INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

FORMULATION AND EVALUATION OF TACROLIMUS LOADED LIQUID CRYSTALLINE NANOPARTICLES

Hiren Kodinariya*, Chintan Aundhia, Avinash Seth, Nirmal Shah, Vinod Ramani, Snehal Patel, Dipti Gohil

Deparatment of Pharmacy, Sumandeep Vidyapeeth, Vadodara.

ARTICLE INFO

Article history
Received 31/12/2017
Available online
31/01/2018

Keywords
Tacrolimus,
Liquid Crystalline Nanoparticles,
Pseudo Binary Mixture Technique,
Particle Size,
Entrapment Efficiency.

ABSTRACT

The use of liquid crystalline nanoparticles is a novel approach in the field of controlled drug delivery. Tacrolimus, being a highly lipophilic drug, is easily incorporated in the hydrophobic core of these nanoparticles. Which are prepare by pseudo binary mixture technique by using polymers like monoolein and poloxomer 407 to extend the drug release for about 32 hours, there by improved bioavailability. Formulation optimization of tacrolimus loaded liquid crystalline nanoparticles was carried out by using different concentration of monoolein and poloxomer 407. Total 10 batches were formulated. The process optimization was carried out at three different stirring speeds i.e. 2200, 2500 and 2800 rpm for three different stirring time period i.e. 05mins, 10mins and 15mins. Out of all the batches S5 showed the spherical shape of liquid crystalline nanoparticles. All 10 batches were evaluated for entrapment efficiency (EE) and particle size (nm). Among all batches S5 shows maximum entrapment efficiency (EE) and uniform particle size and was considered as optimized formulation. Optimized batch S5 was evaluated for Zeta Potential, Particle Size Distribution which show -35.4mV and 126.1nm particle size, TEM Analysis. Batch S5 was charged for stability and were placed in glass vials container and stored at ICH storage condition (30 ± 2°C / 60% ± 5% RH , 40 ± 2°C / 75% ± 5% RH) for a period of 30 days. The samples were analyzed for physical appearance, entrapment efficiency and particle size after 30 days. After 1 months samples were withdrawn and liquid crystalline nanoparticles showed no change in physical appearances, entrapment efficiency and particle size, which indicate that the liquid crystalline nanoparticles were stable. Therefore, study suggests the possible use of tacrolimus-loaded formulation for intradermal or topically delivery can be useful in the treatment of locally affecting autoimmune skin disease such as psoriasis.

Corresponding author
Hiren Kodinariya
Deparatment of Pharmacy, Sumandeep Vidyapeeth,
Vadodara
24/B-1,"Yamuna", Shantinagar-1,
Near Janta Fatak, Jamnagar-361004
+919429801718
hirenkodinariya5678@gmail.com

*Please cite this article in press as **Hiren Kodinariya** et al. Formulation and Evaluation of Tacrolimus Loaded Liquid Crystalline Nanoparticles. Indo American Journal of Pharmaceutical Research.2018:8(01).*

INTRODUCTION

Psoriasis is the most common T cell-mediated inflammatory disease in humans. It is a long-lasting autoimmune disease which is characterized by patches of abnormal skin. These skin patches are typically red, itchy, and scaly. Psoriasis is estimated to affect 2–4% of the population of the western world. Approximately one third of people with psoriasis report being diagnosed before age 20. Psoriasis affects both sexes equally.[1] Tacrolimus is exciting agents in the treatment of skin disease. Their role in the treatment of psoriasis is still being defined but their use will likely increase. Tacrolimus inhibits T-lymphocyte activation by first binding to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin is inhibited. This prevents the DE phosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines. Tacrolimus also inhibits the transcription for genes which encode IL-3, IL-4, IL-5, GM-CSF, and TNF-, all of which are involved in the early stages of T-cell activation.[2, 3] Lipid nanoparticles have taken the lead because of obvious advantages of higher degree of biocompatibility and versatility. These systems are commercially viable to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery. The most frequent role of lipid-based formulations has traditionally been to improve the solubility of sparingly water soluble drugs especially Bio pharmaceutics Classification System (BCS) Classes II & IV drugs. Lipid nanoparticles (e.g. Liquid crystalline nanoparticles, LCNs) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery.[4] LCNs adhere to the stratum corneum forming a film as this film has been shown to possess occlusive properties. It was shown that the degree of crystallinity has a great impact on the extent of occlusion by the formulation. The occlusive effect leads to reduced water loss and increased skin hydration. Highly crystalline LCNs can be used for physical sun protection.[5] Tacolimus has low aqueous solubility and higher degradation, causing a problem in formulating it as a liquid preparation. Another problem associated with the use of tacrolimus is that it has narrow therapeutic index, and, therefore, it is essential to prevent the possible toxic effects of the drug when extended release dosage form is administered. Tacrolimus is a hydrophobic drug which is effective drug for the treatment of atopic dermatitis and has been successfully used for the treatment of psoriasis. Tacrolimus is a biopharmaceutical classification system class II drug (low solubility and high permeability). They are capable of incorporating tacrolimus into the hydrophobic core of the nanoparticles. Being bio adhesive and biocompatible, the use of such nanoparticles can enhance transdermal delivery and alleviate the disease effectively within shorter period of time. Also, these nanoparticles are capable of providing a controlled drug release. Monoolein is used as a penetration enhancer. The skin penetration enhancement of Monoolein results from two mechanisms involving stratum corneum lipid fluidization and phase separation. Monoolein- based nanoparticles with entrapment efficiency of tacrolimus above 99 %. Therefore, it can be considered as a good candidate for the development of a site-specific release formulation. Permeation and retention time can describe liquid crystalline nanoparticles are bio adhesive and are capable of disturbing the lipid phase in the skin thus enhancing the intake of nanoparticles. It is thus, importance to have a drug formulation with extended release dosage form with less toxic effect, which can be achieved by formulating the tacrolimus loaded liquid crystalline nanoparticles.

