



Solid Lipid Nanoparticles of Cyclosporine for the Treatment of Skin Disease

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Abstract

Cyclosporine is one of the shows potential drugs and employ for the treatment of variety of skin diseases like Psoriasis. However, High blood pressure, Swollen or inflamed gums, and other common side effects are tremors, restlessness, stomach upset, nausea, cramps, diarrhea, headache limit its clinical applications.

The reported work pivot on the preparation of solid lipid nanoparticles (SLN) using cyclosporine drug, for the enhancement of their penetration efficacy in the skin. Solid lipid nanoparticles (SLNs) of the drug by the using of many type of lipids, like palmitic acid, glyceryl behenat, cetyl palmitate and glyceryl monostearate. Prepared SLN was validated regarding particle size, zeta potential, percentage entrapment efficiency (EE). *In-vitro* dermatokinetics and drug efficacy evaluated respectively by tape stripping method and using HaCaT cell lines. The particle size of nanoformulation was ranging less than 350 nm and the morphology is showing spherical. HaCaT cell lines were used for the *in-vitro* study that showed higher uptake and efficacy with decrease cell viability for SLNs.

Keywords: Cyclosporine; Fatty Acids; SLN; HaCaT Cell Line

Introduction

Psoriasis is one of the more widespread forms of unending dermal disease in the world. In fact, at hand is no good remedy for psoriasis but there are many special treatment way to reduce the harshness of symptoms. This disease is an auto-immune of skin with frequent portion of hyperkeratosis and inflammation connecting abnormal commencement of T cells, their relocation into the skin and final aggregation [1].

The main remedy therapy in the treatment psoriasis presuppose dermal or topical drug delivery remaining to its benefit of least systemic absorption and consequently with minor toxicity. None of topical drug category is ideal for the management of psoriasis; apart from the main difficulty in combinational drug therapy is the trouble in administration; so, requirements arise to develop

a nanoformulation for the improved pharmacokinetic effect of the drug [2,3].

Cyclosporine (CsA) present anti psoriatic activity outstanding, due to shyness of T cells and its immune responses which are hyper active in psoriasis. It also inhibits IL-2 production which in turn reduces the production of secondary cytokines (TNF α , IL-17, IL-23) which are likely to be the mechanisms of keratinocyte production [4].

Cyclosporine A (BCS Class II, Biopharmaceutical Classification System) is a fat soluble, hydrophobic polypeptide metabolite of fungus *Beauveria nivea* (formerly *Tolypocladium inflatum* Gams) [5]. It is a hydrophobic cyclic peptide built from non-mammalian amino acids with low oral bioavailability, which is one of first line

immunosuppressive drugs used to prevent transplant rejection and to treat autoimmune diseases [6,7].

Materials and Methods

Drug profile

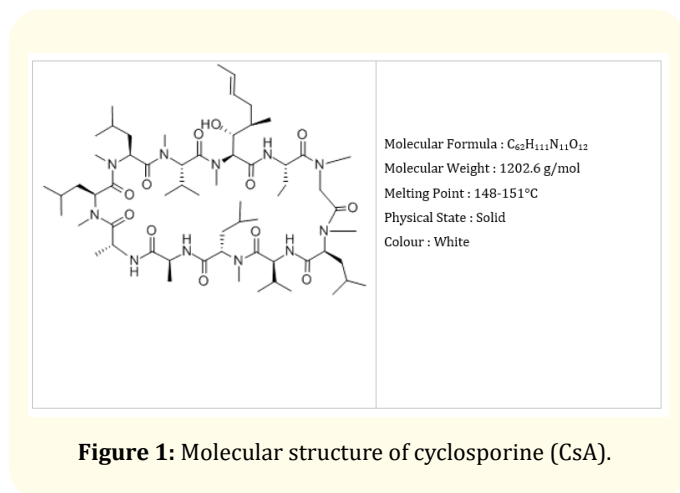


Figure 1: Molecular structure of cyclosporine (CsA).

