RESEARCH PAPER

Solvent-Free Fabrication Of Tacrolimus-Loaded Solid Lipid Nanoparticles Via Continuous Hot-Melt Extrusion: Process Optimization And Characterization

Sushama Sagar Pole[1], Dr. Rajani B. Athawale[2*], Dr. Mullaicharam Bhupathyraaj[3]

[1] Research Scholar, Prin.K.M. Kundnani College Of Pharmacy, Colaba, Mumbai.

[2*] Professor & In Charge Principal, Prin.K.M. Kundnani College Of Pharmacy, Colaba, Mumbai.

[3] Professor, College Of Pharmacy, National University Of Science & Technology, Muscat, Sultanate Of Oman.

Corresponding Author: Dr. Rajani B. Athawale, Professor & In Charge Principal, Prin.K.M. Kundnani College Of Pharmacy, Colaba, Mumbai. Email: sushmapole@gmail.com

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Abstract

Aim: The present study is aimed to develop and evaluate tacrolimus-loaded solid lipid nanoparticles (slns) using stearic acid (hs8) and hydrogenated castor oil (hc5) to enhance solubility, stability, and controlled drug release, addressing the limitations of tacrolimus such as poor aqueous solubility, low oral bioavailability and a narrow therapeutic window.

Objectives: To prepare tacrolimus-loaded slns using hot melt extrusion (hme) followed by lyophilization. To compare the effects of stearic acid and hydrogenated castor oil on particle characteristics, drug encapsulation and release profiles.

Methodology: Slns were prepared by hot melt extrusion, followed by high-pressure homogenization and lyophilization with mannitol as a cryoprotectant. Optimization was performed using a box-behnken design to study the effects of lipid, surfactant, co-surfactant and screw speed on particle size, zeta potential and entrapment efficiency. Formulations were characterized for particle size, pdi, zeta potential, drug content, entrapment efficiency, percentage yield, ftir, dsc, xrd, sem and in-vitro drug release in fasted state simulated intestinal fluid (fassif) and fed state simulated intestinal fluid (fessif).

Results and Discussion: Optimized formulations hs8 and hc5 showed nanosized particles (19–60 nm for hs8; 59–198 nm for hc5), high entrapment efficiency (94.5% and 93.6%) and drug content (97.2% and 96.8%). Hs8 exhibited smaller particle size, higher negative zeta potential and slightly better colloidal stability than hc5. Drug release studies revealed sustained, diffusion-controlled release following the higuchi model, with hs8 and hc5 outperforming the marketed formulation in both fasted and fed-state conditions. Ftir confirmed compatibility, while dsc and xrd indicated partial amorphization, enhancing solubility and dispersion. Sem showed well-formed, spherical nanoparticles for hs8 and irregular but dispersed particles for hc5.

Conclusion: Hs8 and hc5 slns demonstrated improved solubility, sustained drug release and potential for enhanced intestinal absorption, making them promising carriers for effective tacrolimus delivery. Stearic acid-based slns showed slightly superior performance in terms of particle size, stability, and yield.

Keywords: Tacrolimus, Solid Lipid Nanoparticles, Hot Melt Extrusion, Lyophilization, Sustained Release.

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1. INTRODUCTION

Tacrolimus is a potent immunosuppressive agent extensively used in the treatment of autoimmune diseases and for the prevention of organ transplant rejection. However, its therapeutic application is limited by poor aqueous solubility, low and variable oral bioavailability and a narrow therapeutic window. These limitations necessitate the development of advanced drug delivery systems capable of enhancing solubility, stability and controlled drug release while minimizing systemic side effects.[1,2]

Solid lipid nanoparticles (SLNs) have gained considerable attention as an effective lipid-based nanocarrier system for poorly water-soluble drugs. SLNs combine the advantages of polymeric nanoparticles and lipid emulsions, offering biocompatibility, biodegradability, protection of the encapsulated drug and the ability to modulate drug release. The physicochemical properties and performance of SLNs are strongly influenced by the choice of solid lipid, which governs drug loading, entrapment efficiency and release behavior.[3,4]

Stearic acid and hydrogenated castor oil (HCO) are widely used solid lipids due to their safety, biodegradability and suitability for sustained drug delivery. Stearic acid, a long-chain fatty acid, forms a dense crystalline matrix that favors high drug entrapment and prolonged release[5,6]. Hydrogenated castor oil, a waxy lipid with amphiphilic characteristics, provides enhanced matrix flexibility and improved drug dispersion[i]. Comparative evaluation of these lipids enables a better understanding of their role in SLN formulation performance.

The hot melt extrusion (HME) technique is a solvent-free, scalable and reproducible method for SLN preparation. HME ensures uniform drug–lipid mixing at elevated temperatures, reduces batch-to-batch variability and eliminates residual solvent concerns. To further enhance long-term stability and facilitate storage, lyophilization was employed to convert the SLN dispersions into dry, free-flowing powders while preserving their nanoscale properties.[7,8]

In the present study, tacrolimus-loaded SLNs were prepared using stearic acid and hydrogenated castor oil via the hot melt extrusion method, followed by lyophilization. The formulations were characterized for particle size, zeta potential, drug content, entrapment efficiency, in-vitro drug release and solid-state properties. The objective of this work was to investigate the influence of lipid type and freeze-drying on the physicochemical characteristics, stability and release behavior of tacrolimus-loaded SLNs and to identify an optimized formulation for improved drug delivery.