MATERIALS AND METHOD

Tacrolimus was obtained from BDR Pvt. Ltd., Vadodara. Monoolein was obtained from Aatur Instra Chem, Vadodara. Poloxomer 407 was procured from Sulab Laboratory, Vadodara.

Method of Preparation of Liquid crystalline nanoparticles[6, 7]

Pseudo Binary Technique is a new approach to prepare LCNs.

Accurately weighed quantities of drug (Tacrolimus) (15 mg), Monoolein (0.1 or 0.5 or 0.9 mg), Poloxomer 407 (46 or 129 or 212 mg) and Distilled water (49 ml). Preparation of drug polymer melted mixture**:** Monoolein (0.1 or 0.5 or 0.9 mg) and poloxamer 407 (46 or 129 or 212 mg) was taken and melted in water bath at 60 ºC followed by tacrolimus addition with continuous stirring to dissolve completely. In this technique, the drug and polymer was melted in water bath at 60 ºC. Then, the dispersion solution was added drop-by-drop by syringe into distilled water. Resultant emulsion was stirred at 2500 rpm using a propeller-type agitator for 10 minutes. The LCNs are separated by centrifugation process at 14000 rpm for 90 min. That leads to formation of an extremely porous nanoparticles called 'LCNs'. The appearance of the formulation was milky white liquid dispersion.

CHARACTERIZATION OF LCNs

Fourier Transform Infrared Spectroscopy (FTIR)

The LCNs were subjected to Fourier Transform Inferred Spectroscopy (FTIR) studies using (Shimadzu 8400 s). The potassium bromide (KBr) disk method was used for preparation of sample. The spectrum was compared with the infrared spectra of plain drug and polymer and checked for the drug-polymer interaction.

Drug Entrapment Efficiency

Separation of free drug: Analysis of Tacrolimus from LCNs was done by separating free drug from the LCNs dispersion. The separation was done by filtration (Whatmann filter paper) of LCNs. Then, the LCNs and filtrate were separated out.

Indirect method:

In this method, analysis of drug from LCNs was done by appropriately diluting filtrate in methanol and absorbance was taken at 295 nm against methanol as a blank on UV-Visible Spectrophotometer. To find out % entrapment following equation was used.

$$\% \text{ EE} = \frac{\text{Weight of total drug} - \text{Weight of free drug in filtrate}}{\text{Weight of total drug}}$$

Measurement of Particle size and Zeta potential

Zeta potential of LCNs reflects the electric potential of particles and is used to characterize the surface charge properties and to determine whether the charged particle is encapsulated within the Centre or adsorbed on to the surface of LCNs. The Particle size and Zeta potential of LCNs was recorded using Zetasizer. The optimized formulation was subjected to particle size and zeta potential analysis.

Transmission electron microscopy (TEM)

TEM is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a charge-coupled device. The morphology of LCNs was determined using TEM.

In Vitro Diffusion Studies

In vitro release of drug studies by using dialysis tubes with molecular weight of 12000—14000 Da. dialysis membrane. The developed Tacrolimus LCNs equivalent to 15 mg of drug were dispersed in 5 ml of 0.005% hydroxypropyl cellulose (pH 4.5) and lead to dialysis by dipping the tube of dialysis to the acceptor medium contain 30 ml of 0.005% hydroxypropyl cellulose (pH 4.5). Using of magnetic stirrer acceptor medium was agitated continuously at 37 ± 0.5 °C. Aliquot quantity of sample from acceptor medium was withdrawn at different break of time up to 32 hrs. and each time it was make up with aliquot quantity of new 0.005% hydroxypropyl cellulose (pH 4.5). The released drug quantity was determined by spectrophotometric ally at 295 nm.

Release Kinetics[8]

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equation a namely zero order (%release vs. t), first order (log% unreleased vs. t), and higuchi matrix (%release vs. square root of time). In order to define model which will represent a better fit for the formulation, drug release data further analyzed by korsmeyer peppas equation, Mt/M is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

Stability Study

The stability study was carried out for optimized formulation of Tacrolimus loaded LCNs as per ICH guidelines. The LCNs of the best formulation were placed in glass vials and stored at ICH storage condition (30± 2°C / 60% ± 5% RH and 40 ± 2°C / 75% ± 5% RH) for a period of 30 days. The samples were analyzed for physical appearance, entrapment efficiency and particle size after 30 days.

RESULTS AND DISCUSSION

LCNs of Tacrolimus in different ratio were designed and prepared by Pseudo binary mixture technique. Drug-polymer compatibility studies were carried out using FTIR spectroscopy to establish any possible interaction of Tacrolimus with the polymer used in the formulation. Thus results indicate that the characteristic absorption peak due to pure Tacrolimus have appeared in the formulated LCNs without any significant change in their position indicating no chemical interaction between Tacrolimus and polymers (Fig. 1-4).