Solubility study of cyclosporine in different oils, surfactants and co-surfactants:

The solubility study of Cyclosporine was studied in different oils, surfactants and co-surfactants, including: Carpryol 90, Lauroglyol 90, Peceol, Labrafac Lipophile, Span 80, PEG-400, Labrafil, PEG35 Castor oil, PEG-40 Hydrogenate Castor Oil, Propylene Glycol, Labrasol [8,9]. Cyclosporine A was mixed in 10 ml vial with such amounts of each of the above solvents in order to produce supersaturated solutions. The mixtures were shaken at constant vibration under ambient temperature for 2 days for equilibrium. The obtained suspensions were centrifuged at 5000 rpm for 20 minutes. Then an accurately weighed quantity of supernatant was further diluted with methanol and analyzed using an HPLC method for its drug content.

Preparation of cyclosporine solid lipid nanoparticles (SLNs)

The Cyclosporine was dissolved in the hot molten lipid matrix (palmitic acid, glyceryl behenat, cetyl palmitate and glyceryl monostearate) which is then dispersed in the hot aqueous surfactant solution using high speed stirrer (e.g. ultra turrax) to form a coarse pre emulsion which is then homogenized using high pressure homogenizer at appropriate pressures with sufficient number of cycles (homogenization method). After this the o/w nanoemulsion is cooled to room temperature followed by lipid crystallization which leads to the formation of solid lipid nanoparticles [10,11].

Oils, surfactants and co-surfactants	Solubility(mg/g)
Carpryol 90	496.0 ± 1.3
Lauroglyol 90	469.2 ± 2.2
Peceol	228.2 ± 2.8
Labrafac Lipophile	302.4 ± 2.4
Span 80	152.4 ± 2.2
PEG-400	287.6 ± 3.2
PEG35 Castor oil	161.8 ± 1.2
Labrasol	152.2 ± 2.1
PEG-40	172.2 ± 3.8
Hydrogenate Castor Oil	205.2 ± 1.3
Propylene Glycol	185.4 ± 1.2

Table 1: Solubility of cyclosporine A in different oils, surfactants and co-surfactants.

Characterization of SLNs

The surface morphological examinations of SLNs were carried out with scanning electron microscopy (Model JSM 5610 LV SEM, Japan).

Laser diffraction particle size analyzer (Zetasizer Nano S90, Malvern, UK) were used for determination of the particle size as well as particle size distribution of the SLNs.

Determination of entrapment efficiency (EE)

Cyclosporine in the SLNs was analyzed by dissolving the SLNs in 0.5% Polysorbate-20 in PBS (pH 5.8). The concentration of SLNs in 0.5% Polysorbate-20 in PBS (pH 5.8) was 05 mg/ml. The mixture was stirred continuously overnight at room temperature. Solutions were analyzed by above-mentioned high-performance liquid chromatograph (HPLC) at a wavelength of 212 nm for cyclosporine A content (Shimadzu LC10AD) with suitable dilutions. Percent EE was calculated using following equation [12,13].

$$\%EE = \left(\frac{\text{Amount of drug entrapped}}{\text{Total amount of drug taken}} \right) \times 100$$

Stability Study of SLNs

The changes in the particle size and EE of SLNs were widely used as indicators of storage stability. Optimized batch of SLNs were stored at 4°C and 25°C for 3 months. The physical stability of the samples were evaluated on 0 days, 3rd month and 6th month for any change in particle size, PDI and % EE [14,15].

Drug release kinetics

Dialysis methods are an appealing alternative to investigate release of drug from *in-situ* depot forming system and other formulations including implants. The *in vitro* release studies of Cyclosporine from the formulation were studied using the dialysis Cassettes (10000 MWCO, Thermo Scientific). A dialysis Cassettes previously soaked overnight in the diffusion medium and a defined amount of drug-loaded nanoparticles containing 0.1 mg Cyclosporine was accurately inserted using syringe into dialysis Cassettes. The dialysis Cassettes was suspended in a beaker

containing 100 mL of PBS (pH 5.8) at (37± 0.5) °C. This assembly was kept on magnetic stirrer at 50 rpm. At fixed intervals (2.25 h, 2.5 h, 2.75 h, 3 h, 3.5 h, 4 h, 6 h, 8 h, 12 h and 24 h) 3 mL samples were withdrawn over 24 hrs and were refilled with fresh dissolution medium. Withdrawn samples were analyzed by above-mentioned high-performance liquid chromatograph (HPLC) at a wavelength of 212 nm for cyclosporine A content (Shimadzu LC10AD). *In-vitro* drug release data were fitted to zero-order, first-order, Higuchi equation, Korsmeyer–Peppas equations and hixon crowel model, as per in Table 2, and regression analysis was performed to find the best fitted one [16-22].

