger vesicles splitting into smaller vesicles. The size range was found to be between 67.8 nm and 121.6 nm. It was evident that due to increase in the concentration of cholesterol the vesicle is increasing due to increasing the hydrophobicity in the formulation. Among them batch NF6 121.6 nm followed by NF5 111.2 nm and 100.3 nm. Whereas batch NF1 was found to have smallest particle size of niosomes 67.8 nm

**Table 3:** Formulation niosomes vesicle size

| Formulation code | Particle size (nm) |
|---|---|
| NF1 | 67.8 |
| NF2 | 69.2 |
| NF3 | 72.6 |
| NF4 | 100.3 |
| NF5 | 111.2 |
| NF6 | 121.6 |
| NF7 | 98.6 |
| NF8 | 98.2 |
| NF9 | 97.4 |

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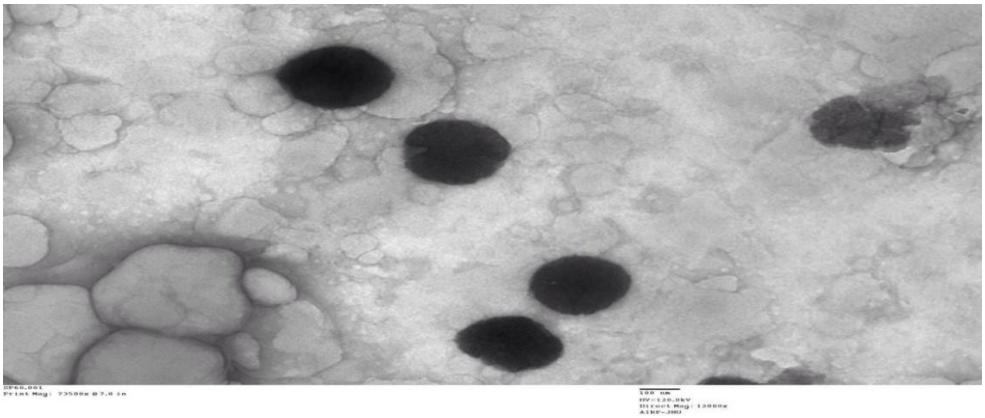

**Fig 3**: Scanning Electron Microscopy of Niosome prepared

### 7.1.2 Drug Contents:

All the batches of niosomes prepared were evaluated for yield of drug content, results are given in Table 4.3. Higher vesicle size given higher drug content. Niosomes formulated with Span 20 gave drug content 83.1±1.1 to 91.8±2.6 mg; Span 60 gave 90.3±1.6 to 93.9 ±1.5 mg and Span 80 gave 93.1±1.5 to 85.1±1.0 mg. All the assessments were done in replicate. Among all the nine batches, NF5 batch was found to have maximum drug content i.e. 99.2 ± 1.2 mg.

**Table 4:** Drug content of formulation batches of niosomal batches

| Formulation code | Drug content Mg |
|---|---|
| NF1 | 83.1±1.1 |
| NF2 | 89.6±1.6 |
| NF3 | 91.8±2.6 |
| NF4 | 90.3±1.6 |
| NF5 | 99.2±1.2 |
| NF6 | 93.9±1.5 |
| NF7 | 93.1±1.5 |
| NF8 | 89.9±1.8 |
| NF9 | 85.1±1.0 |

Data is represented as Mean ± SD, where N = 2

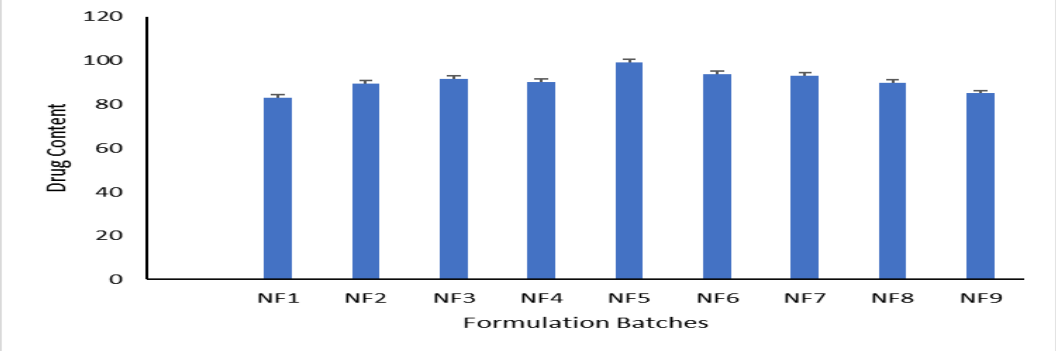


**Fig 4**: Drug Content of different formulated niosomal batches.

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### 7.1.3 Drug Entrapment Efficiency:

