ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.6.P21-33

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Development and Invitro Evaluation of Solid Lipid Nano Particles Loaded Topical Gel Containing Combination of Drugs Used In The Better Therapy of Psoriasis

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Abstract: The present research work was aimed to develop a Solid lipid nanoparticles (SLNs) based topical gel for the treatment of psoriasis. SLNs were prepared and then incorporated in a topical gel as a carrier. High-Pressure Homogenization method was used to improved drug loading capacity and drug release properties. Excipients like Compritol 888 ATO, Tween 80,Precirol ATO5, Poloxamer407, Cremophor RH40, Carpobol 934, Methyl Paraben, TEA, Distilled water were used. The optimized formulations were based on Zeta potential, analysis of particle size, differential scanning Colorimetery, scanning electron microscopy and study of Invitro drug release. The present research study revealed that the SLNs based Gel containing F4 formulation could potentially exploit as a carrier with improved drug loading capacity and drug release properties. Thus, tacrolimus loaded SLNs formulation can be beneficial in the treatment of psoriasis. It was concluded that the prepared formulation can be used for treatment of psoriasis by using the topical therapy of nanogel and this will attempt to increase the efficacy of the drug at the site of action.

Key Words: High-Pressure Homogenization (HPH), Tacrolimus, Finasteride, Nanogel, Solid lipid nano carrier.

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Received On 23 March 2021
Revised On 15 October 2021
Accepted On 19 October 2021
Published On 05 November 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Suryakumari Chalakanti, Narender Malothu * , Development and Invitro Evaluation of Solid Lipid Nano Particles Loaded Topical Gel Containing Combination of Drugs Used In The Better Therapy of Psoriasis.(2021).Int. J. Life Sci. Pharma Res.11(6), P21-33 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.6.P21-33

1. INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder typically characterized by erythematous plaques covered with silver or white scale on the surface by proliferation of epidermis with parakeratosis, polymorph nuclear leukocyte infiltration in the stratum corneum, dilation of superficial blood vessels.[1]Whereas atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by an itchy, erythematous and intensely pruritic rash with periods of exacerbation and remission.[2]Treatment with topical corticosteroids, although effective, may be associated with several local and systemic adverse events, such as thinning of the skin and adrenal gland suppression. [3]Patients' fears about the safety profile of topical corticosteroids also have important implications for adherence to treatment, and knowledge on differentiating weak preparations from strong preparations is poor. [4] Therefore, there is a need for an alternative treatment that is efficacious and free from the long-term side effects were associated with corticosteroids.[5] The use of corticosteroids is regarded as a first line therapy for chronic patients, however the more prevalent and popular therapy is that of Tacrolimus and other drugs which can be considered as second line therapy in this case.[6]Targeted delivery of drug molecules to specific organ sites such as the skin or eye is one of the most challenging areas of research in pharmaceutical development. The skin is the largest organ of the body and functions as a protective layer skin having large surface area (1.8m^2) and easy accessibility of skin make it an attractive route for drug delivery. However, the unique structure of skin limits the transport of molecules through it. The skin is broadly categorized into the non-viable epidermis called stratumcorneum, viable epidermis, and dermis but is a complex structure with sweat glands, hair follicles and blood vessels (Figure 1).

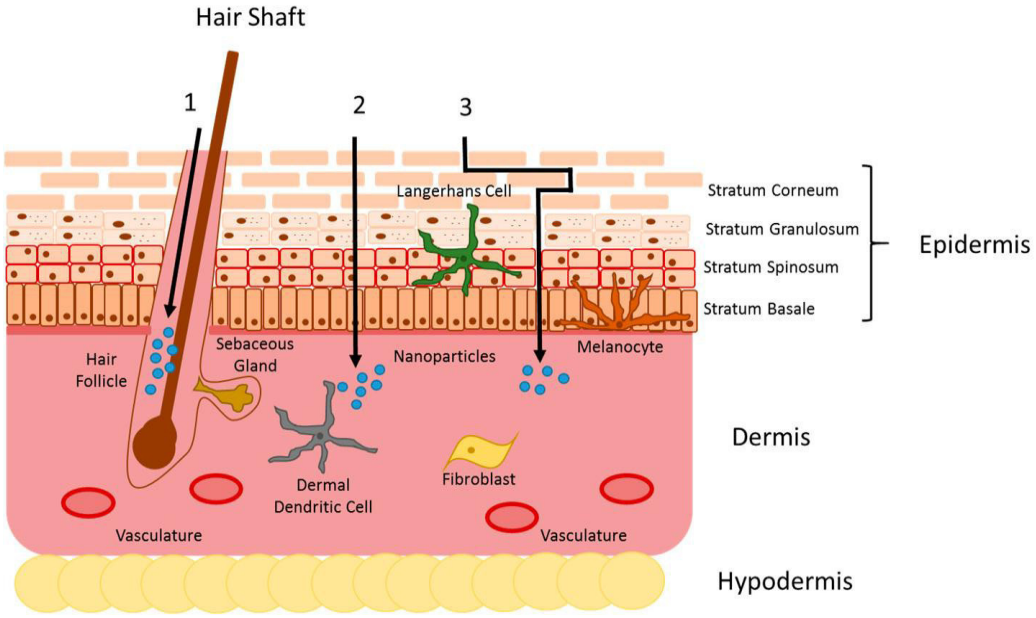


Fig 1. Illustration of nanoparticle skin penetrations.[4]