Effect of stearic acid as lipid on tacrolimus in SLN preparation: In solid lipid nanoparticles (SLNs) for oral delivery, stearic acid protects tacrolimus primarily by acting as a solid lipophilic matrix that encapsulates the drug, shielding it from the harsh gastrointestinal (GI) environment. As a long-chain fatty acid, stearic acid provides high stability and reduces the degradation rate of the incorporated drug. By keeping the highly lipophilic tacrolimus in an amorphous state within the lipid core, stearic acid improves its solubility and facilitates absorption through the intestinal lymphatic pathway, which bypasses first-pass metabolism. Its long-chain structure is more stable against lipase-driven breakdown compared to shorter-chain lipids, which helps maintain the integrity of the nanoparticle in the intestine. Stearic acid provides a rigid matrix that prevents premature drug leakage, a common issue in oral drug delivery. Safety profile shows that it is non-toxic and biodegradable, ensuring low cytotoxicity in oral formulations. In the present formulation, Stearic Acid (SA) is a strong choice, it combines with other surfactants (e.g., Poloxamer, Tween) to improve nanoparticle stabilization.

Effects of hydrogenated castor oil as lipid on tacrolimus in SLN preparation: As a solid lipid, HCO forms a stable matrix that entrapped tacrolimus, a poorly water-soluble (BCS Class II) drug with high efficiency. Its triglyceride structure provided a lipophilic environment that kept the drug in an amorphous state, preventing crystallization and improved payload capacity. By encapsulating tacrolimus in an HCO matrix, the drug's oral bioavailability was enhanced through several mechanisms like lymphatic transport: solid lipid nanoparticles absorbed via the intestinal lymphatic system, bypassing hepatic first-pass metabolism. HCO provided a robust matrix structure that prevented the initial "burst release" in the formulation, and allowed a sustained release profile of tacrolimus. HCO-based SLNs typically exhibited a sustained or gradual release of tacrolimus over a 24-hour period. This slow release is attributed to the solid nature of the lipid at body temperature, which requires enzymatic degradation or slow diffusion for drug liberation. Reduced side effects: Hydrogenated castor oil as a solid lipid in a nanoparticle matrix allowed for a more controlled, lower-dose approach, which helped in minimizing the side effects associated with high levels of surfactants.

2. Materials and Methods

2.1 Materials

Drug Tacrolimus was gifted by Concord Biotech, Ahmedabad. Stearic acid and Hydrogenated castor oil which were used as solid lipids were gifted by BASF, Navi Mumbai and Surfactants such as

poloxamer 188 and Tween 80 were gifted by BASF, Navi Mumbai and soya lecithin was purchased from Hyma Synthesis Pvt. Ltd. All other reagents were of analytical grade.

2.2 Method

Hot Melt Extrusion Method

SLNs were prepared by mixing a lipid and aqueous phase containing surfactants inside the extruder barrel at temperatures 10–15°C above the melting point of the lipid which resulted in the formation of an emulsion. The selected lipids and Tacrolimus geometric mixture were fed into the co-rotating twin-screw extruder (VJHME-01) using a volumetric feeder. Concentrations of the surfactant tween 80 and co-surfactant poloxamer 188 aqueous solutions, equivalent to the extrusion temperature, was injected into the extruder barrel either zone 3 or zone 4 through an injection port using a peristaltic pump. The barrel temperature for zone 2 was either 150 or 120°C; zone 3 and the rest of the zones including the die temperature were kept at 90°C. Screw speed for all of the formulations were either 800-1000 rpm. The hot pre-emulsion that resulted from hot-melt extrusion was passed through an insulated tube connecting to the HME die and sample holder of the high-pressure homogenizer for size reduction. The HPH was performed at 85°C and 1000 bar pressure with two repeated cycles to reduce the particle size of pre-emulsion resulting from melt extrusion[9]. The HPH parameters were constant for all of the batches. Further, the size-reduced emulsion was cooled at room temperature to obtain SLN[10].

Independent variables	**Low value(-1)**	**Medium (0)**	**High value(+)**
Stearic acid (mg) (A)	10	11	12
Poloxamer 188 (mg) (B)	2.5	2.75	3
Soya lecithin (mg) (C)	5	5.5	6
Screw speed (RPM) (D)	800	900	1000
Dependent variables	Constraints		
Entrapment efficiency (%)	Maximize		
Particle Size (nm)	Minimum		
Zeta Potential (mV)	Maximize		
Drug release (%)	Maximize		

Lyophilization Method:

The Tacrolimus -SLNs were lyophilized using a freeze dryer (Germany). Various amounts of cryoprotectant (Mannitol 50%) were added to Tacrolimus -SLNs suspensions before freezing. Each sample was rapidly precooled at -25˚C, and lyophilized for 24 hours at a temperature of -50˚C and a vacuum of 3 mmHg.[11]

Experimental design for SLN with Stearic acid

Response Surface Methodology (RSM) was applied using a Quality by Design approach to develop polynomial models with fewer experimental runs. A 3-factor, 3-level Box-Behnken Design was used to study the effect of independent variables—Stearic acid (A), Poloxamer 188 (B), Soya lecithin (C) and Screw speed (D) on dependent responses: Entrapment Efficiency (Y1),

Particle Size (Y2), and Zeta Potential (Y3). Each variable was studied at low, medium, and high levels. The finalized independent and response variables are summarized in the table1.