S. No.	Model	Graph	Equations
1.	Zero order model	Time versus % Drug release	$Q_0 - Q_t = K_0 t$
2.	First order model	Time versus Log % Drug unreleased	$\log C = \log C_0 - K_{t/2.303}$
3.	Higuchi model	Square root of time versus cumulative % release	$F_1 = Q = A\sqrt{D(2C - CS)CS}t$
4.	Korsmeyer peppas model	Time versus log % cumulative drug release	$M_t / M_\infty = Kt^n$

Table 2: Various Kinetic Models for Drug Release [23,24].

***In-vitro* HaCaT cell line studies [25-27]**

The *in-vitro* efficacy studies were carried out using HaCaT cell lines, as they mimic the hyperproliferative and impaired differentiation of impaired psoriatic epidermal keratinocytes. HaCaT cells were placed in 24 well plates at a density of 4 x 10⁵ cells/well and were allowed to grow and attach for 24 hr before treatment with free drug mixture, blank formulation and formulation loaded with Cyclosporine A. After UV A radiation, CoQ10 were added to wells. Before MTT test, each well was washed with PBS, 760 1 fresh medium and 0.5% MTT 40 were added and then were incubated for 3 hr. Finally, DMSO was added to dissolve purple formazan and absorption was determined by the absorption in an enzyme linked immunosorbent assay plate reader at 490 nm and reference wavelength at 630 nm. The results were expressed in terms of % growth inhibition. These cell line studies are used to assess the *in vitro* anti psoriatic activity as these mimic the hyperproliferation and impaired characterization of epidermal keratinocytes. These cell lines efficiently predict the uptake of formulations by the cells.

Results

Standard plot of cyclosporine in ethanol

In the studied range of 1-8 µg/mL of DTX-PL in ethanol, the observed λmax was 214 nm. The standard calibration curve of cyclosporine was obtained by plotting Absorbance vs. Concentration.

S. No.	Concentration (µg/ml)	Mean Absorbance (±) SD
1.	1	0.092 ± 0.01
2.	2	0.198 ± 0.02
3.	3	0.295 ± 0.01
4.	4	0.402 ± 0.03
5.	5	0.565 ± 0.04
6.	6	0.620 ± 0.03
7.	7	0.789 ± 0.04
8.	8	0.878 ± 0.02

Table 3: Standard plot of cyclosporine in ethanol.

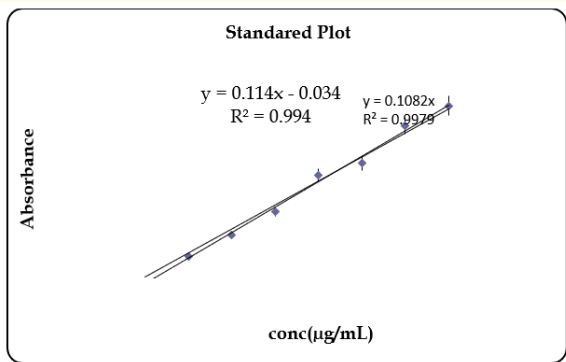


Figure 2: Calibration curve of Cyclosporine in Ethanol.

Morphology of SLNs

Scanning electron microscopy (SEM) was utilized to evaluate the morphology of the SLNs. Figure 3 display SEM photographs for Cyclosporine loaded SLNs. SEM images of solid lipid nanoparticles clearly indicated the interaction of drug with respective carrier material and concluded the incorporation of drug in matrix material [28-30].