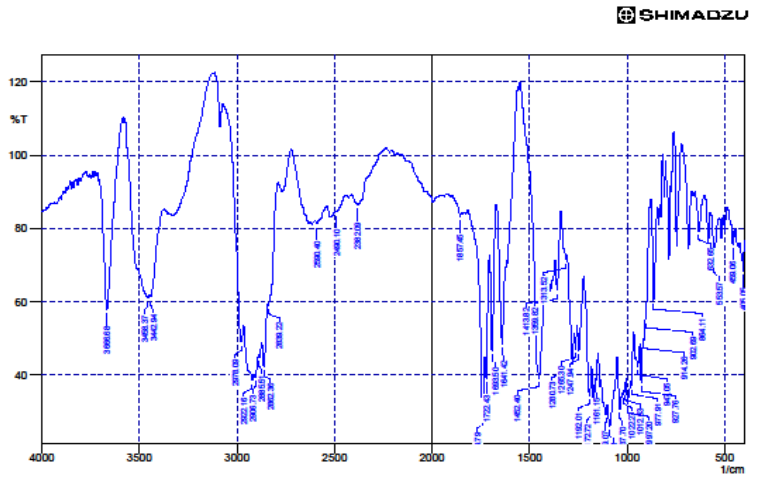


Figure 1: FTIR Spectra of Tacrolimus.

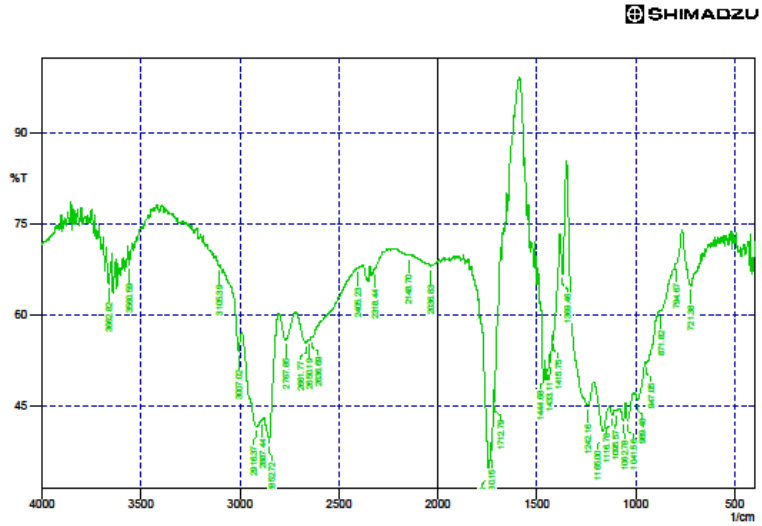


Figure 2: FTIR Spectra of Monoolein

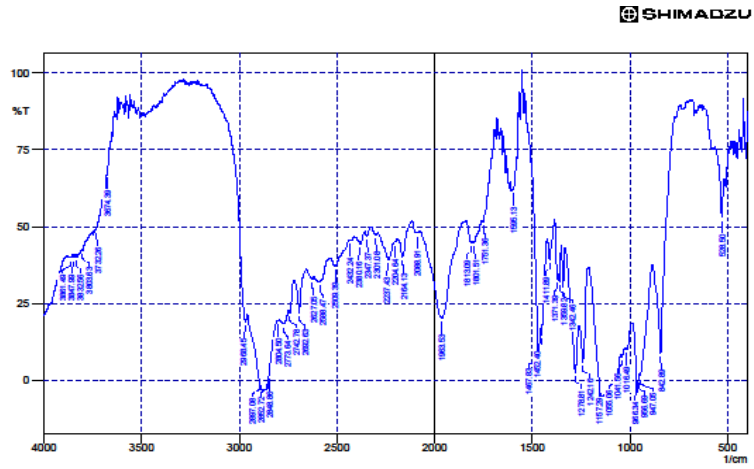


Figure 3: FTIR Spectra of Poloxomer 407.

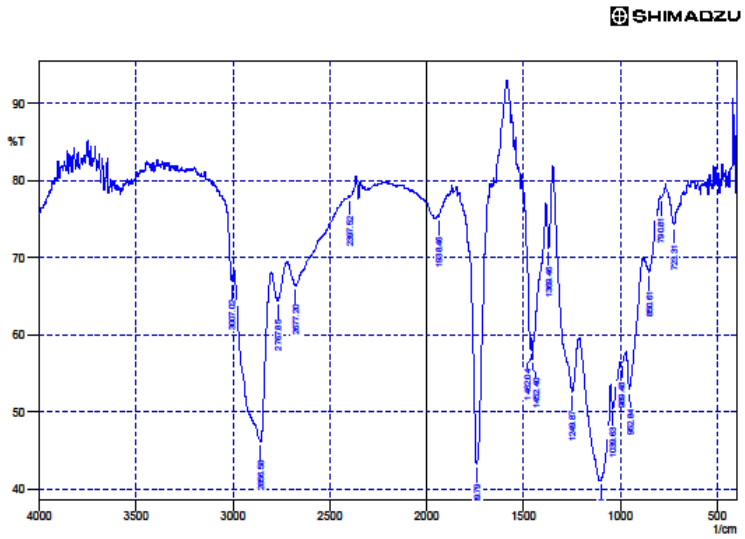


Figure 4: FTIR Spectra of LCNs Formulation.