Table 1: List of Independent and Dependent variables for SLNs with Stearic acid in Box–Behnken design

Experimental design for SLN with Hydrogenated castor oil

Response Surface Methodology (RSM) was applied using a Quality by Design approach to construct polynomial models with fewer experimental runs. A Box-Behnken Design with 3 factors and 3 levels was used to study the effect of independent variables—hydrogenated castor oil (A), Poloxamer 188 (B), Soya lecithin (C), and Screw speed (D) on dependent responses: Entrapment Efficiency (Y1), Particle Size (Y2), and Zeta Potential (Y3). The finalized independent and response variables are summarized in the table 2.

Table 2: List of Independent and Dependent variables for SLN with Hydrogenated castor oil in Box–Behnken design

Independent variables	Low value (-1)	Medium (0)	High value (+)
Hydrogenated castor oil (mg) (A)	10	12.5	15
Poloxamer 188 (mg) (B)	2.5	3.125	3.75
Soya lecithin (mg) (C)	5	6.25	7.5
Screw speed (RPM) (D)	800	900	1000
Dependent variables			
Entrapment efficiency (%)	Constraints Maximize		
Particle Size (nm)	Minimum Maximize		
Zeta Potential (mV)			

3. Evaluation and characterization of Solid Lipid nanoparticles

The drug was screened for its solubility in various solid lipids such as Hydrogenated castor oil, Stearic acid, Cetyl palmitate and Compritol 888 ATO, etc. by melting them 5–10 °C above its melting point. 1 gram of selected solid lipids were melted and drug was added. The lipid showing maximum drug solubility is considered suitable for the formulation of solid lipid nanoparticles[12,13].

Tween 80, Tween 20, Tween 40, Tween 60, Span 60 were selected as surfactants for the study[14,15]. Poloxamer 407 and Poloxamer 188 were selected as co - surfactants for the study.

Particle Size, PDI and Zeta potential analysis were done for the SLN formulation[16,17].

For determining the drug content, 5 mg of SLN powder of HS8 and HC5 was weighed accurately and transferred to a 10 ml conical flask, followed by addition of 10 ml of methanol and sonication for 10 min. The following solution was filtered and analyzed spectrophotometrically at 293 nm for drug content[18,19,21].

The entrapment efficiency of Tacrolimus loaded SLN formulation was determined by centrifugation method, in which 5 mg in 5 ml of methanol the prepared SLN suspension was placed in the Eppendorf tube for centrifugation (RM-12) at 10000 rpm for 30 min. After centrifugation, the supernatant liquid was separated, containing the un-entrapped drug, diluted with methanol and analyzed using UV spectrophotometer at 293nm. By using that absorbance entrapment efficiency (%EE)[ii]. Percentage yield was calculated for SLN batches[22].

In vitro drug diffusion studies of Tacrolimus - SLN formulations were performed by using dialysis method in pH 6.8 phosphate buffer for 24 h. During in vitro release studies, dialysis membrane with a molecular weight cut-off ranging from 12,000 Da was used. Prior to the study, the dialysis membrane was soaked by keeping it in double distilled water overnight. The apparatus had two compartments, a donor and a receptor compartment. In donor compartment, about equivalent weight formulation was taken for releasing study, which consisted of a boiling tube with opening at one end and tied with dialysis membrane at the other end[23]. A beaker of 250 ml capacity was used as receptor compartment with 100 ml release medium and maintained at the temperature of 37±0.5°C. About 2 ml of samples were withdrawn from receiver compartment and were replaced with fresh medium periodically at predetermined time intervals and analysed for drug content by using UV-visible spectrophotometry (Jasco v-630) at 293 nm[24].

Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) were prepared for the invitro fasted and fed dissolution study. It was carried out for tacrolimus SLN optimized formulation.

In order to define the release mechanism that gives the best description of the release pattern; the in vitro release data for HS8 and HC5 were fitted to kinetic equations models. The kinetic equations were used i.e., zero, first-order and Higuchi model. Both the kinetic rate constant (k) and the determination coefficient (R2) were calculated and presented in graphs.

The kinetic equations were used i.e., zero, first-order and Higuchi model. Both the kinetic rate constant (k) and the determination coefficient (R2) were calculated and presented in below graphs.

The drug excipients compatibility study was performed by FTIR technique. The Optimized batches HS8 and HC5 samples were scanned over wavenumber range of 500-4000 cm^{-1} with diffraction reflectance scanning technique[24].