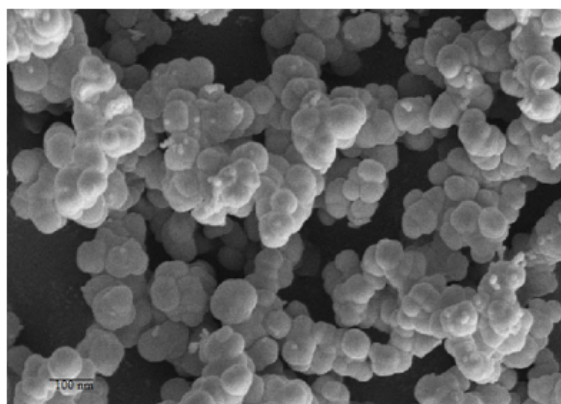


Figure 3: SEM photographs for Cyclosporine loaded SLNs.

Particle size, surface charge analysis entrapment efficacy (EE)

Table 4 represent the data of the particle size, zeta potential and PDI along with drug entrapment efficacy for all the developed SLNs loaded with cyclosporine, viz. palmitic acid derived SLNs (Pal-SLN),

cetyl palmitate-derived SLNs (Cpal-SLN), glycerol monostearate-based SLNs (Gcms-SLN) and glyceryl behenat -based SLNs (Gcb-SLN). Model of size was observed as Gcms-SLN > Pal-SLN > Cpal-SLN > Gcb-SLN [30].

S. No.	Name of Sample	Particle size (nm)	PDI	Zeta potential (mV)	% EE
1.	Pal-SLN	239.80 ± 12.01	0.302	- 14.9	89.16 ± 0.25
2.	Cpal-SLN	183.78 ± 15.84	0.290	- 21.9	92.09 ± 0.78
3.	Gcms-SLN	321.00 ± 14.89	0.367	- 29.3	94.70 ± 0.17
4.	Gcb-SLN	152.07 ± 10.52	0.367	- 8.9	91.38 ± 0.53

Table 4: The obtained data pertaining to particle size, surface charge and drug entrapment studied SLNs.

The EE of the CAs in all the SLNs was near by 90%. The PDI values of developed SLNs confirmed the reliability of the obtained particle size distribution range (≤4).

In-vitro Drug Release Studies and Release Kinetics

The findings indicate that the developed SLNs own drug release controlling latent, which is the most preferred characteristic of nanocarrier-based drug delivery. The results of drug release have been shown in figure 4.

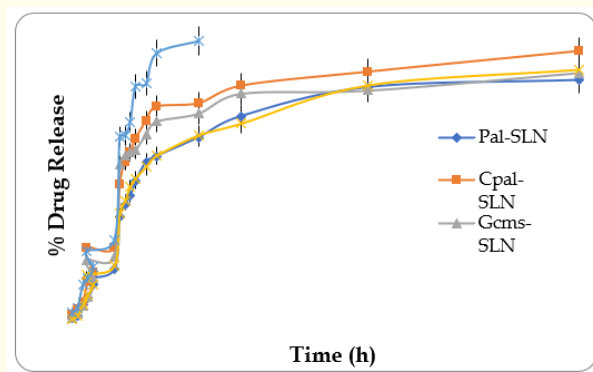


Figure 4: Drug release plot of CAs from the developed SLNs in PBS pH 5.8 for 24 h.

The drug release data were showing to drug release kinetics employing the mostly working models like zero-order, first-order, Higuchi and Korsmeyer-Peppas. The evolution data of the modeling have been represented in table 5.

Kinetic Model	Plain CAs		Pal-SLN		Cpal-SLN		Gcms-SLN		Gcb-SLN	
	R ²	Equation	R ²	Equation	R ²	Equation	R ²	Equation	R ²	Equation
Zero-order	0.872	18.33x+7.346	0.899	11.99x+4.145	0.867	14.49x+7.591	0.83	14.21x+6.427	0.9	12.05x + 4.602
First-order	0.779	0.223x+1.041	0.796	0.224x+0.843	0.768	0.215x+0.984	0.749	0.235x+0.895	0.782	0.226x + 0.848
Higuchi	0.863	46.44x- 14.74	0.876	30.14x- 10.00	0.879	37.17x- 10.47	0.851	36.67x- 11.58	0.886	30.47x - 9.847
Korsmeyer-Peppas	0.802	0.984x+1.305	0.831	1.000x+1.107	0.813	0.967x+1.236	0.801	1.060x + 1.17	0.814	1.003x + 1.115

Table 5: Drug release kinetics of CAs and its solid lipid nanoparticles (SLNs).