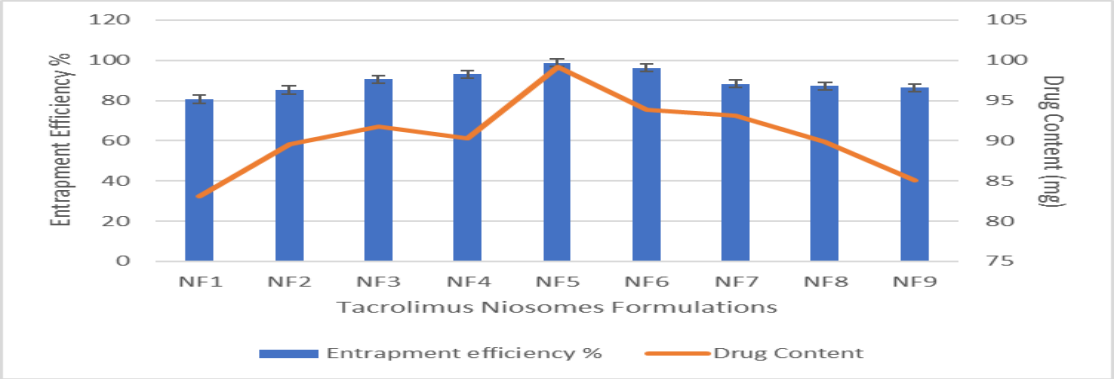
The entanglement productivity of the niosomal definitions were estimated by centrifugation strategy. The components, for example, HLB worth and Phase progress temperature of the surfactant influence the entanglement productivity. Low HLB and high change temperature increment the capture proficiency. Among all the details, NF5 (Span 60/cholesterol S/C 0.5:1.0) indicated most extreme capture proficiency when contrasted and different definitions as appeared in the Table 4.4. It was due to its low HLB value and high transition temperature. NF5 formulation corresponds to the higher drug content and entrapment efficiency 98.686% shown in Fig 4.13.

**Table 5**: Entrapment efficiency of prepared niosomes formulations

| Formulation code | Entrapment efficiency % |
|---|---|
| NF1 | 80.652± 1.20 |
| NF2 | 85.254± 2.30 |
| NF3 | 90.582± 2.50 |
| NF4 | 93.147± 1.80 |
| NF5 | 98.686± 2.30 |
| NF6 | 96.258± 2.80 |
| NF7 | 88.369± 2.60 |
| NF8 | 87.159± 1.60 |
| NF9 | 86.357± 1.90 |

Data was represented as Mean ± SD, N =3

**Fig 5:** Comparison of Drug Content and Drug Entrapment Efficiency



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## 8. FORMULATION OF NIOSOMAL GEL FORMULATION.

From the results of evaluation of batches of niosomal formulations, batch NF5 is found to have with optimized results and suitable found suitable for further process. Niosomes of batch NF5 is used for the formation of gel using different compositions as given in Table 3

### 8.1 EVALUATION OF TACROLIMUS NIOSOMAL GEL FORMULATION:

### 8.1.1 Clarity, Spreadibility and Homogeneity:

All the nine batches of Tacrolimus noisome batches were subjected to prepration of niosomal gel. And Gel preparations were than evaluated as per the clarity, its spreadability and homogeneity. Among all the formulations the gel formed F5 and F6 were found to be clearest with better spreadability and with excellent homogeneity.