Differential scanning calorimetric measurements were carried out on a modulated DSC (Mettler Toledo, SW STARe, and USA). The Optimized batch STF 6 and GTF8 were weighed (2-8mg), the aluminum pans were

used and hermetically covered with lid. The heating range was 0-250 °C for sample with constant increasing rate of temperature at 10°C /min under nitrogen atmosphere (50-60ml/min). The resultant thermograms of formulation was obtained.[25]

The data obtained from XRD was used to determine whether newly formed compounds were crystalline or amorphous. The following conditions were used for the measurement: target metals Cu, filter K, 40kV voltage, and 30 mA current. Optimized batch HS8 and HC5 samples were scanned over a two-degree range of 10–90°C with a 0.2° phase scale[26]. Scanning electron microscopy was used to determine the morphology of fractured, surface topography and texture. The surface morphology of optimized batches was determined.

4. Results and Discussion

4.1. Drug lipid Solubility

The solubility study showed that tacrolimus exhibited the highest solubility in stearic acid (25.0 mg/g), followed by hydrogenated castor oil (21.5 mg/g). Cetyl palmitate demonstrated moderate solubility (12.8 mg/g), whereas Compritol® 888 ATO showed the lowest solubility (5.5 mg/g). Higher solubility in stearic acid and hydrogenated castor oil indicates better drug–lipid compatibility and greater potential for efficient drug entrapment. Based on these results, stearic acid and hydrogenated castor oil were identified as suitable solid lipids for SLN formulation.

4.2. Screening of Surfactants and co-surfactants

The surfactant solubility study showed that Tween 80 exhibited the highest solubility (0.2606 mg/ml), followed by Tween 40 (0.1547 mg/ml) and Tween 60 (0.1191 mg/ml). Tween 20 showed lower solubility (0.0878 mg/ml), while Span 60 exhibited the least solubility (0.0641 mg/ml). The higher solubility of Tween 80 indicates better drug–surfactant compatibility and suitability for enhancing drug solubilization in SLN formulations. The co-surfactant solubility study showed that Poloxamer 188 exhibited slightly higher solubility (0.0606 mg/ml) compared to Poloxamer 407 (0.0518 mg/ml). The improved solubility of Poloxamer 188 suggests better drug–co-surfactant compatibility and enhanced potential for stabilizing the SLN system. Based on these results,

Poloxamer 188 was selected as the preferred co-surfactant for formulation development.

4.3. Optimization by Box Behnken Design

4.3.1 Optimization of SLN with Stearic acid by Box Behnken Design shown in table 3:

Table 3: Optimization of SLN with Stearic acid by Box Behnken Design

Formulation Code	Stearic acid(mg)	Poloxamer 188 (mg)	Soya lecithin (mg)	Screw speed (RPM)	Entrapment efficiency (%)	Particle Size (nm)	Zeta Potential (mV)
HS1	11	2.5	5	900	90 ±0.026	192.3±0.196	-24.3±0.173
HS2	11	2.5	6	900	86±0.012	196.5±0.02	-14.2±0.2
HS3	12	2.75	6	900	80.12±0.03	213.1±0.3	-17.8±0.34
HS4	11	2.5	5.5	800	88.45±0.045	197.8±0.46	-21.8±0.051
HS5	10	2.75	6	900	87.26±0.02	212.4±0.07	-16.8±0.042
HS6	11	2.75	6	1000	86.24±0.031	236.2±0.094	-15.2±0.009
HS7	11	2.75	6	800	86.2±0.07	209.4±0.23	-23.4±0.073
HS8	10	2.5	5.5	900	94.5±0.02	185.6±0.10	-28.5±0.046
HS9	11	3	6	900	85.14±0.042	214.6±0.025	-24.7±0.3
HS10	10	2.75	5	900	92.12±0.03	189.7±0.034	-27.9±0.37
HS11	12	2.5	5.5	900	87.25±0.036	216.7±0.009	-23.8±0.4
HS12	12	2.75	5.5	1000	89±0.04	208.7±0.010	-18.2±0.069
HS13	12	2.75	5	900	91.2±0.03	197.5±0.055	-26.4±0.134

Effect of independent variables on Entrapment efficiency

The entrapment efficiency of formulations HS1–HS13 ranged from 80.12% to 94.5%, indicating effective incorporation of the drug within the carrier system. The 3D response surface plot of the effects of independent variables on entrapment efficiency is shown in Figure1.

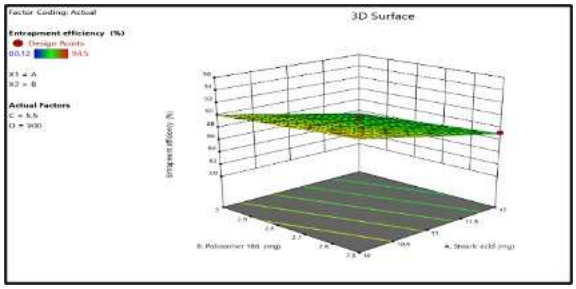


Figure 1: 3D surface plot for entrapment efficiency

Effect of independent variables on particle size

The particle size of formulations HS1–HS13 ranged from 185.6 nm to 236.2 nm. The 3D response surface plot of the effects of independent variables on particle size is shown in Figure 2.

