Original Article

INTERNATIONAL JOURNAL OF CLINICAL PHARMACOKINETICS AND MEDICAL SCIENCES

Published by Pharma Springs Publication Journal Home Page: https://pharmasprings.com/ijcpms

Development and bioavailability improvement of Resiquimod in the form of solid lipid nanoparticles

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Article History:

Received on: 26 Jun 2024
Revised on: 20 Sep 2024
Accepted on: 25 Sep 2024

Keywords:

Resiquimod,
Solid Lipid Nanoparticles,
Bioavailability,
Development

Abstract

The present study aims to develop Resiquimod's Solid Lipid Nanoparticles (SLNs) for topical drug delivery. To evaluate the physicochemical characterization of Resiquimod, lipids, polymers, surfactants & other additives. Formulation & Evaluation of SLNs for particle size analysis, Zeta potential, entrapment efficiency, polydispersity index (PDI), and % yield of produced SLNs. Based on the current studies, it can be said that Resiquimod solid lipid nanoparticles were effectively created and optimized. Hydrogels of prepared SLNs can be applied topically to treat skin conditions such as actinic keratoses (AKs). Different concentrations of lipids and surfactants were used to design SLNs. Mannitol showed much effectiveness as a Cryoprotectant. The drug's excipient Research on the compatibility of drugs, lipids, polymers, and their combinations using FTIR and DSC confirms that the drugs and excipients do not interact chemically. The Nanoparticles in the SLN droplets were intact, non-aggregated, and almost spherical according to scanning electron microscopy (SEM) pictures. The particle size of SLNs loaded with Resiquimod ranges from 208 to 503 nm. This could contribute to the SLNs' more extended blood circulation period. It was discovered that the PDI was less than 0.6. Adequate stability was demonstrated by the negative charge of the zeta potential in the formulation of SLNs. The results are encouraging and represent a novel contribution of Resiquimod SLNs in Topical Drug Delivery.

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eISSN: 2583-0953
DOI: https://doi.org/10.26452/ijcpms.v4i4.664

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INTRODUCTION

Common skin lesions known as actinic keratoses (AKs) indicate a higher chance of acquiring squamous cell carcinoma (SCC) and other skin malignancies. They are caused chiefly by excessive UV exposure. They're primarily found in fair-skinned people, and they're becoming more of an issue for immunocompromised people. AKs can spontaneously regress, stay stable, or develop into invasive SCC. With more than 5 AKs, the risk of SCC increases, and AKs cause the majority of SCCs [1].

Inflammation and oxidative stress are the two main factors that cause AK to occur.

Immunosuppression, apoptotic impairment, mutagenesis, cell growth and proliferation dysregulation, and tissue remodeling are all examples of immunosuppression. The formation of some AKs has also been linked to the human papillomavirus. Understanding these mechanisms guides the logic for the currently available treatments for AKs. Field cancerization is one of the primary factors underlying the therapy of AKs [2].

As we age, large regions of our skin are exposed to more UV rays and other environmental irritants. This is particularly true in the head, neck, and forearms. These insults not only attack individual lesions on the skin but also vast areas where AK crops may emerge. The skin between lesions is exposed to the same stresses and will likely have preclinical lesions or dysplastic cell regions that are still invisible. The 'field' refers to the entire impacted region. As a result, treatment is separated into two categories: lesion-directed and field-directed therapies [3].

Actinic keratoses (AK), known as sun keratoses or senile keratoses, are benign intraepithelial neoplasms caused by abnormal keratinocyte proliferation. They are widespread in photo-exposed areas of adults and older people with fair skin. These are the most common precancerous lesions in humans, and UV radiation is typically the cause (UVR). They are the most prevalent pre-malignant lesions and can potentially progress into squamous cell carcinoma (SCC). They were first characterized in 1826 by Dubreuilh, and their SCC antecedent nature has been known for more than a century. Deep, rest wrinkles, loss of skin suppleness, atrophy, telangiectasia, and pigmentation alterations are phenotypic expressions of cutaneous photo-aging; they can also suggest individuals/chronic photo exposure [4].