Topically applied nanoparticles can penetrate the skin in one of three different ways: (1) Through the appendageal route, (2) through the intracellular route, or (3) Through the intercellular route. The appendageal route involves nanoparticles entering hair follicles, sweat glands, or skin furrows for either penetration to the dermis or retention for increased drug release. The intracellular route involves a direct path through the cell membrane of multiple layers of the epidermis. The intercellular route involves a more tortuous path between epidermal cells. The pathway taken likely depends on the nanoparticle size, charge, morphology, and material. The term 'nanogel' is defined as the nano sized particles formed by physically or chemically cross linked polymer networks that is swelling in a good solvent. [8]The term "nanogel" (Nanogel™)was first introduced to define cross-linked bifunctional networks of a polyion and a nonionic polymer for delivery of Poly Nucleotides. [9] Spontaneous outbreak in the field of nanotechnology have introduced the need for developing nanogelsystems which proven their potential to deliver drugs in controlled, sustained and targetable manner with the emerging field of polymer sciences and it has now become inevitable to prepare smart nano-systems which can prove effective for treatment as well as clinical trials Progress.[10]

1.1 Solid lipid nanoparticles(SLNs)

The first generation of lipid nanoparticles is composed of Lipids which is solid at room temperature and are covered by a surfactant shell that stabilizes their dispersion. [11] The first SLNs were developed in the early 1990s. SLNs are considered to have all the advantages of liposomes when used as topical carriers, and can also improve the physical and chemical stability of loaded drugs. SLNs have been used as topical carriers for many lipophilic drugs, such as isotretinoin, retinyl palmitate, prednicarbate, tacrolimus, penciclovir, clotrimazole, and antioxidants including lutein, curcuminoids, quercetin, and idebenone .[12]

1.2 Tacrolimus (FK-506)

Tacrolimus (FK-506)is a powerful macrolide immuno-suppressant, isolated from the fermentation broth of Streptomycin tsukubaensis and has shown notable efficacy as a rescue or primary immunosuppressant therapy for immune-inflammatory conditions including atopic dermatitis. It inhibits early T-cell activation in gene transcription such as interleukin, which result from its inhibition of phosphatase

(calcinuerin) in activated T-cells.[13]It binds to cytoplasmic receptor FKBP- immunophilin and gain ability to associate with calcinuerin and inhibits its phosphatase activity resulting in inhibition of T-lymphocyte (i.e. immunosuppressant) and thus suppresses information. [14] However, Tacrolimus has proven to be effective for the treatment of skin diseases like atopic dermatitis or psoriasis. The main target for immunosuppressant drug Tacrolimus is the dermis with its lymphocytes. [15]On the other hand, the Formulation already available in the market are in ointment form which is again has disadvantage of greasy nature of dosage form and difficult to remove with low penetration across stratum corneum which gets extended in psoriatic skin. Therefore, it becomes a challenge to reach the dermis sufficient active drug concentrations because of the high molecular size of Tacrolimus (822.05 $gmol^{-1}$), structure and lipophilicity (log 3.96 ±0.83) with an ointment base. Therefore, the objective of our study become very clear to develop some alternative formulation which can carry the drug up to dermis layer of skin to improve the bioavailability and should be non-greasy with the advantage of ease of applicability.[16]Generally the formulations of Tacrolimus commercially available are in oral and ointment form. More recently, a topical gel formulation will be introduced specifically for the treatment of localized painful and inflammatory condition, such as soft tissue musculoskeletal disorders and osteoarthritis. So in the present study, formulation and evaluation of Tacrolimus transdermal gel will be attempted to increase the efficacy of the drug at the site of action[1].

1.3 *Finasteride (FIN)*

Finasteride (FIN)reduces androgenetic alopecia through the specific and competitive inhibition of 5α-reductase enzyme. This enzyme is responsible for the dihydro testosterone (DHT) production in hair follicles.[18] Accordingly, FIN decreases the DHT levels in the hair follicles and in the blood. Although the FDA has approved FIN to use in oral formulations, the administration of FIN by this route has led to undesirable systemic side effects[19] .The undesirable effects includes mood disturbance, gynecomastia, decreased libido, erectile dysfunction, and ejaculation disorder. Consequently, Successful topical administration of FIN could improve FIN penetration and increases its accumulation in the skin layers and hair follicles and could possibly reduce its major side effects resulting from oral administration.

2. MATERIALS AND METHODS

2.1 *Materials*

Tacrolimus received as a gift sample from the company Yacht Parma, Hyderabad. Finasteride was obtained from Carbanio chemicals industries (Biocon, Bangalore).Compritol 888 ATO, Labrasol, Poloxamer 407, Cremophor RH 40,Precirol ATO5 , Carpobol 934,Methyl Paraben, Triethanolamine from Carbanio chemicals industries. All the chemicals were of analytical grade and were used without further purification.

2.2 *Method Preparation of Transdermal Gels*

2.3 High-Pressure Homogenization (HPH)

HPH is a reliable and powerful technique, used for production of solid lipid nanoparticles. High-pressure homogenizers push liquid at high pressures (100 – 2000bar), through a narrow gap (in the range of few microns).The fluid accelerates over a very short distance under very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated.[20]

2.4 *Procedure*

Accurately weighed amount of Polymers (Compritol 888ATO, Precirol and Poloxamer 407, Camophar RH40) in four different ratios was placed in known amount of distilled water (Eight formulations were prepared using varying concentrations of Compritol 888ATO, Precirol and Poloxamer 407, Camophar RH40). After complete dispersion, the polymer solution was kept in 24 hours for complete swelling. Accurately weighed amount of Tacrolimus was dissolved in a specified quantity of suitable solvent. The drug solution is added slowly to the aqueous dispersion of polymer with the help of High-pressure homogenizers push liquid at high pressures (100 – 2000bar), and high speed stirrer (20000 rpm) taking precaution that air did not entrap. Finally, the remaining ingredients were added to obtain a homogeneous dispersion of gel[21]

Table: 1 Composition of SLNs loaded with Tacrolimus and Finasteride-SLNs topical gels (0.5% w/w).