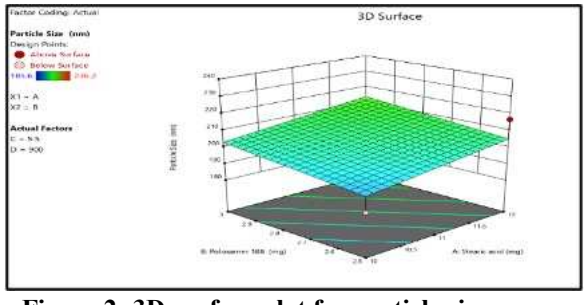


Figure 2: 3D surface plot for particle size

Effect of independent variables on Zeta potential

The zeta potential values of formulations HS1–HS13 ranged between –14.2 mV and –28.5 mV. The 3D response surface plot of the effects of independent variables on Zeta potential is shown in Figure 3.

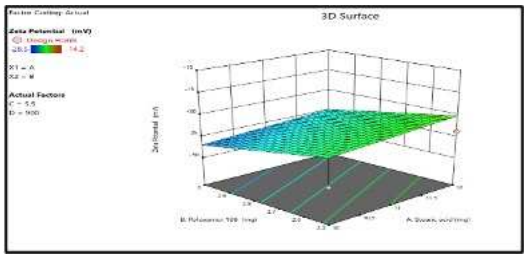


Figure 3: Effect of independent variables on Zeta potential

4.3.2 Optimization of SLN with Hydrogenated castor oil by Box Behnken Design shown in table 4:

Table 4: Optimization of SLN with Hydrogenated castor oil by Box Behnken Design

Formulation Code	Hydrogenated castor oil (mg)	Poloxamer 188 (mg)	Soya lecithin (mg)	Screw speed (RPM)	Entrapment efficiency (%)	Particle Size (nm)	Zeta Potential (mV)
HC1	15	3.125	7.5	900	82.5±0.15	230.5±0.2	-19.5±0.45
HC2	15	3.125	6.25	800	85.2±0.10	250.6±0.35	-15.2±0.16
HC3	10	3.125	6.25	1000	84.62±0.06	219.5±0.01	-26.3±0.2
HC4	10	3.75	6.25	900	92.1±0.031	238.4±0.08	-23.9±0.2
HC5	15	3.125	6.25	1000	93.6±0.1	215.6±0.15	-29.6±0.34
HC6	12.5	3.75	6.25	1000	89.72±0.03	226.2±0.01	-25.8±0.07
HC7	12.5	2.5	6.25	1000	88.54±0.04	251.3±0.09	-23.4±0.14
HC8	10	3.125	7.5	900	90.12±0.045	248.7±0.15	-18.5±0.5
HC9	12.5	3.75	7.5	900	84.56±0.05	243.4±0.16	-22.6±0.08
HC10	12.5	3.125	5	800	87.7±0.036	238.1±0.14	-26.7±0.1
HC11	12.5	2.5	7.5	900	86.12±0.46	255.2±0.033	-17.3±0.09
HC12	12.5	3.75	6.25	800	89.25±0.23	252.4±0.001	-19.4±0.11
HC13	12.5	3.125	5	1000	81.45±0.32	219.8±0.0054	-27.2±0.5

Effects of independent variables on Entrapment efficiency

The entrapment efficiency of formulations HC1–HC13 ranged from 81.45% to 93.6%, indicating effective incorporation of the drug within the carrier matrix. The 3D response surface plot of the effects of independent variables on entrapment efficiency is shown in Figure 4.

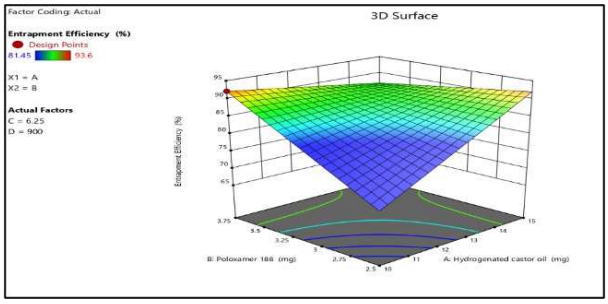


Figure 4: 3D surface plot for entrapment efficiency

Effects of independent variables on particle size

The particle size of the formulations HC1 – HC13 ranged from 215.6 nm to 255.2 nm. The 3D response surface plot of the effects of independent variables on particle size is shown in Figure 5.

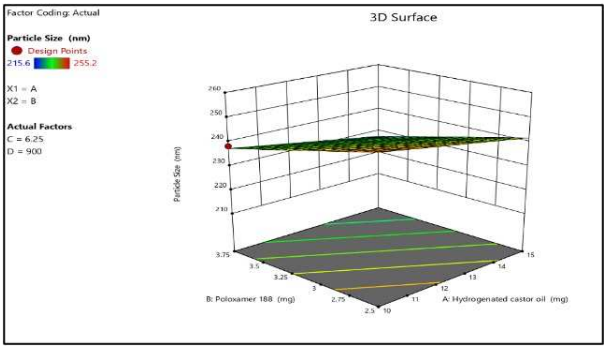
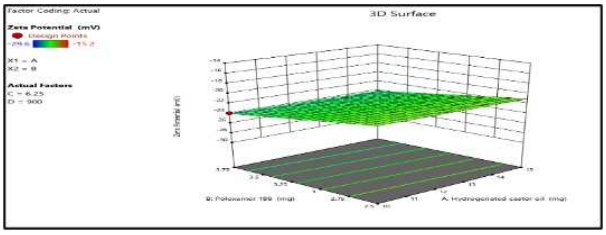


Figure 5: 3D surface plot for particle size

Effects of independent variables on Zeta potential

The zeta potential values of the formulations (HC1–HC13) ranged from –15.2 mV to –29.6 mV. The 3D response surface plot of the effects of independent variables on Zeta potential is shown in Figure 6.