**Table 6**: Evaluation of Tacrolimus Niosomal Gel formulations

| Formulation code | Clarity | Spreadability | Homogeneity |
|---|---|---|---|
| **F1** | good | better | excellent |
| **F2** | good | better | excellent |
| **F3** | good | good | very good |
| **F4** | good | good | very good |
| **F5** | very clear | better | excellent |
| **F6** | very clear | Better | excellent |
| **F7** | turbid | good | good |
| **F8** | turbid | good | good |
| **F9** | turbid | average | good |
| **F10** | turbid | poor | good |

### 8.1.2 Viscosity:

Viscosity of all the Tacrolimus niosomal gel formulation was evaluated. Formulation F6 and F5 were found to be highest viscosity among all the other formulation batches i.e. 8166 cPs and 8023 cPs respectively. This evident the better retention of gel formulation on the skin. Formulation F3 and F4 found to be least viscous with viscosity 5106 cPs and 5158 cPs respectively. F6 was reported with highest viscosity and would have higher retention time on the skin.

**Table 7** : Viscosity of Niosomal Gel formulations

| Formulation code | Viscosity (cPs) |
|---|---|
| F1 | 7870 |
| F2 | 6975 |
| F3 | 5106 |
| F4 | 5158 |

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| | |
|---|---|
| F5 | 8023 |
| F6 | 8166 |
| F7 | 6582 |
| F8 | 5921 |
| F9 | 5236 |
| F10 | 5235 |