FK 506&FIN-SLNs dispersions(% w/w)					FK 506& FIN-SLNs topical gels (% w/w)				
Formulation code	Compritol 888 ATO (%w/w)	Precirol ATO5 (%w/w)	Poloxamer 407 (%w/w)	Cremophor RsH40 (%w/w)	Carpobol 934 (%w/w)	Methyl Paraben (mg)	TEA[b](ml)	Distilled water up (ml)	Gel code
SLN (F1)	4	–	0.5	–	2%	0.1	2	100	Gel 1
SLN(F2)	6	–	0.5	–	2%	0.1	2	100	Gel 2
SLN (F3)	8	–	0.5	–	2%	0.1	2	100	Gel 3
SLN(F 4)	10	–	0.5	–	2%	0.1	2	100	Gel 4
SLN (F5)	–	4	–	0.5	2%	0.1	2	100	Gel 5
SLN(F 6)	–	6	–	0.5	2%	0.1	2	100	Gel 6
SLN(F7)	–	8	–	0.5	2%	0.1	2	100	Gel 7
SLN(F8)	–	10	–	0.5	2%	0.1	2	100	Gel 8

Tacrolimus concentration is 0.5% w/w,finasteride.0.005%

TEA: Triethanolamine.

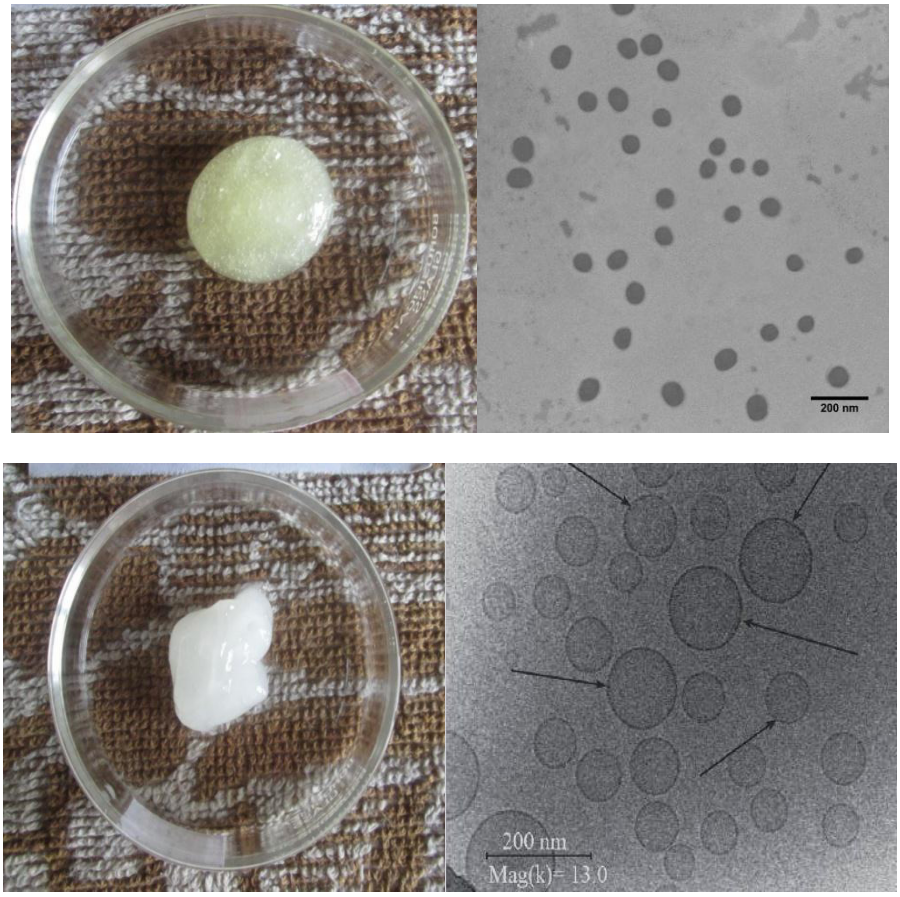


Fig 2&3 SEM images of formulated Tacrolimus nanogel, Scanning electron microscopy

2.4. *Characterization of Tacrolimus SLNs*

Total 8 different SLN (F1 to F8) formulations were prepared for the evaluations of clarity, pH, viscosity, Spreadability, Extrudability, skin irritation test, percentage drug content, *in-vitro* diffusion studies, *in-vitro* drug release kinetic study, *ex-vivo* permeation studies using rat abdominal skin and stability studies by using standard procedure. All studies were carried out in triplicate and average values were reported[22].

2.5. *Clarity*

Clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows[23]:turbid +; clear ++; very clear (glassy) +++.