.**Figure 6: 3D surface plot for zeta potential**

4.4 Drug content:

The drug content of HS1–HS13 ranged from 75.7% to 97.2%, indicated good batch uniformity, with HS8 showing the highest drug content (97.2%) and identified as the optimized formulation. Similarly, HC1–HC13 exhibited uniform drug distribution with values between 88.1 ± 0.046% and 96.8 ± 0.042%, thereby reflected good reproducibility of the formulation process. Among HC batches, HC5 showed the maximum drug content (96.8 ± 0.042%), indicating efficient drug incorporation. Overall, both HS8 and HC5 demonstrated superior drug loading and formulation efficiency.

4.5 Percentage yield:

Practical yield studies showed satisfactory recovery for both stearic acid and hydrogenated castor oil based SLNs. Stearic acid SLNs (HS) ranged from 78.50–97.56% (highest for HS8), while hydrogenated castor oil SLNs (HC) ranged from 85.75–94.35% (highest for HC5). Overall, stearic acid SLNs achieved slightly higher peak yield and both lipid systems demonstrated reproducible and scalable formulations, supporting stearic acid for optimized SLN preparation.

4.6. In vitro drug release

The in-vitro release profiles of HS and HC formulations showed sustained and time-dependent drug release over 24 h, confirming effective controlled-release behavior of the SLN system with minimal initial burst release. Among HS batches, HS8 exhibited the highest cumulative drug release (98.30%), indicating optimal particle characteristics and efficient drug diffusion which is shown in figure 7. Similarly, HC5 showed the maximum drug release (97.42%), reflecting optimal formulation parameters which is shown in figure 8. Overall, HS8 and HC5 were identified as the optimized formulations with superior and consistent sustained drug delivery performance.

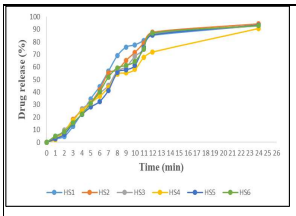


Figure 7(A): Drug Release of HS1-HS6

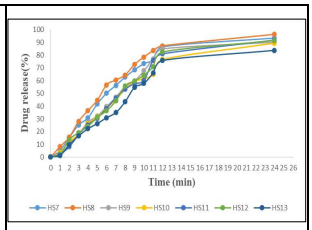


Figure 7(B): Drug Release of HS7-HS13

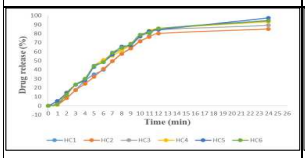


Figure 8 (A): Drug Release of HC1-HC6

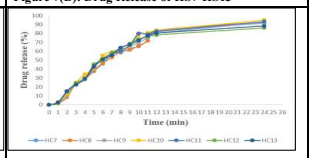


Figure 8 (B): Drug Release of HC7-HC13

4.6.1 The in-vitro fasted and fed dissolution study

The in-vitro drug release study in Fasted State Simulated Intestinal Fluid (FaSSIF) demonstrated enhanced drug release from the optimized formulations compared to the marketed product. HS8 showed the highest cumulative drug release (99.89% at 8 h), followed closely by HC5 (99.54%), indicating improved dissolution behavior under fasted intestinal conditions. Both HS8 and HC5 achieved faster and more complete drug release than the marketed formulation. Overall, the results confirm the superior release performance of HS8 and HC5 in FaSSIF, highlighting their potential for improved intestinal drug absorption shown in table 5.

Table 5: Drug release from Fasted State Simulated Intestinal Fluid (FaSSIF) of HS8, HC5 and marketed formulation

Time (hr)	Drug released (%)		
	HS8	HC5	Marketed Formulation
0	0	0	0
1	26.03±0.34	20.13±0.11	19.72±0.35
2	31.95±0.14	29.10±0.17	30.12±0.15
3	47.14±0.14	45.30±0.04	39.61±0.05
4	55.73±0.06	52.47±0.12	50.43±0.04
5	72.87±0.09	65.03±0.13	62.38±0.15
6	81.39±0.19	74.25±0.07	81.05±0.05
7	93.07±0.25	87.35±0.06	89.28±0.10
8	99.89±0.04	99.54±0.17	93.24±0.15

The in-vitro drug release study in Fed State Simulated Intestinal Fluid demonstrated enhanced and faster drug release from the optimized formulations compared to the marketed product. HS8 showed the highest cumulative drug release (99.57% at 6 h), followed by HC5 (98.11%), indicating superior dissolution behavior under fed intestinal conditions. Both HS8 and HC5 achieved higher drug release at earlier time points than the marketed formulation shown in table 6. Overall, the results confirm that the optimized formulations provide improved and consistent drug release in fed-state conditions, highlighting their potential for enhanced intestinal absorption.