Process Optimization

Screening of stirring speed

Table 1: Screening of stirring speed.

Sr no.	Stirring speed (RPM)	Result
1	2200	Lump formation
2	2500	Spherical particles
3	2800	Ruptured particles

During screening of process parameter i.e. stirring speed, it was observed that lump formation was occurred at 2200 RPM. This may be due to insufficient rotation speed at which the LCNs were unable to get separated out. While at 2800 RPM, due to high stirring speed, the morphology of LCNs got disturbed and resulted into ruptured particles. As a result, 2500 RPM was selected for preparation of LCNs as it showed discrete spherical particles.

Screening of stirring time

Table 2: Screening of stirring time.

Sr no.	Stirring time (mins.)	Result
1	05	Irregular particles
2	10	Spherical particles
3	15	Ruptured particles

With stirring time, it was observed that at 05 minutes, the solvent was unable to get proper interaction and thus resulted into irregular particle formation. While at 15 minutes due to over stirring time, the morphology of LCNs got disturbed and resulted into ruptured particles. As a result 10 minutes was selected for further study, as it was found to be optimum for formation of complete spherical particles.

Formulation Optimization

Formulation optimization has been done by 3^2 factorial designs

Table 3: Factors (independent variables), factor levels used in 3^2factorial experimental design.

Factors	Factor level used		
	-1	0	1
X_1=Concentration of Monooelin(ml)	0.1	0.5	0.9
X_2=Concentration of Poloxomer 407 (mg)	46	129	212

Table 4: Formulation code for preparation of various LCNs compositions by 3^2 design.

Batch Code	Variable level in coded form		Variable level in Decoded form	
	X_1	X_2	X_1	X_2
S 1	-1	-1	0.1	46
S 2	0	-1	0.5	46
S 3	1	-1	0.9	46
S 4	-1	0	0.1	129
S 5	0	0	0.5	129
S 6	1	0	0.9	129
S 7	-1	1	0.1	212
S 8	0	1	0.5	212
S 9	1	1	0.9	212
S10	0	0	0.5	129

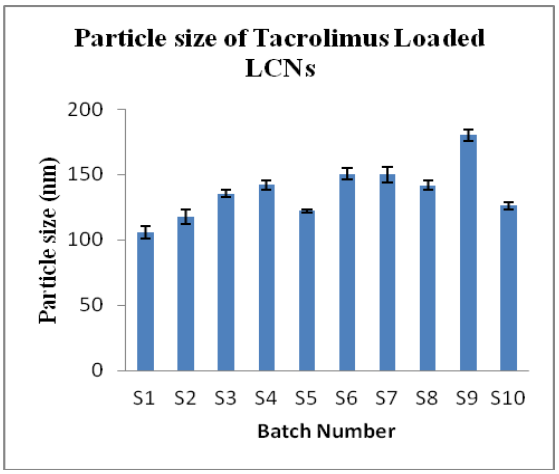
%Entrapment efficiency (% EE) and Particle size (nm)

Percentage entrapment efficiency (% EE) and particle size are shown in the Table 5 and represented graphically in Figure 5, Figure 6.

Table 5:% Entrapment efficiency and Particle size of Tacrolimus loaded LCNs.

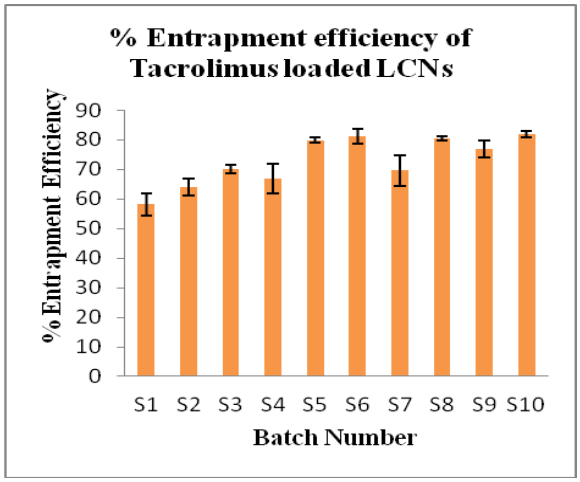
Formulation	Particle size (nm) ± SD	%Entrapment efficiency± SD
S1	105.9 ±4.54	58.13 ±3.67
S2	117.8 ±5.82	63.87 ±2.86
S3	135.4 ±2.64	70.13 ±1.48
S4	142.1 ±3.41	66.80 ±4.96
S5	122.1 ±1.37	79.86 ±0.87
S6	150.5 ±4.24	81.20 ±2.58
S7	150.0 ±5.79	69.67 ±5.22
S8	141.8 ±3.62	80.53 ±0.76
S9	180.2 ±4.36	77.00 ±2.85
S10	126.3 ±2.81	81.87 ±1.07

*n=3.



*n=3

Figure 5: Particle size of Tacrolimus loaded LCNsFigure.



*n=3

6: % Entrapment efficiency of Tacrolimus loaded LCNs.

Contour plots and Response surface plots

Contour plots were established between X_1 and X_2 at fixed level of -1, 0 and 1. And they are diagrammatically represented in Figure 7 and 9. By establishment of two dimensional contour plots, the relationship between independent and dependent variables can be explained. Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables. This is shown in Figure 8 and 10.