2.6. *Homogeneity*

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.[24]

2.7. *Consistency*

The estimation of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fixed distance of 10cm in other way that it should fall down on the centre of the glass cup which was filled with the gel. The penetration by the cone was accurately measured from the surface of the gel to the tip of the cone inside of the gel. The distance traveled by cone in the period was noted down after 10sec.[25]

2.8. *Spreadability*

It was determined by wooden block and glass slide apparatus. For the determination of Spreadability, excess of sample was applied in between two glass slides and then was compressed to uniform thickness. The weight (50gm) was added to pan. The time required to separate the two slides i.e., the time in which upper glass slide moves over the lower plates was taken as a measure of Spreadability(S). It is calculated by using the formula[26]:

$$S = m \cdot l / t$$

m – weight tied on upper slide
l – length of glass slide
t – time in s

2.9. *Extrudability*

Extrudability test was carried out by using Pfizer hardness tester. 15gm of gel was filled in collapsible aluminium tube. The plunger was adjusted to hold the tube properly and the pressure of 1kg/cm^2 was applied for 30 sec. The quantity of the gel extruded was weighed. The procedure was repeated at three equidistance places of the tube. The test was carried out in triplets.[27]

2.10. *Surface pH*

2.5 gm of gel was accurately weighed and dispersed in 25ml of distilled water. The pH of the dispersion was determined by using digital pH meter.

2.11. *Viscosity*

Viscosity was determined by using Brookfield viscometer. Viscosity measurements were carried out at room temperature (25- 27°C) using a Brookfield viscometer (Model RVTDV II, Brookfield Laboratories, Hyd).

2.12. *Drug Excipient compatibility studies*

The drug polymer and polymer-polymer interaction was studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr. The mixture was grounded to a fine powder using an agate mortar and then compressed into a KBr Disc in a hydraulic press at a pressure of 1000psi.[28]Each KBr disc was scanned 16times at 2 mm/sec at a resolution of 4 cm^{-1} using cosine apodization. The characteristic peaks we rerecorded

2.13. *Drug content*

A specified quantity (100mg) of developed gel and marketed gel were taken and dissolved in 100ml of phosphate buffer of pH 6.8 separately. The volumetric flask containing gel solution was shaken for the period of 2hr on mechanical shaker in order to get absolute solubility of drug. This solution was filtered and estimated spectrophotometrically at 294.nm using phosphate buffer (pH 6.8) as blank.[29]

2.14. *Stability studies*

The optimized formulation F4 was subjected to a stability testing for the period of three months as per ICH norms at a temperature of 25°±2°C with relative humidity RH= 60±5% and 40° ± 2°C with relative humidity RH= 75±5%. The optimized formulation F4 was analyzed for the changes in appearance, pH, percentage of drug content and *in-vitro* diffusion study by procedure stated earlier.[30]

2.15 *Zeta potential and particle size*

Zeta potential was assessed for colloidal dispersions to know the physical stability of gels using the measure of the magnitude of the electrostatic or charge repulsion or attraction between particles using dynamic light scattering method(Zeta Sizer Nano-ZS: Malvern Instruments Ltd, United Kingdom).. Particle size was analyzed to establish the physical stability of optimized SLNs formulations.[31]

2.16. *Scanning electron microscopy (SEM)*

The Tacrolimus SLNs surface and shape characteristics were determined by using gold sputter technique in SEM SLNs drugs were loaded with fixed on subtitling double-sided tape. The gels containing the sample were coated with gold using JEOL fine coat (JFC-1100F ion sputtering device).[32]

2.17. *Determination of encapsulation efficiency (EE) and loading capacity (LC)*

Percent values were expressed as the percentage of trapped drug compared to the initially added drug or to the used lipid, encapsulation efficiency and loading efficiency of Tacrolimus-loaded SLNs formulations were determined by first separation of the un-encapsulated drug by centrifugation method using an AmiconUltra-15 -30 K tube (Millipore, Germany) (at 6000 rpm for 20 min) and measurement of the concentration of free drug in the lower chamber. The formulations in the upper chamber of Amicon Ultra tube were rinsed three times by hydro alcoholic solution to eliminate unloaded drug and were used for the subsequent experiments. Drug concentration was determinate by the percent amount of drug was determined by spectrophotometer[33].

2.18. *X-ray diffraction study (XRD)*

XRD analysis is a unique method in determination of Crystallinity of a compound and properly interpreted, by comparison with drug XRD pattern before formulation, and this allows the identification of the drug crystalline changes. X-ray diffract to grams of pure Tacrolimus, Methyl Paraben, Poloxamer 188 as well as physical mixture and freeze dried powder of optimized SLNs formulation were obtained using the X-ray diffract meter.[34]

2.19. *Differential Scanning Calorimetry (DSC)*

The Crystallinity rate depends on using DSC which is estimated by melting enthalpy/g comparison of the bulk material with the melting enthalpy/g of the dispersion.The DSC thermo grams of the drug was estimated by using instrument (Diamond DSC, Perkin) and lyophilized SLNs was recorded at heating rate temperature is 10°C/min from temperature 0-250°C under N_2flow.[35]

2.20 *In vitro* release study

Rats weighing 135-160 gm were used to obtain freshly excised full thickness skin. Animal was sacrificed by spinal dislocation. Hairs from abdominal regions was removed by means of surgical and razor taking care not to damage the epidermal surface and subcutaneous fats was removed carefully without damaging the skin.[36]