MATERIALS AND METHODS:

MATERIALS

The gift sample Resiquimod is from Drugs India Pvt Ltd, Hyderabad India and other polymers such as Glycerol Monostearate, Glyceryl Behenate, Glyceryl Trimyristate, Dioleyl Trimethyl Ammonium Propane, Poloxamer 188, Sodium Taurocholate, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween 80), Polyethylene Glycol (PEG) and Fructose, Maltose, Mannitol Sorbitol.

METHODS

FT-IR Analysis of Pure Drug

Fourier Transform Infrared (FT-IR) is a crucial supplemental technique for the solid-state characterization of medicinal materials. Infrared spectroscopy and an Alpha Bruker FTIR spectrophotometer were used to identify the material. The sample was prepared using the disc method. To create a delicate and uniform mixture, the drug was triturated with potassium bromide in a mortar and pestle (about 5 mg sample with 100 mg dry potassium bromide). The powder was compressed using a potassium bromide press set to 20 psi for ten minutes to make the pellets. After being ready, the sample disc was placed into the sample compartment. The sample was scanned in transmission mode within 4000-400 cm-1. The obtained infrared spectra were contrasted with the standard spectrum of pure drugs [5].

DSC Study

The melting point of 5 Fluorouracil was determined using differential scanning calorimetry (DSC). DSC Thermograms are created by utilizing a DSC instrument. The sample was placed in aluminum pans and pressed to seal. The aluminum pan and blank aluminum pan were heated from 30°C to 300°C at ten °C/min under nitrogen atmospheric conditions. Indium is used to calibrate instruments [6].

Analytical Method [7]

Preparation of Calibration Curve in Phosphate Buffer pH 6.8

The standard Resiquimod and buffer solution (pH 6.8) was diluted to make the series of drug solutions (2.5-25 µg/ml). Using a UV Spectrophotometer at 249 nm, the graph plotted between concentration and absorbance.

Pre-formulation Study [8-9]

Collection and Selection of Necessary Materials

To prepare SLN, necessary excipients like drugs, lipids, surfactants, and gelling agents are purchased from different sources. All other excipients used were pure and analytical grade.

Physiochemical Properties of Drug and Excipients

Color, odor, physical form, and melting point of physiochemical parameters were determined using appropriate procedures. These choices were made based on the solubility of the safety profiles and the component's approval status.

Physical Interaction of Drug and Excipients Study

A drug interaction study investigated the physical changes of pure drugs in combination with additives. The stability of drugs is affected by the formulation additives that were used for the preparation of SLNs loaded hydrogel. Pure drug and different mixture of drug and additives kept for 6th week. Then, vials were observed for any physical changes in drugs or additives.

Drug Lipid Interaction Studies

A drug interaction study investigated physicochemical changes of pure drugs in combination with additives. The stability of drugs is affected by the formulation additives. Additives are carefully selected and stable, depending on patient compliance, promote constant release, enhance the bioavailability, and protect pharmaceutical actives from degradation. For stability study, these combinations have been stored for a month in combinations (Drug + Lipid).

Samples of drugs were weighed, and it was mixed with different types of lipids/emulsifiers. These mixers are then put into vials and capped with low-density polyethylene (LDPE). Aluminum caps are used for the sealing and closures. These vials are kept under different conditions: 5°C, 40°C/60% RH, and 60°C/75% RH for at least four weeks. This vial was observed every week and recorded if there was any physical change. The interaction study was performed using the DSC method.

Drug Excipients Compatibility Study

FTIR (Fourier Transform Infrared) Spectrophotometry

FTIR (Fourier Transform Infrared Spectrophotometry) determines the physicochemical interaction of different formulation components. This was applicable for the stable, acceptable, and compatible component formulation. For pure drugs and other excipients, IR spectra were compared with a physical mixture of drugs and excipients [10].

Differential Scanning Calorimetry

The Melting Point of Resiquimod and other additives was determined using differential Scanning Calorimetry (DSC). DSC Thermogram is created by utilizing DSC equipment. The sample is placed in aluminum pans and pushed down to seal. The aluminum pan and blank aluminum pan were heated from 30°C to 300°C at a rate of 10°C/min in a nitrogen atmosphere. Indium is used for instrument calibration.