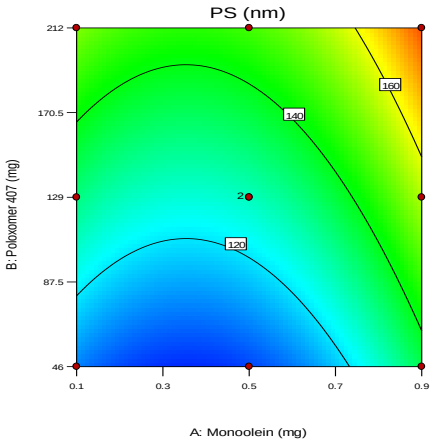


Figure 7: Contour plot showing the effect of Amount of monoolein (X_1) and poloxomer 407 (X_2) on response Y_1(Particle size).

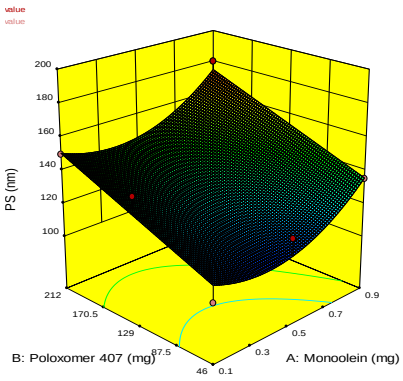


Figure 8: Response Surface plot showing the effect of Amount of monoolein (X_1) and poloxomer 407 (X_2) on response Y_1 (Particle size).

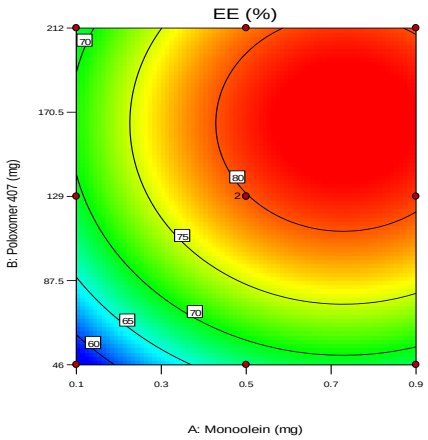


Figure 9: Contour plot showing the effect of Amount of monoolein (X_1) and poloxomer 407 (X_2) on response Y_2 (%Entrapment efficiency).

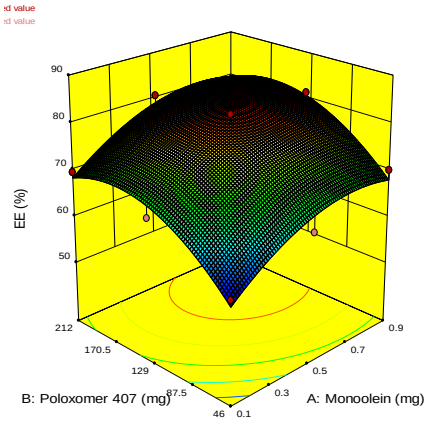


Figure 10: Response Surface plot showing the effect of Amount of monoolein (X_1) and poloxomer 407 (X_2) on response Y_2 (%Entrapment efficiency).

Overlay Plot

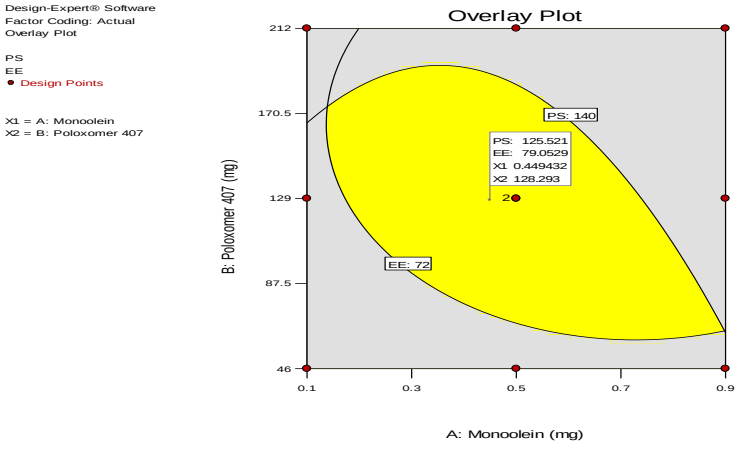


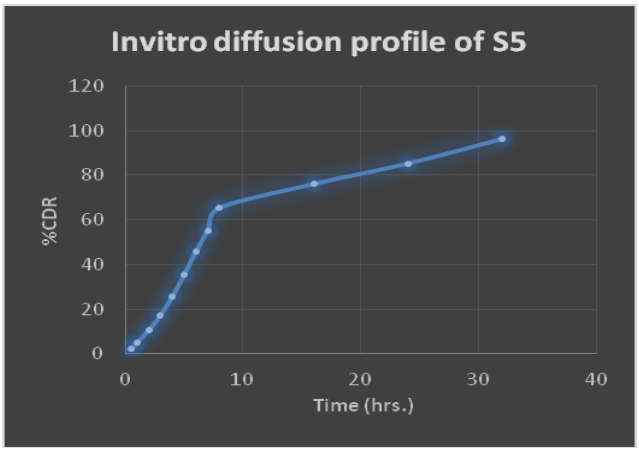
Figure 11: Overlay plot.