2.21 In vitro drug permeation through rat abdominal skin membrane

In vitro permeation of transdermal gel was studied through the rat abdominal skin membrane. The skin membrane was mounted between the donor and receptor compartment of the standard Franz diffusion cell with a diffusion area of2.1 cm^2 and the acceptor compartment volume of 21ml.The two chambers were tied with the help of springs so that the

skin membrane did not move from its place. The phosphate buffer pH 6.8 in the acceptor compartment was continuously stirred at 600rpm using a magnetic stirrer. The entire setup was placed over a magnetic stirrer and the temperature was maintainedat37°±0.5°C by placing the diffusion cell in a water bath. The selected gel (F4) containing 0.5mg of Tacrolimus was placed into the donor compartment.[37]The amount of drug permeated through the membrane was determined by removing aliquots from the receptor compartment and by replacing the same volume of buffer. The amount of Tacrolimus in the diffusion samples was estimated by the HPLC method and the flux (J) through the membrane was calculated by using the equation. J = dQ / Adt Where 'J' is flux (mg $h^{-1}cm^{-2}$); dQ / dt is the slope obtained from the steady-state portion of the curve and A is the area of diffusion (cm^2)

3. *Drug release kinetic studies*

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data was fitted into zero-order, first order, Higuchi and Korsmeyer Pappas release model, to study the drug release from the dosage form. After the triplicate drug release study, the results were expressed by a graph of percentage cumulative release at different time (in hrs) intervals and calculated the kinetics of drug release from the nanogel. The results were described in mathematical models such as first-order, zero-order, and Higuchi were used[37.]

3.1 *Zero-order model*

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q0 - Qt = K0\ t \quad (1)$$

Rearrangement of equation (1) yields

$$Qt = Q0 + K0\ t \quad (2)$$

Where Qt is the amount of drug dissolved in time t,

3.2 *First order model*

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order rate constant expressed by the equation:

$$\text{Log } C = \log C0 - Kt / 2.303$$

Where C0 is the initial concentration of drug, k is the first order rate constant, and t is the time

3.3 *Higuchi model*

Graph was plotted between cumulative percentages of drug released vs. square root of time.

$$Q = K1/2$$

Where K is the constant reflecting the design variables of the system and t is the time in hours

3.4 *Korsmeyer-Peppas model*

Korsmeyer*et al.* (1983) derived a simple relationship which described drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model

$$Mt / M\infty = Ktn$$

Where $Mt / M\infty$ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent

3.5. *Skin Irritation Test*

The hair on the dorsal side of Rabbits was removed by clipping1day before the experiment. The rabbits were divided in to 3groups. Group1served as control; Group2receivedoptimizedformulation;Group3received0.8 %v/vaqueoussolutionofformalinasastandardirritant.[37]Finally ,theapplication sites were graded according to visual scoring scale.

STATISTICAL ANALYSIS

All values obtained were expressed as mean± standard error mean(SEM). Statistical comparisons were performed by analysis of variance and Student's *t* test using SAS Version 8.0 software.[37]

4. RESULTS AND DISCUSSIONS

An important parameter affecting drug incorporation is the polymorphic modification of the lipid particle matrix. In general, the process of production of nano particles can change the type of modification of their respective fraction. In order to select the optimized formulation, various evaluation parameters were checked and subjected to *in vitro* diffusion study and their release kinetic study were observed and optimized formulation was further developed for *ex-vivo* permeation using rat abdominal skin.Compritol 888ATO gels were found to be sparkling and transparent, Camophar RH40 gels were found to be translucent. All gels were free from presence of particles. All developed gels (F1-F8) showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent. The value of Spreadability indicates that the gel is easily spreadable by small amount of shear. In formulations F1 to F4, Spreadability of Compritol 888 ATO gel was in the range 18.75- 27.39 g.cm/sec. In formulations F5 to F8, Spreadability of Poloxamer gel was in the range 20.06- 24.27 g.cm/sec. F4 was good i.e. 27.39 g. cm/sec as compared. The extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly, and hence suitable consistency is required in order to extrude the gel from the tube. Extrudability of Compritol888 ATO gel i.e. F4 formulation was found to be Excellent when compared to other formulations. The results were expressed in (Table 2).

Table 2: Results of the evaluated parameters clarity, homogeneity, Spreadability, Extrudability

Formulation Code	Clarity	Homogeneity	Spreadability	Extrudability
F1	+	Satisfactory	12.14	+
F2	++	Good	17.10	++
F3	++	Good	21.46	++
F4	+++	Excellent	23.22	+++
F5	++	Good	20.13	+
F6	++	Good	19.05	++
F7	++	Good	22.11	++
F8	++	Good	20.31	+ +

The pH value of all developed formulations of Compritol888 ATO (F1-F4) were in the range of 5.71- 6.27, Poloxamer 407 gels (F5-F8) were in the range of 6.45- 6.82 Hence, it was concluded that all the formulations could not produce any local irritation to the skin. The Viscosity of the formulations i.e. F1-F4 containing drug and Compritol888 ATO were in the range of 1, 92,000-3, 10,000 cps, whereas the formulations i.e. F5-F8 containing drug and Poloxamer407 were in the range of 1, 36,000 – 1, 47,000 cps, whereas formulations From the results it was found that the formulation F1 showed maximum viscosity i.e. 3, 20,000 cps and formulation F8 showed minimum viscosity i.e. 1, 36,000cps.The percentage drug content of all prepared gel formulations i.e. F1 to F8 were found to be in the range of 95.21±0.18 to 98.46±0.26%. The percentage drug content of formulations was found to be within the I.P limits. Hence methods adopted for gels formulations were found suitable. The results are shown in (Table 3).