In-vitro HaCaT cell line studies

It was observed from the figure 5, that 50% of the cells were inhibited by Cyclosporine SLNs when dose of 50 µg/mL was given and cell viability decreased progressively as the dose was increased. On the other hand free Cyclosporine were not able to inhibit 50% of the growth when highest dose of 100 µg/ml was given, thereby proving the fact that Cyclosporine SLNs had better effect on cell growth as compared to the free drug which was in turn due to higher uptake by the cells. It was also observed that cell viability was not affected with blank SLNs, which proved that there was no effect of surfactant concentration and surfactant type on cell viability causing no cell death [31,32].

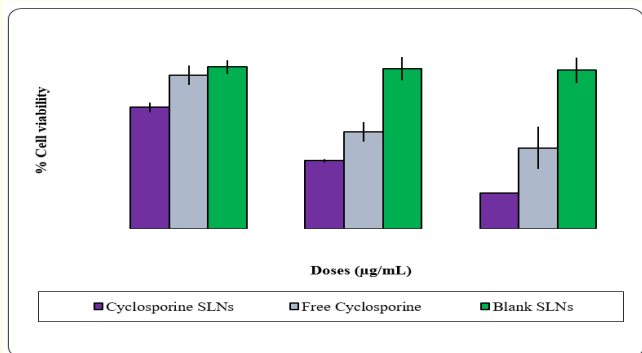


Figure 5: Comparative evaluation of % Cell viability of free drug, blank SLNs, and Cyclosporine SLNs. Each value is expressed as mean± S.D (n = 3).

Stability studies

To picture the stability prospective of the developed SLNs, was performed shown in table 6. It was establish that there was less increase in the particle size which can be credited to the development of slight aggregates. But there was no important effect in the entrapment efficacy of the SLNs. This guaranteed that the prepared SLNs were appropriate to be stored at the room temperature, at lease for 6 months.

Pal-SLN				
Months	Particle size (nm)	Zeta Potential (mV)	PDI	% EE
Initial	239.80 ± 12.01	- 14.9	0.302	89.16 ± 0.25
3	245.05 ± 11.01	- 13.7	0.309	86.18 ± 0.21
6	256.60 ± 13.01	- 15.8	0.323	83.78 ± 0.19
Cpal-SLN				
Initial	183.78 ± 15.84	- 21.9	0.290	92.09 ± 0.78
3	193.89 ± 14.87	-20.8	0.298	95.00 ± 0.68
6	198.78 ± 16.89	-20.8	0.311	89.04 ± 0.58
Gcms-SLN				
Initial	321.00 ± 14.89	- 29.3	0.367	94.70 ± 0.17
3	351.00 ± 13.89	- 36.3	0.378	91.60 ± 0.15
6	371.60 ± 17.89	- 36.9	0.379	84.70 ± 0.20
Gcb-SLN				
Initial	152.07 ± 10.52	- 8.90	0.367	91.38 ± 0.53
3	162.17 ± 09.52	-9.90	0.389	87.13 ± 0.57
6	188.57 ± 08.52	-11.03	0.403	84.45 ± 0.35

Table 6: Stability studies observation of the developed SLNs.

Conclusion

The existing studies effectively embarked upon the preparation and characterization of solid lipid nanoparticles of Cyclosporine. The appearance of studies novel drug delivery systems and its added development has concerned the curiosity of researchers in psoriasis. The lipid-based nano-carriers resulted in approving interactions with the cell lines, and found to be enhanced tolerated. This study not only begins upon with the intend space and the perfect formulation, but also well-known the cause and result relationship along-with mechanistic role SLN characteristics. Thus, it can be concluded that SLNs offer a better option for the delivery of more side effect having and less skin penetrate molecules like Cyclosporin.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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