In Vitro Release Study

The in vitro release study carried out for a period of 0.5 to 32 hrs.

Table 6: In vitro diffusion profile of S5.

Time (hrs.)	%CDR± SD	Time (hrs.)	%CDR± SD
0.5	2.03 ±0.25	6	45.82 ±2.99
1	4.95 ±1.67	7	55.31 ±3.42
2	10.56 ±1.08	8	65.39 ±3.58
3	17.36 ±1.54	16	76.08 ±4.31
4	25.65 ±1.98	24	85.27 ±5.79
5	35.44 ±2.63	32	96.25 ±5.03



***n=3**

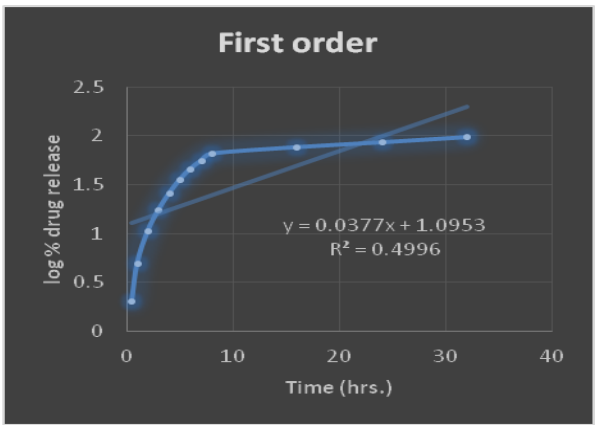
Figure 12: In vitro diffusion profile of S5.

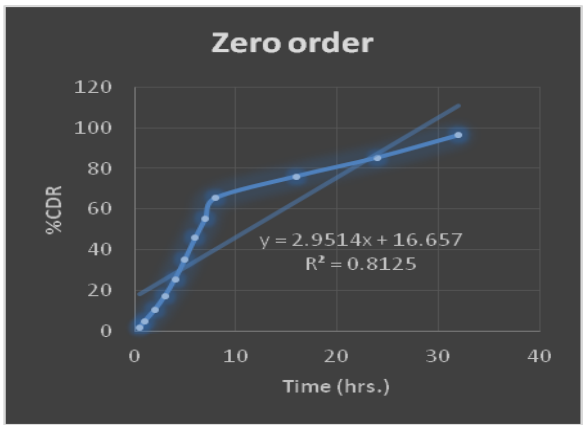
Release Kinetics of Final Optimized Formulation

Various values of the square of regression coefficient for various kinetic models are given in Table 7 and represented graphically in Figure 13, Figure 14, Figure 15 and Figure 16.

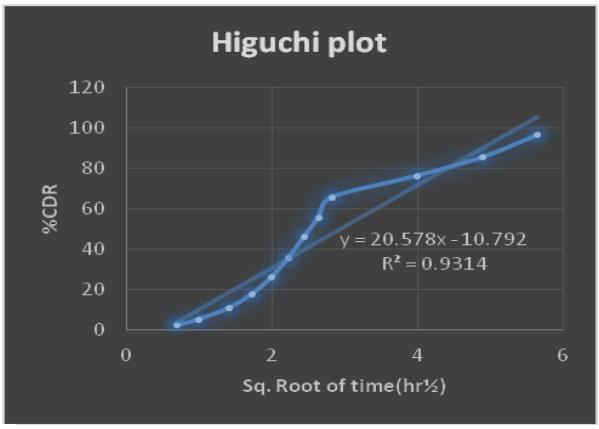
Table 7: Release Kinetics of optimized formulation of Tacrolimus loaded LCNs.

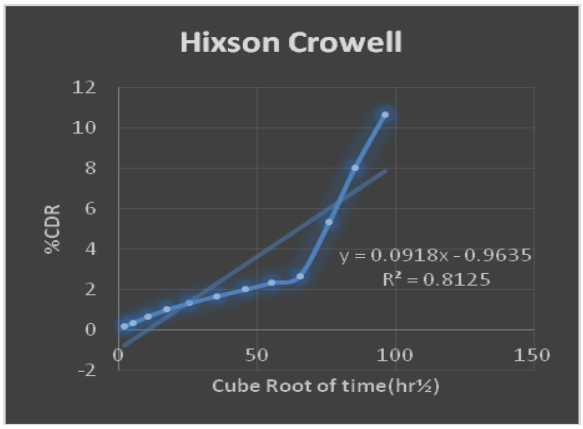
Optimized formulation	Higuchi (R^2)	Zero order (R^2)	First order (R^2)	Hixson Crowell (R^2)
HK01	0.9314	0.8125	0.4996	0.8125





***n=3** **Figure 13: Zero Order**





Transmssion Electron Microscopy.