Table 3: pH, Viscosity and Drug Content (%)

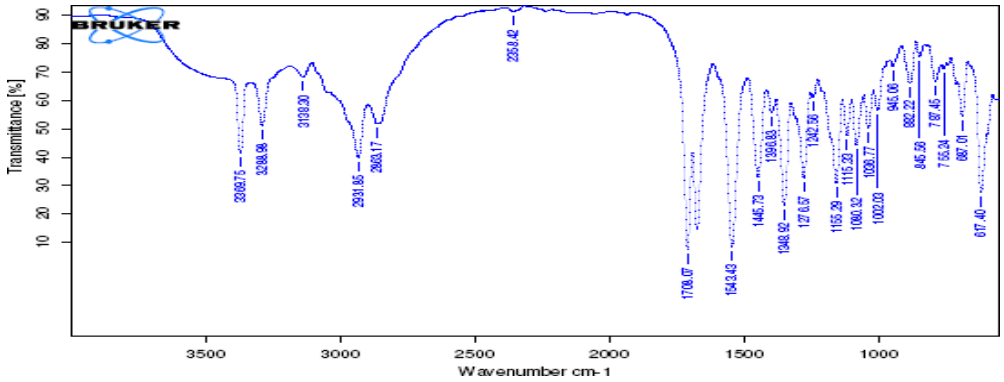
Formulation code	pH	Viscosity (cps)	Drug Content (%)
F1	5.67±0.01	3,09,000	91.14±0.11
F2	5.70±0.14	1,70,000	90.11±0.10
F3	6.12±0.03	2,61,000	94.86±0.21
F4	6.32±0.06	3,11,000	96.10±0.13
F5	6.24±0.04	1,44,000	94.46±0.18
F6	6.15±0.03	1,41,000	92.92±0.17
F7	6.80±0.01	1,36,000	94.12±0.13
F8	6.66±0.05	1,31,000	93.35±0.15

Accelerated stability studies was conducted in optimized formulation(F4) , according to ICH guidelines i.e. 25˚±2˚C/60±5%RH for first 30 days and 40˚±2˚C/75±5%RH up to 90 days. The results indicates, that there was no much change in appearance, pH, and drug content and in-vitro drug release studies. The results are shown in (Table 4).

Table 4: Stability studies of formulation F4

Formulation	Days	Temperature and Relative Humidity	Appearance	pH	Drug content	In-vitro drug release
F4	0	25˚±2˚C/60±5% RH	Clear	6.12	97.3	96.18
F4	15	25˚±2˚C/60±5% RH	Clear	6.10	99.2	97.10
F4	30	25˚±2˚C/60±5% RH	Clear	6.17	98.6	97.42
F4	60	40˚±2˚C/75±5% RH	Clear	6.11	98.5	98.15
F4	90	40˚±2˚C/75±5% RH	Clear	6.14	96.1	98.22

4.1 *Drug Excipient compatibility studies*



By FTIR spectroscopy, the incompatibility between drug and excipients were studied and the results indicated that there were no chemical incompatibility between drug and excipients in the formulation. The results are shown in [Fig 4&5].

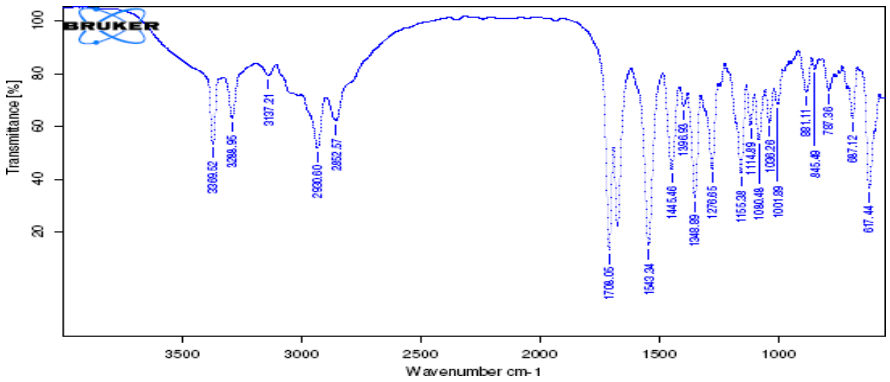
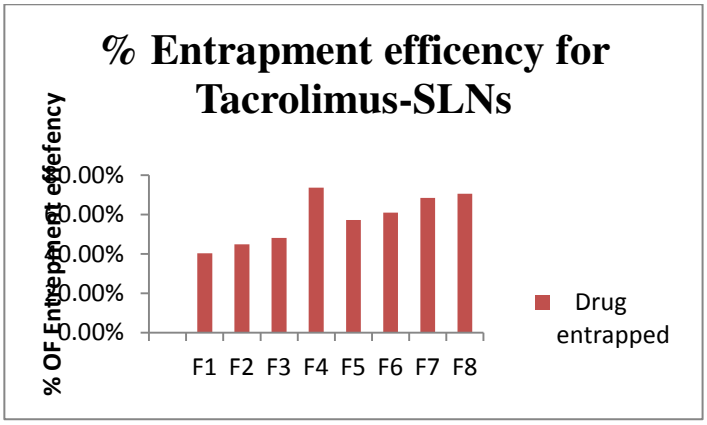


Fig:4&5 FT-IR Spectra of Tacrolimus &Compritol 888 ATO, Poloxamer 407, Cremophor RH 40, Precirol ATO5Carpobol 934

Table 5: Particle size distribution of formulation F1 to F8

Formulation Code	Particle Size (nm)
F1	2400-3200
F2	1700-2400
F3	930-950
F4	1500-1700
F5	820-960
F6	785-850
F7	733-740
F8	630-670

Table 6: Percentage drug entrapped in formulation F4 to F8.	
Formulation Code	**Drug entrapped**
F1	41.11 %
F2	40.70 %
F3	42.16 %
F4	70.60 %
F5	56.10 %
F6	61.81 %
F7	67.33 %
F8	72.11 %



Each value represents the mean ± SD (n=3).