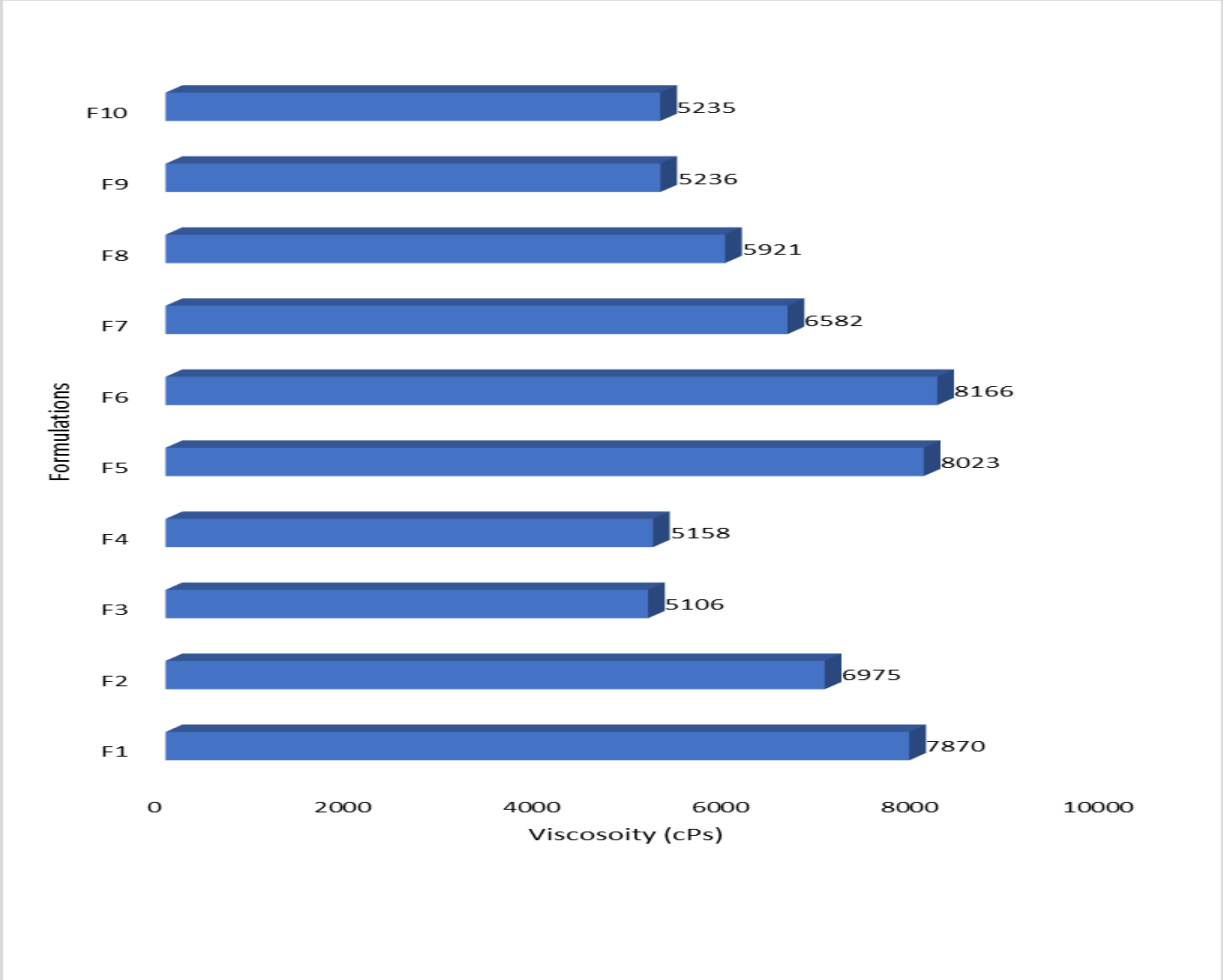


**Fig 6:** Viscosity of Tacrolimus Niosomal Gel Formulations.

### 8.1.3 Extrudability study:

Through the test it was evident though all the formulations showed good extrudability properties but among them formulations F6, F5, F4 and F3 showed excellent extrudability property.

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**Table 8**: Extrudability properties of formulations

| Formulation code | Extrudability |
|---|---|
| F1 | ++ |
| F2 | ++ |
| F3 | +++ |
| F4 | +++ |
| F5 | +++ |
| F6 | +++ |
| F7 | ++ |
| F8 | ++ |
| F9 | ++ |
| F10 | ++ |

++ = Good, +++ = Excellent

### 8.1.4 Skin Irritation study:

All the planned clumps of Tacrolimus Niosomal Gel were exposed to skin disturbance test on dermal tissue. Applications were made for 7 successive days. For every creature, dermal reaction scores at 1 h, 24 h, 48 and 72 h after evacuation of the test materials. added and afterward separated by three to acquire a mean aggravation score for every time point. The outcomes were contrasted with those of the control creatures which got refined water. The mean scores were added and arrived at the midpoint of to acquire the essential disturbance file. Upon overall mean value and observation, the score is classified as A: No reaction, B: Slight erythema, C: Moderate erythema. Any sign of inflammation, eczema and erythema is not observed at different interval as shown in fig 4.15.

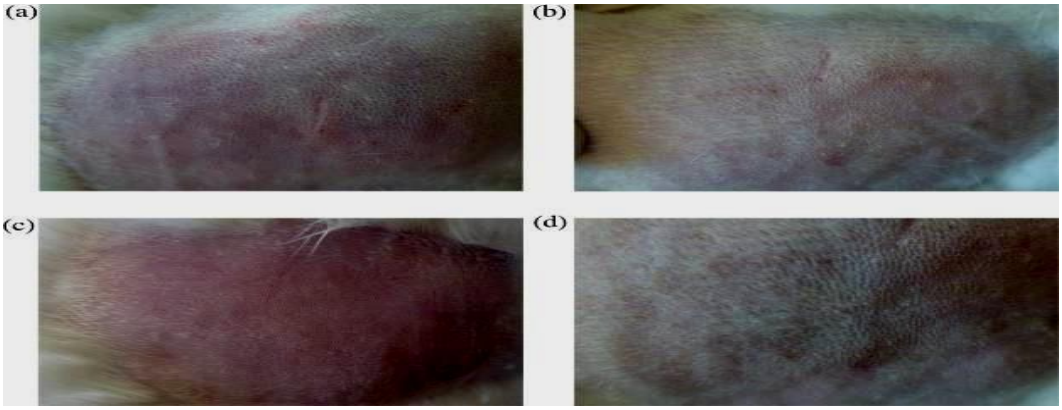


**Fig 7**: Dermal skin irritation studies on skin at different interval by of optimized F6 batch.
a. Dermal in singled group before administration. b. Dermal in singled group at 72 h after administration. c. Dermal in repeated group before administration. d. Dermal in repeated group at 72 h after last administration.