Scanning Electron Microscopy (SEM)

SEM (Scanning Electron Microscopy) is used to examine the morphology of SLN. The samples for Scanning Electron Microscopy were created by lightly dusting nanoparticles onto a double adhesive carbon tape that was adhered to an aluminum stub. The stub was then coated with gold to a thickness of 200 to 500 in an argon environment, using a gold sputter module in a high vacuum evaporator. The samples were then scanned, and photomicrographs were obtained at 27000 times magnification.

Transmission Electron Microscopy (TEM)

TEM (Transmission Electron Microscopy) is used to study the morphology of SLN. Distilled water in a 1:10 ratio was used to dilute SLNs. One drop of the diluted solution was then placed on a carbon-coated copper grid. The excess liquid was collected with filter paper and left to stand for 10 meters. The grid was then dyed with 1% phosphotungstic acid (PTA) and air-dried for 5 m. The sample was subsequently examined under a Transmission Electron Microscope (TEM), and photomicrographs were taken [11].

Formulation of SLNs

Formulation Methodology

Selection of Formulation Technique

SLN formulation strategies include high shear and ultrasonic homogenization, high-pressure homogenization, solvent emulsification, and evaporation, microemulsion-based SLN preparation technologies, and others. The technique was chosen based on the particle size, PDI, and entrapment effectiveness of the nanoparticles obtained using regularly used and

proven to be dependable and powerful techniques using trial batches.

Estimation of Lipid-Lipid Ratio

Different Ratio lipids (1:0, 0:1,1:1,2:1,3:1,4:1,5:1,1:2,1:3,1:4 and 1:5) of Glycerol Monostearate, Glyceryl Behenate(Compritol 888ATO), Glyceryl Trimyristate (Trimyristin) and Dioleyl Trimethyl Ammonium Propane were screened. The lipid ratio has been optimized using the most minor particle size mean.

Drug Lipid Ratio Estimation

For the optimization of the Drug-Lipid Ratio, 5 Fluorouracil was weighed and dispersed into Lipid-Lipid Ratio (1:3) and Different Drug Lipids Ratio of 1:1,1:2,1:3,1:4 and 1:5. A minimum quantity of Ethanol is used for the dissolution of Poloxamer 188 and Sodium Taurocholate for the ratio of 1:1(w/w). The lipid ratio has been optimized by the Smallest mean of Particle Size, PDI, and % EE [12].

Surfactant and Co-Surfactant Ratio Estimation

Poloxamer 188 is used as a surfactant, and Sodium Taurocholate is used as a co-surfactant. It was decreasing the surfactant uses. It has higher HLB values. Emulsification of the selected lipids will be required. For the optimum HLB values, the concentration of both surfactant and co-surfactant was varied from 0.5 to 3 % (w/w). Ethanol is used as a solvent for solubilizing Poloxamer 188 and Sodium Taurocholate.

These are non-toxic, polarity, and water miscibility. 2% w/w ethanol concentration was used for surfactants' depreciation and HLB values with different ratios [13].

HLB (blend) = (% HLB1/100) HLB1 + (% HLB2/100) HLB2 + (% HLB3/100) HLB3

Estimation of Cryoprotectant Concentration

For cryoprotectants, Mannitol, Maltose, and Fructose are used. It may decrease the osmotic activity of water and form the crystallization. It favors the glassy state of the frozen sample. These may prevent the aggregation properties. The concentration (5 % w/v) of mannitol, Maltose, and Fructose was used, and the parameters were optimized for stabilization.