Fig: 6 Percentage entrapment efficiency for Tacrolimus-SLNs.

4.2. *In-vitro* drug diffusion studies

In-vitro drug release study of different gel formulations i.e. F1 to F8 were carried out through dialysis sac (cellophane membrane) and were plotted (Fig 7). The percentage drug release for the formulations containing drug and Compritol888 ATO i.e. F1 to F4 were found to be in the range of 82.88% to 98.68% in 6 hours. Among these formulations, formulation F4 containing drug and Compritol888 ATO in the ratio1:2 showed high percentage of drug release i.e. 98.68%in 6 hours. The results indicates that the drug release property were directly proportional to concentration. The percentage drug release for the formulations containing drug and Poloxamer 407 i.e. F5-F8 were in the range of 79.59 – 87.72% in 6 hours. Among these, formulation F8 containing drug and Poloxamer 407 in the ratio 1:4 showed highest percentage of drug release i.e. 87.72% in 6 hours. The comparison of *in-vitro* drug release studies were conducted for the formulations F4, and F8(Fig 8-9). The *In-vitro* studies revealed that the formulation F4 containing drug and Compritol888 ATO in the ratio 1:2 showed highest percentage drug release i.e.98.68% in 6 hours.

4.3. *Drug release kinetics*

According to Zero order, First order, Higuchi and Korsemeyer-Peppas equation were used to ascertain the mechanism of drug release. Among the zero-order and first-order, the R^2 values were found to be higher in zero-order. All the formulations followed zero-order. From the Higuchi and Korsemeyer-Peppas equation, the R^2 value were found to be higher in Korsemeyer-Peppas equation and release exponent "n" value less than 1 i.e. (n > 0.5). This indicates that all the formulations followed non-Fickian diffusion. Hence it was concluded that all the formulations followed zero-order drug release with non-Fickian diffusion.

Table 7: Drug release kinetics of all the formulations (F1 – F8)					
Formulation code	**Zero order**	**First order**	**Korsmeyer-Pappas**		**Higuchi**
	R^2	**R^2**	**R^2**	**N**	**R^2**
F1	0.986	0.893	0.994	0.780	0.957
F2	0.990	0.871	0.993	0.782	0.945
F3	0.988	0.872	0.991	0.765	0.957
F4	0.990	0.936	0.998	0.781	0.959
F5	0.990	0.924	0.996	0.783	0.952
F6	0.991	0.902	0.993	0.785	0.957
F7	0.981	0.979	0.941	0.783	0.924
F8	0.980	0.983	0.970	0.801	0.931

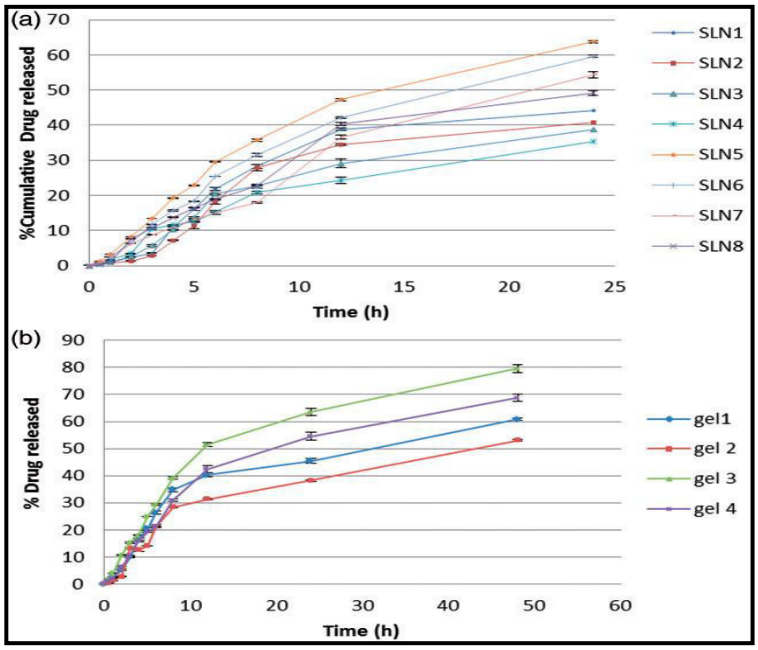


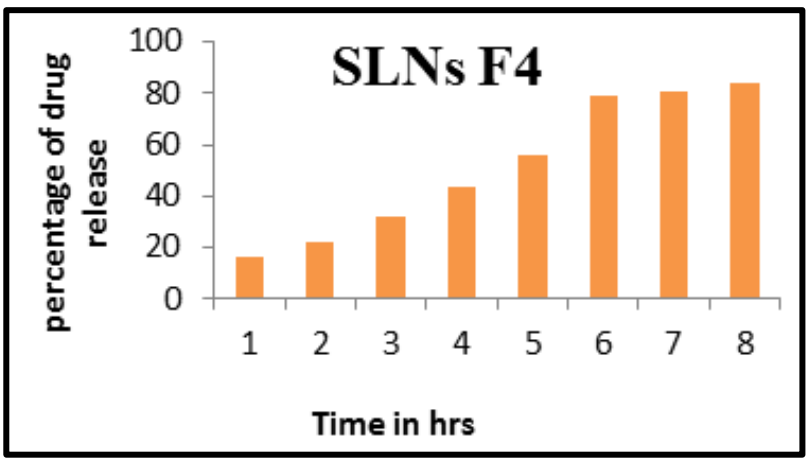
Fig 7. In vitro release profiles of Tacrolimus from (a) prepared SLNs and (b) relevant SLNs gels.