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### 8.1.4 *In vitro* release study

Tacrolimus Niosomal gel batch 6 is released *in vitro* and shows the release profiles. Tacrolimus gel, however, demonstrated a controlled release profile (92.46 ± 2.16) over the 48 h period. Controlled release profile of Tacrolimus niosome gel may be due to slow diffusion of Tacrolimus in the Carbopol matrix from lipid niosome. Controlled release phenotype of Tacrolimus Niosome Gel will avoid the fast delivery of the drug to the exfoliated psoriatic skin, which is sensitive to accelerated absorption of drugs and can thus avoid toxicity.

Tacrolimus Niosomal gel batch F6 which contains carbopol 4% showed release profiles. Tacrolimus gel, however, demonstrated a controlled release profile (92.46 ± 2.16) over the 48 h period. Controlled release profile of Tacrolimus niosomal gel may be due to slow diffusion of Tacrolimus in the Carbopol matrix from lipid niosomes. Controlled release phenotype of Tacrolimus Niosomal Gel will avoid the fast delivery of the drug to the exfoliated psoriatic skin, which is sensitive to accelerated absorption of drugs and can thus avoid toxicity. Where batch F9 containing gaur gum 2 % as gelling agent showed lowest drug release in same period of time.

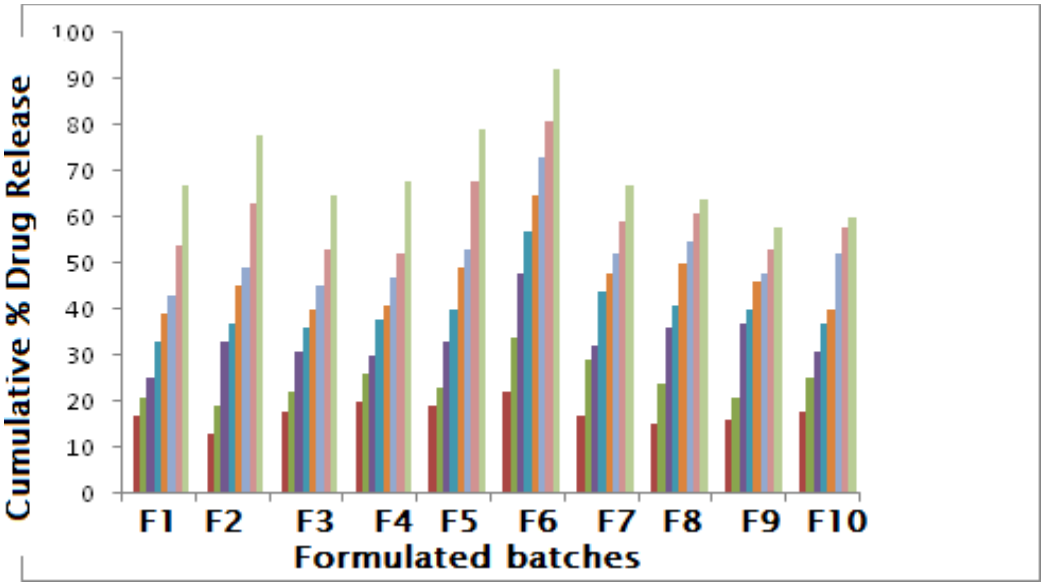


**Fig 8: % Cumulative drug release of Tacrolimus Niosomal gel batches F1 – F10.**

### 8.1.5 *In vivo* antipsoriatic activity

**Table 9**: PASI Score of anti-psoriatic studies of Tacrolimus Niosome Gel formulation batch F6.

| Groups | Erythema | | Scaling | | Thickness | | Average PASI score | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 11 | Day 1 | Day 11 | Day 1 | Day 11 | Day 1 | Day 11 |
| Group I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Group II | 1 | 1 | 2 | 2 | 3 | 3 | 2 | 2 |
| Group III | 1 | 1 | 3 | 2 | 2 | 2 | 2 | 1.6 |
| Group IV | 1 | 0 | 2 | 0 | 3 | 1 | 2 | 0.5 |

Group IV that contains the treatment with the best formulation (F6) of Niosomal gel of Tacrolimus showed lowest PASI score among the treatment group. Along with the when it is compared with that of negative group it is found in nearest value after treatment i.e. 0.5.