Preparation of SLNs

SLNs were created using the Wang approach, with some alterations. In summary, the organic phase was made by changing the ratios of Resiquimod, Glycerol Monostearate, Glyceryl Behenate (Compritol 888ATO), Glyceryl Trimyristate (Trimyristin), and Dioleyl Trimethyl Ammonium Propane in ethyl alcohol (10 mL, 93 percent V/V). Variable amounts of Poloxamer 188 were dissolved in water to make the aqueous phase (50 mL). Using a high-shear homogenizer, the organic phase was dumped into the aqueous phase at a consistent rate during homogenization (8000 rpm, 15 min). The suspension was immediately transferred to 50 mL of cold distilled water and maintained in an ice bath for 1 hour while stirring at 800 rpm. Two formulae were created as controls: one without and the other without Poloxamer 188. The SLN was centrifuged for 1 hour at 50,000 rpm. The residue was suspended in mannitol solution (10 percent w/v) after being rinsed twice with double-distilled water. The suspension was lyophilized and stored at a constant temperature of 40C for later usage.

Evaluation of SLNs [14]

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to determine evaluation criteria such as particle size, PDI, Zeta Potential, percentage encapsulation efficiency, and percentage drug loading capacity for optimized formulation. In vitro, drug release and kinetics were also investigated.

Particle Size, PDI, Zeta Potential

The particle size and zeta potential were determined using the built-in dynamic light scattering, DLS, and Laser Doppler Electrophoresis. The materials were placed in 'folded capillary cells,' and their size, PDI, and zeta-potential values were measured. Lyophilized SLNs were redispersed with distilled water. These samples were placed in a cuvette and analyzed at 90°C.

% Entrapment Efficiency and % Drug Loading Capacity

The percentage of entrapment efficiency was calculated by measuring the amount of free drug in the supernatant at 265 nm after centrifugation

of a known amount of nanoparticulate dispersion at 10000 RPM with a freeze centrifuge (BL135 R).

Percentage (%) Yield

30mg of Resiquimod loaded SLNs were transferred into 30 ml of volumetric flasks and 15 ml of dimethyl Sulphoxide (DMSO) and then sonicated for 30 to 35 minutes. DMSO adjusted the volume by stirring for 15 minutes. After that, this was used for HPLC analysis, and the AUC curve was plotted. AUC is used to measure the total drug content. The following equations are used:

***In-vitro* Drug Diffusion Profiles**

This investigation was conducted using the bag diffusion method. This bag membrane should hold the nanoparticles while allowing free medicines to enter the dissolving fluid at a molecular weight cutoff 15000. Double distilled water was used to soak this bag. 3ml of pH 6.8 PBS was used to disperse 200mg of lyophilization SLNs. The solution was then placed in the membrane bag and secured with clips at both ends. The bag is placed in a conical flask containing 60ml of PBS pH 6.8. The conical flask was placed on a thermostatic magnetic stirrer at 380 degrees Celsius and 100 revolutions per minute. At regular intervals, 2 to 3 ml of media were removed and replaced with new medium volumes. Filtration at 0.22 µm is performed and evaluated using UV Spectrophotometry [15].

RESULTS AND DISCUSSION

FTIR Study:

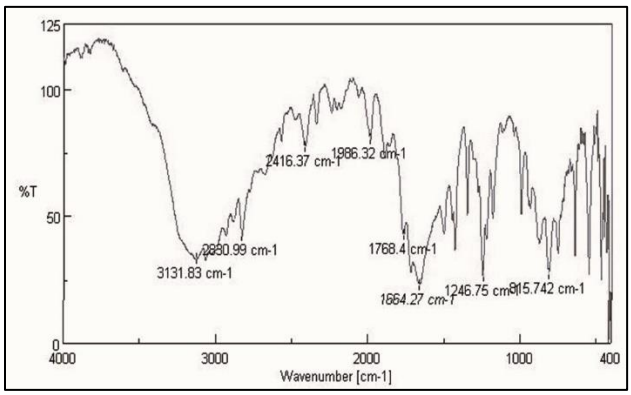

Figure 1 FTIR of Resiquimod (Drug sample)

The sample is confirmed to be Resiquimod according to its conformity to the criteria. Fourier-transform infrared (FT-IR) spectroscopy was utilized to identify the various functional groups in the powder medication sample, which were then compared to Resiquimod's standard spectra for confirmation. Figure 32 depicts the observed and reported IR spectra of Resiquimod.