Table 6: Drug release from Fed State Simulated Intestinal Fluid (FaSSIF) of HS8, HC5 and marketed formulation

Time (hr.)	Drug released (%)		
	HS8	HC5	Marketed Formulation
0	0	0	0
1	38.43±0.16	29.89±0.07	29.08±0.05
2	49.76±0.025	42.94±0.09	43.95±0.10
3	57.95± 0.13	53.76±0.04	56.92±0.01
4	71.13± 0.11	67.96±0.16	70.70± 0.02
5	89.81± 0.1	81.45±0.08	85.42± 0.03
6	99.57± 0.16	98.11±0.15	95.38±0.12

Kinetic analysis of drug release-

The in-vitro drug release kinetics of optimized formulations HS8 and HC5 were best described by the Higuchi model, showing the highest correlation coefficients (R^2 = 0.9232 for HS8 and R^2 = 0.9218 for HC5). This indicates that drug release from both formulations is predominantly diffusion-controlled through the polymeric/lipid matrix. The lower R^2 values for zero-order and first-order models suggest that the release is neither constant nor concentration-dependent.

Overall, the results confirm diffusion-controlled sustained drug release from the optimized formulations.

4.7 FTIR spectroscopy:

The FTIR spectra of optimized formulations HS8 and HC5 confirmed successful incorporation of the drug into the lipid matrix without any significant chemical interaction. In HS8, characteristic peaks corresponding to O–H, C–H, C=O, C=C, and C–O–C functional groups were retained, indicating the presence of lipid and excipient components shown in figure 9. Similarly, HC5 showed prominent peaks for O–H stretching, aliphatic C–H stretching, ester C=O, and C–O–C vibrations shown in figure 10. The absence of peak shifting or disappearance in both formulations suggests good compatibility between the drug and excipients. Overall, the FTIR results confirm physical dispersion of the drug and chemical stability within both optimized formulations.

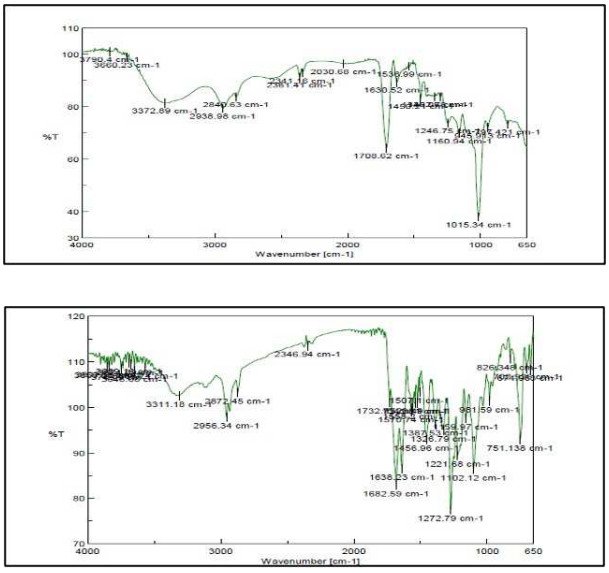


Figure 9: FTIR Spectrum of HS8

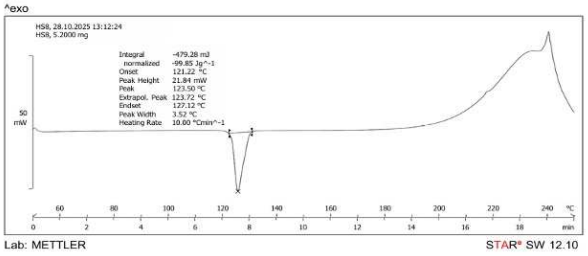


Figure 10: FTIR Spectrum of HC5

4.8 Differential Scanning Calorimetry (DSC)

DSC analysis of HS8 and HC5 confirmed thermal stability and partial amorphization of the drug within the lipid matrix. HS8 showed a sharp peak at 123.5 °C, indicating some retained crystallinity shown in figure 11, while HC5 exhibited a slightly broader peak at 122.9 °C, suggesting a greater shift toward the amorphous state. Overall, both formulations demonstrate partial amorphization with uniform component dispersion and acceptable thermal stability.

Figure 11: DSC thermogram of HS8

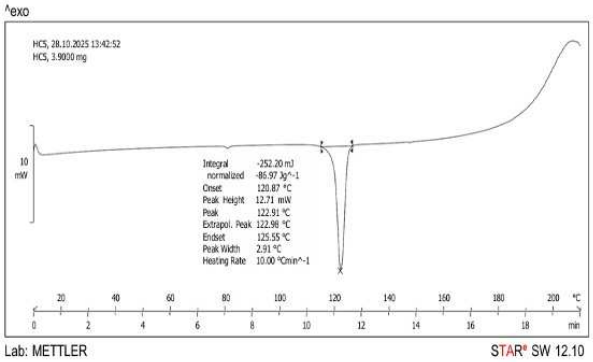


Figure 12: DSC thermogram of HC5

4.9 X-ray Diffraction Study

The XRD analysis of HS8 and HC5 indicated reduced crystallinity and increased amorphous character, reflecting good dispersion of the drug within the lipid matrix. HS8 showed a predominantly amorphous pattern which is shown in figure 13, while HC5 exhibited broadened and less intense crystalline peaks, suggesting partial amorphization which is shown in figure 14. These results confirm the successful formulation and enhanced physical stability of both optimized batches.