***n=3 Figure 15: Higuchi plot.** ***n=3 Figure 16: Hixson Crowell plot.**

TEM analysis

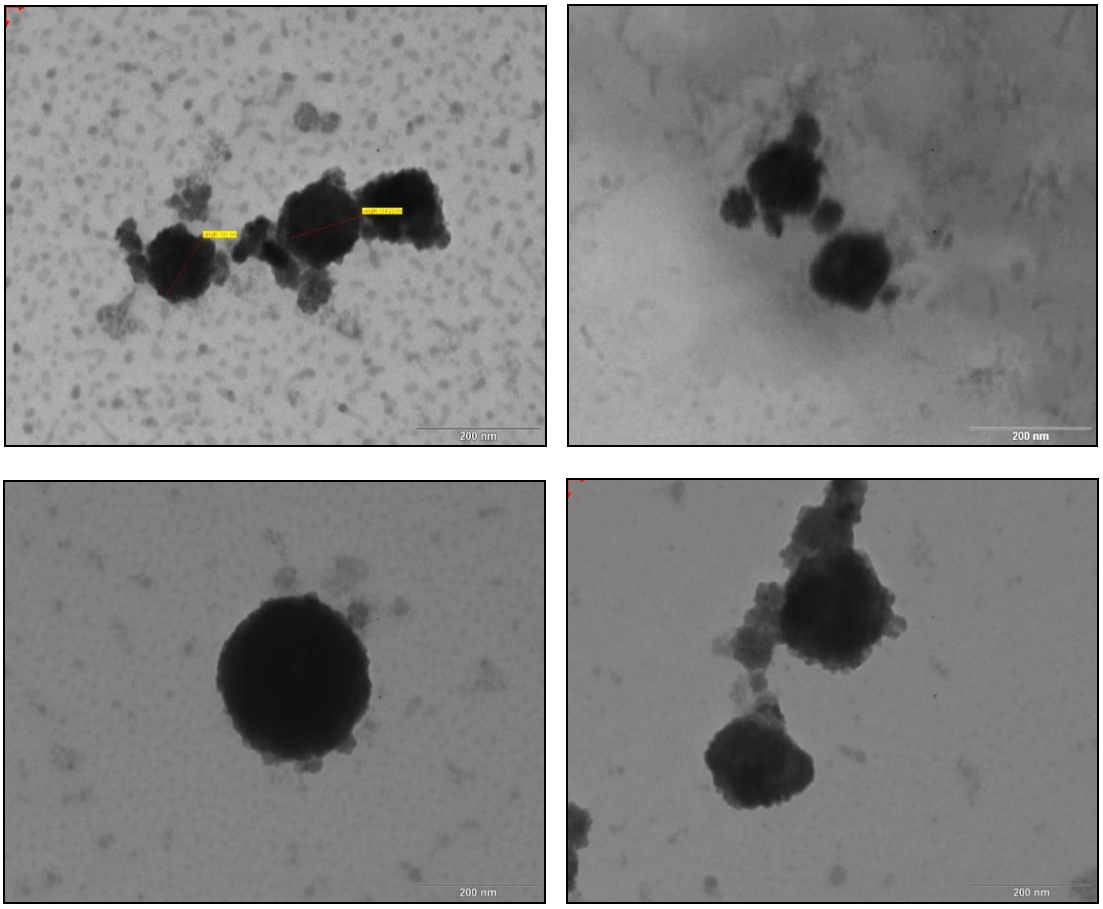


Figure 17: TEM analysis of Tacrolimus loaded LCNs.

Zeta Potential and Particle Size Distribution

Optimized Formulation S5 was evaluated for Zeta Potential and Particle Size Distribution which was obtained as -35.4mV and 126.1 nm respectively.

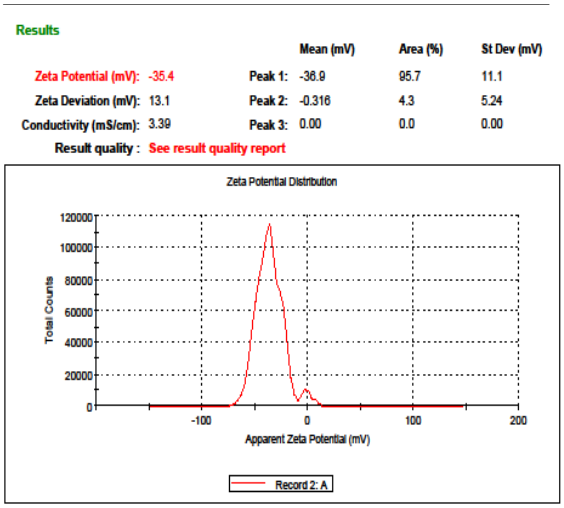


Figure 18: Zeta Potential.

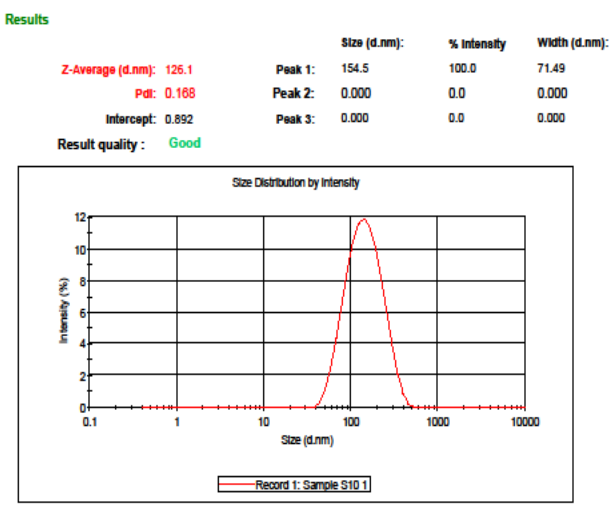


Figure 19: Particle Size.

Stability study

On the basis of this study it was considered that there was no significant change in the formulation and so we can conclude that formulation was stable after 1-month study at accelerated stability study.