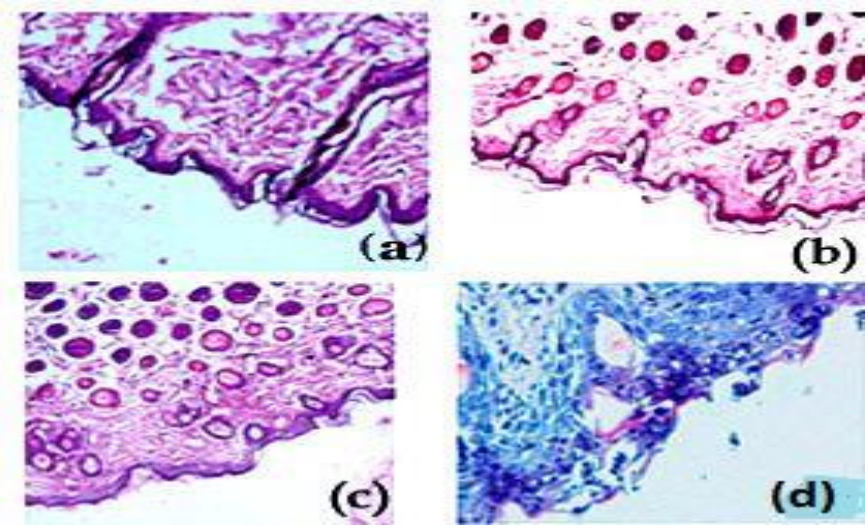


**Figure 4.17**. Histopathology of skin samples (200_, H&E staining). Negative control (a), positive control (b), blank gel (c), Tacrolimus Niosomal gel

**Morphology of optimized gel formulation:**

The optimized batch of noisome gel formulation was subjected to determination of morphological characters of it which is determined by Transmission electron microscopy as shown in fig 4.18. From the study it was found that particles were evenly distributed and was spherical in shape.

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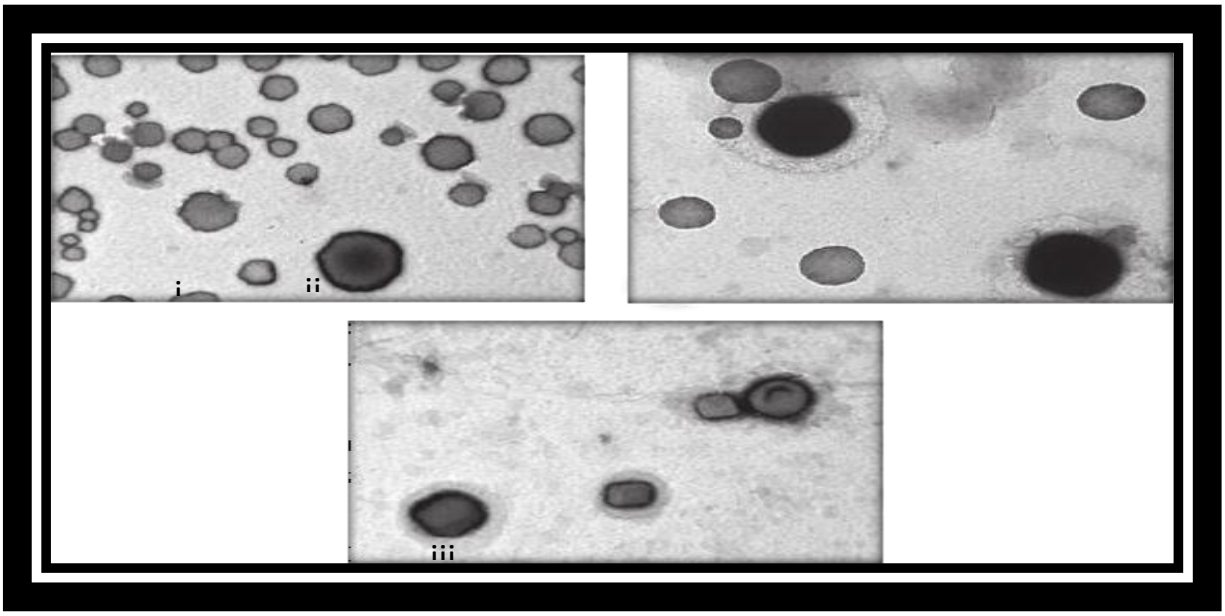