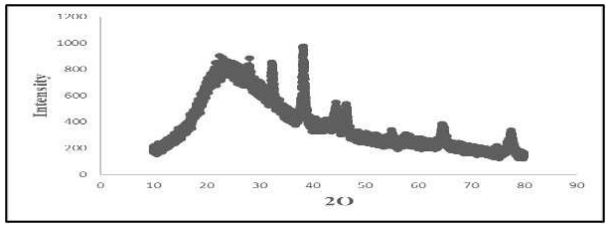


Figure 13: X ray diffractogram of HS8

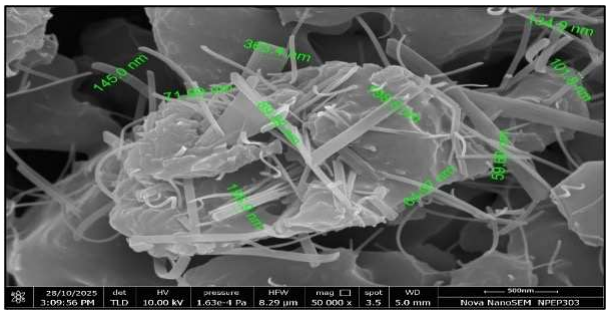


Figure 14: X ray diffractogram of HC5

4.10 Scanning Electron Microscopy

SEM analysis of HS8 and HC5 confirmed their nanoparticulate nature. HS8 showed predominantly spherical, uniform, and well-dispersed particles (19.45–60.63 nm) with smooth surfaces, indicating good encapsulation and formulation stability shown in figure 15. HC5 displayed irregular particles (59–198 nm) with some aggregation and mixed smooth and rough surfaces, confirming successful formation of the lipid matrix shown in figure 16. Both formulations exhibited nanoscale dimensions, supporting improved drug release and bioavailability.

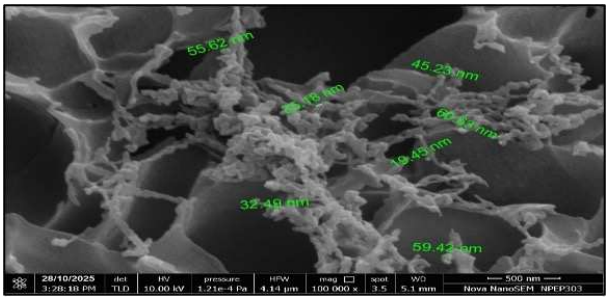
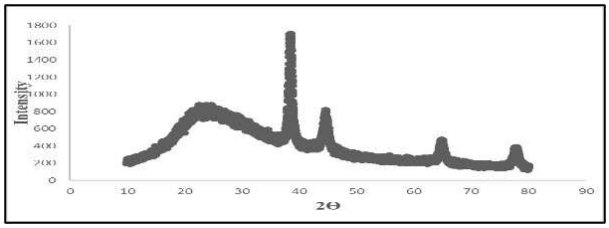


Figure 15: SEM of HS8

Figure 16: SEM of HC5



CONCLUSION

Tacrolimus-loaded solid lipid nanoparticles (SLNs) were successfully formulated using stearic acid (HS8) and hydrogenated castor oil (HC5) via hot melt extrusion followed by lyophilization. Both optimized formulations exhibited nanosized particles with uniform dispersion and acceptable PDI values, confirming successful nanoparticle formation. HS8 showed smaller particle size and higher negative zeta potential compared to HC5, indicating superior colloidal stability. Drug content and entrapment efficiency were high for both formulations, with HS8 achieving 97.2% drug content and 94.5% entrapment efficiency, while HC5 showed 96.8% and 93.6%, respectively. Practical yield was also satisfactory, with HS8 at 97.56% and HC5 at 94.35%. In vitro drug release studies in Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) demonstrated sustained and time-dependent drug release, with HS8 and HC5 releasing more rapidly and completely than the marketed formulation. Kinetic analysis indicated that drug release followed the Higuchi model, confirming a diffusion-controlled mechanism. FTIR spectroscopy showed no significant chemical interaction between the drug and lipids, suggesting compatibility and stability. DSC and XRD studies indicated partial amorphization of tacrolimus within the lipid matrices, improving drug solubility and dispersion while maintaining thermal stability. SEM analysis revealed spherical and uniform particles for HS8 and slightly irregular but well-dispersed particles for HC5, supporting proper encapsulation and formulation stability. Overall, HS8 and HC5 were identified as optimized SLN formulations with improved solubility, stability, controlled drug release, and enhanced intestinal absorption, making them promising carriers for effective tacrolimus delivery. In conclusion, stearic acid serves as an excellent solid matrix component for producing tacrolimus-loaded SLNs for oral administration by acting as a biocompatible matrix to enhance the oral delivery of tacrolimus. It formed a stable, hydrophobic core that encapsulates tacrolimus, improving its stability, protecting it from degradation, and enabling sustained release.

Conflict of Interest: None

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