Table 8: Stability Study.

Sr. No.	Parameter	Before storage	After 1 month storage (40 ± 2°C / 75% ± 5% RH)	After 1 month storage (30± 2°C / 60% ± 5% RH)
1	Particle size (nm)	126.80 ±1.04	127.42 ±4.88	128.95 ±5.27
2	% Entrapment efficiency	79.21 ±2.54	78.94 ±3.51	78.14 ±4.43

Composition of final optimized Formulation

Table 9: Final optimized Formulation.

Final optimized formulation	
Ingredient	**Quantity**
Tacrolimus	15 mg
Monoolein	0.5 ml
Poloxomer 407	129 mg
Distilled water	49 ml

Evaluation of Final Optimized Formulation S5

Table 10: Evaluation of Final Optimized Formulation S5.

Parameters	Results
Particle size	126.1nm
PdI	0.168
Zeta Potential	-35.4 mV
% Entrapment	79.72
% drug release at 32 hours	96.25

CONCLUSION

The present research work concludes that the permeability and bioavailability of BCS class III drugs can be improved if delivered in the form of LCNs. This controlled release of tacrolimus may be attributable to high entrapment efficiency of the drug and high hydrophobicity of the drug which causes strong hydrophobic binding between the drug and inner structure of the nanoparticles. Poloxamer 407 provides better access for lipase to attack internal structure of the crystalline nanoparticles. This suggests that resistance of the crystalline structure of the nanoparticles against lipase attack could be controlled by using appropriate manipulation of the ratio between surfactant versus monoolein. In this work tacrolimus was selected to treat psoriasis effectively by formulating LCNs. An effort was made to formulate LCNs of tacrolimus for improving the permeability and controlled release. In this work pseudo binary system technique was used to develop tacrolimus loaded LCNs. The research work further concludes that the concentration of polymer monoolein 0.5ml and surfactant poloxomer 407 129mg play a key role in the optimization of the formula. The particle size and % drug entrapment efficiency vary with the different concentration of the polymer and the surfactant. The full 3^2 factorial design of the formula optimization represent that the LCNs of the tacrolimus showed particle size 126.1nm and high % entrapment efficiency 79.72. The properties of the prepared liquid crystalline nanoparticles containing tacrolimus suggest the possible development of the formulations for the intradermal delivery in autoimmune diseases like psoriasis so that the dosing frequency and adverse effects can be subsequently minimized. This finding suggests that the ability of resistance of the crystalline structure of the nanoparticles against lipase attack could be controlled by surfactant ratio and addition of hydrophobic fatty acids into the nanoparticles. Therefore, the properties of the prepared liquid crystalline nanoparticles containing tacrolimus suggest the possible development of the formulations for the intradermal delivery in autoimmune diseases like psoriasis so that the dosing frequency and adverse effects can be subsequently minimized.

LIST OF ABBREVIATIONS

1	API	Active Pharmaceutical Ingredients
2	Log	Logarithm
3	EE	Entrapment Efficiency
4	µg	Microgram
5	mg	Milligram
6	gm.	Gram
7	nm	Nanometer
8	pH	Hydrogen Ion Concentration
9	rpm	Revolution Per Minute
10	±	Plus or Minus
11	ºC	Degree Centigrade
12	Conc.	Concentration
13	Abs	Absorbance
14	ICH	International Council for Harmonisation
15	FKBP	Binding Protein
16	IL	Interleukin
17	TNF	Tumor Necrosis Factor
18	e.g.	For Example
19	i.e.	That is
20	TEM	Transmission Electron Microscopy
21	LCNs	Liquid Crystalline Nanoparticles
22	BCS	Bio pharmaceutics Classification System
23	PS	Particle Size
24	%CDR	Percentage Cumulative Drug Release
25	SD	Standard Deviation
26	RH	Relative Humidity
27	CDR	Cumulative Drug Release
28	λ_{max}	Absorption maxima
29	%w/v	Percentage Weight/Volume
30	$t_{½}$	Half life
31	FTIR	Fourier transmission infra-red spectroscopy
32	no	Number
33	ANOVA	Analysis of Variance
34	*n	Number of Trials

REFERENCES

1. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, et al. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature. 2005;437(7057):369-75.
2. Thomson A, Bonham C, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Therapeutic drug monitoring. 1995;17(6):584-91.
3. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug metabolism and pharmacokinetics. 2007;22(5):328-35.
4. Stamatoiu O, Mirzaei J, Feng X, Hegmann T. Nanoparticles in liquid crystals and liquid crystalline nanoparticles. Liquid Crystals: Springer; 2011. p. 331-93.
5. Attama AA, Momoh MA, Builders PF. Lipid nanoparticulate drug delivery systems: a revolution in dosage form design and development. Recent advances in novel drug carrier systems: Intech; 2012.
6. Thapa RK, Baskaran R, Madheswaran T, Kim JO, Yong CS, Yoo BK. Preparation, characterization, and release study of tacrolimus-loaded liquid crystalline nanoparticles. Journal of Dispersion Science and Technology. 2013;34(1):72-7.
7. Thapa R, Baskaran R, Madheswaran T, Kim J, Yong C, Yoo B. In vitro release and skin permeation of tacrolimus from monoolein-based liquid crystalline nanoparticles. Journal of Drug Delivery Science and Technology. 2012;22(6):479-84.
8. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.