4.4. *Ex-vivo permeation studies*

From the results of drug permeation from optimized formulation (F4),exvivo permeation study (Table 8) through the rat abdominal skin revealed that tacrolimus was released from the optimized formulation and s through the rat abdominal membrane and believed could possibly permeate through the human abdominal membrane. The drug permeation from F4 was slow and steady and 0.89gm of Tacrolimus could permeate through the skin membrane with a flux of 0.071 gm hr-1 cm-2. The results are shown in (Table 8and figure 10).

Table 8: *Ex-vivo* drug permeation of optimized Formulation F4

Time (h)	Cumulative drug permeated (gm)
0	0
1	0.18
2	0.30
3	0.46
4	0.61
5	0.79
6	0.87



Each value represents the mean ± SD (n= 3).

Fig 8: *In vitro* release profile of Tacrolimus from SLNs.

4.5. *Skin irritation Test*

Skin irritation test was performed based on *in-vitro* diffusion study optimized formulation F4 containing drug and Compritol888 ATO in the ratio 1:2 and with optimized formulation F4 in white rabbits. The animals were divided into 3 groups. It was found that the gel F4 causes no irritation or Erythema.

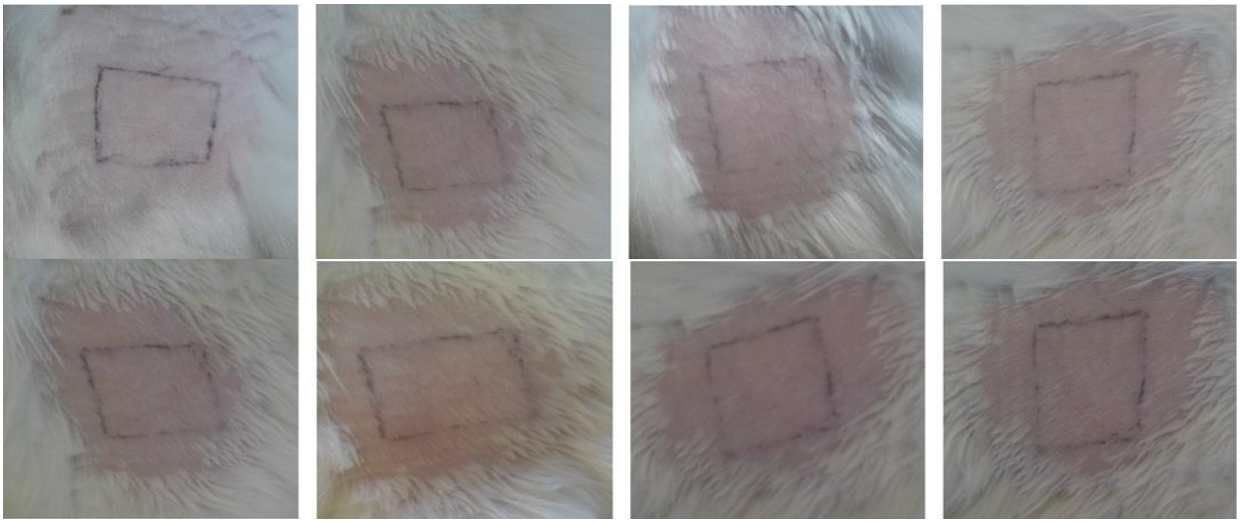


Fig.9. Primary skin irritation photographs of rabbit skin; 1 denotes skin treated with T-FIN SLNs (F4) and 2: reference; intact (A) and abraded (B) after 24 h; intact (C) and abrade (D) after 72 h.

5. CONCLUSIONS

Compritol888 ATO gel containing Tacrolimus (SLNs) in 1:2 ratios (F4) produced better Spreadability and consistency as compared to other formulations. The developed F4 gel showed good homogeneity, suitable at pH, absence of skin irritation and good stability and consistency. The maximum percentage of drug release was found to be 98.68% in 6 hours in formulation F4. The drug permeation from optimized (F4) formulation was slow and steady and 0.89 gm of Tacrolimus could permeate through rat abdominal skin membrane with a flux 0.071 $gmhr^{-1}$ cm^{-2} and could possibly permeate through human abdominal membrane. SLNs based gel had better diffusion and retention rates than the Ointment based gel and also overcomes the problems associated with Ointment based gel. The present research study revealed that the SLNs based Gel containing formulation can be beneficial in the treatment of psoriasis.

6. ACKNOWLEDGEMENT

The authors would like to acknowledge Yarrow chem Products, Mumbai, for providing drug Sample of tacrolimus, and Acharya Nagarjuna University, Guntur, for carrying out spectral analysis studies. We are also thankful to the Principal, the authors would also like to acknowledge *Department of Pharmaceutics,* KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, *AP, India*, for providing a facility for experimentations.

6. AUTHORS CONTRIBUTION STATEMENT

Experimental design, guidance for the Research work and writing of this manuscript was done by Ch. Surya Kumari. Second author Narender Malothu supported to draft manuscript design and correction of data, performed the experiments, analysed spectra and interpreted the result. All authors played an equal role in completing this research work.

7. CONFLICT OF INTERESTS

Conflict of interest declared none

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