**Fig 9**: TEM of Tacrolimus Niosomal Gel Formulation F6 **i.** at 0.5 µm, **ii.** 1 µm and **iii**. At 2 µm

9. **Stability Studies:**

The optimized Niosomal Gel formulation F6 was subjected to stability studies over the period of 6 months. The formulation was evaluated for Drug content, pH and % drug release over the period. The formulation was attributed to have average 93.9 ± 1.5 mg drug content at the starting 0 month, which found to be 93.8 ±1.8 at the 6$^{th}$ month. There is no significant difference between the drug content in mean time interval. This justifies the standardized parameters of evaluation and there is no engulfing and loss of drug content. In the duration of the study the pH and physical appearance of the formulation remained constant. Also there is no significant change observed in the % drug release for the drug formulation. At the day 0 it was found to be 64 ± 1.24 and at 6$^{th}$ month it was found to be 63.17 ± 1.9%. The results are shown in Table 4.10.

**Table 10**: Stability Studies of Optimized Niosome Gel formulation

| **Parameters** | **Months** | | | | |
|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **6** |
| **Drug content (mg)** | 93.9 ± 1.5 | 94.1 ± 0.50 | 93.7 ± 1.2 | 94.01± 0.43 | 93.8 ± 1.8 |
| **pH** | 6.2 | 6.1 | 6.2 | 6.1 | 6.3 |
| **(%) Drug release** | 64 ± 1.24 | 66.17± 0.23 | 65.24± 1.24 | 65.8 ± 1.8 | 63.17 ± 1.9 |

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| | |
|---|---|
| **Physical Appearance** | **Transparent Consistent Gel** |

Data is expressed as Mean ± SD, N= 3 $p<0.05$

## CONCLUSION

- Depending the evidences given in literature drug Tacrolimus was selected for the study. The drug was procured and subjected to physical characterisation and through spectral methods like UV and IR.
- Drug was standardised to check the purity and found that the calibration curve of UV absorption ranges in 291 nm. The drug solution of 10 different concentration showed linear relationship with an R2 value of 0.997. Characterization of the drug through FTIR showed corresponding peaks structural elements of Tacrolimus, which also confirmed the tacrolimus.
- DSC thermogram of pure drug was obtained which showed an endothermic peak at 130.11$^{o}$C. Tacrolimus mixture with polymers like Carbopol, HPMC and SPAN were subjected to the analysis by FTIR and DSC for their interaction with drug. The results showed that there was no interaction found between the two.
- Niosomes of the drug tacrolimus were prepared using various surfactant: cholesterol ratio and total 9 batches of niosomes were prepared. The prepared batched were than subjected to characterization in order to identify the optimized batch for further use of preparation of Niosomal gel. And as per the results of particle size, drug content and entrapment efficiency the NF-5 batch was found most suitable and further used for the preparation of gel batches.
- Total 10 batches of the niosomal gel were formulated and characterized. As per the characterization parameters batch F-6 was found as optimized formulation, which is further subjected to stability studies and pharmacological studies.
- The stability studies showed a non-changing parameters over the course of study of 6 months. There was no significant change observed.
- For anti-psoriatic study the PASI score of the formulation were determined experimenting on Balb/c mice. And found that the animals treated with formulation showed a lowest score among the treatment groups and the average PASI score was found to be reduced from 2 to 0.5 after treatment. Also the blank gel formulation was subjected to evaluation in Group III which showed no significant changes in the score before and after the course of treatment.
- This evident the good penetration of drug through the corresponding formulation and apart from the API the other formulating agent remains inactive for their pharmacodynamic properties. Also from the in-vitro release study it was evident that optimized formulation of Tacrolimus gel, demonstrated a controlled release profile (92.46 ± 2.16) over the 48 h period. Controlled release profile of Tacrolimus niosome

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gel may be due to slow diffusion of Tacrolimus in the Carbopol matrix from lipid noisome. This evident the proposed composition using API Tacrolimus can be used as novel topical formulation for the treatment of Psoriasis.

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