DSC Study

Resiquimod's thermal behavior was determined using Differential Scanning Calorimetry (DSC). It was done at temperatures ranging from 30°C to 300°C. The DSC thermogram of Resiquimod peaks at 283°C, confirming the clean drug sample.

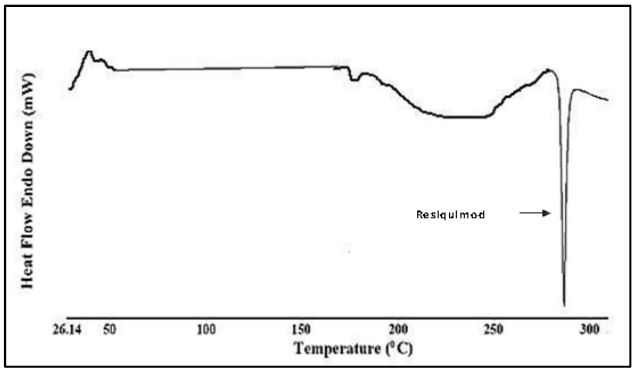

Figure 2 DSC Thermogram of Resiquimod

Development of Analytical Method

Analytical Method for Resiquimod by UV Spectroscopy

The standard Resiquimod and buffer solution (pH 6.8) was diluted to make the series of drug solutions (2.5-25 µg/ml). Using a UV Spectrophotometer at 244 nm, the graph plotted between concentration and absorbance. The regression coefficient was found to be 0.999.

Pre-Formulation Study

Physicochemical Characterization:

The drug samples of Resiquimod were procured as a gift sample from Drugs India Pvt Hyderabad. This sample has been characterized for its physicochemical properties.

Physical Interaction of Drug and Excipients Study:

No physical changes (colors) are observed in Resiquimod & Excipients compared with controls.

Drug Lipid Interaction Studies

The physical mixture of drugs and Lipid were examined. This should be kept in different conditions for six weeks. Observation of these

Table 1 Physical Observation of Resiquimod and Excipients

API and Excipients	Ratio	2nd week	4th week	6th week
Resiquimod	-	NC	NC	NC
Resiquimod + Glycerol Monostearate	1:1	NC	NC	NC
Resiquimod + Dioleyl Trimethyl Ammonium Propane	1:1	NC	NC	NC
Resiquimod + Glyceryl Behenate (Compritol 888ATO)	1:1	NC	NC	NC
Resiquimod + Glyceryl Trimyristate(Trimyristin)	1:1	NC	NC	NC
Resiquimod + Chitosan	1:1	NC	NC	NC

Table 2 DSC Studies of Pure Drugs and Lipid

S. No	DSC Studies of Pure APIs/Physical Mixtures	Experimental Melting Point
1	Resiquimod	292^{0}C
2	Glycerol Monostearate	57^{0}C
3	Glyceryl Behenate (Compritol 888ATO)	70^{0}C
4	Glyceryl Trimyristate (Trimyristin)	56^{0}C
5	Dioleyl Trimethyl Ammonium Propane	38^{0}C

vials was done every week. There seemed to be no change in any physical state compared with control. An interaction study was performed using a DSC analyzer. The Pure drug Resiquimod and various Lipids were subjected to DSC evaluation. The thermal behavior of Resiquimod and different Lipids were studied. These studies show the compatibility between Resiquimod and Lipids.

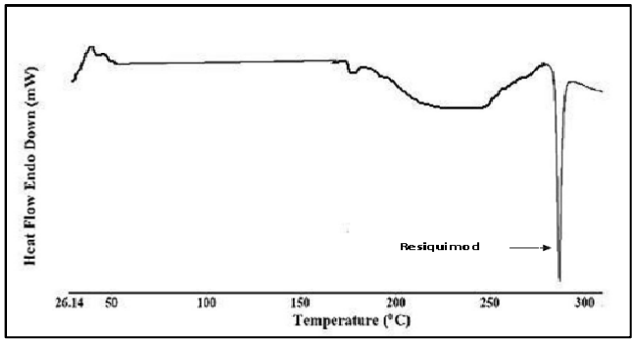

Figure 3 DSC Thermogram of Pure Drug Resiquimod

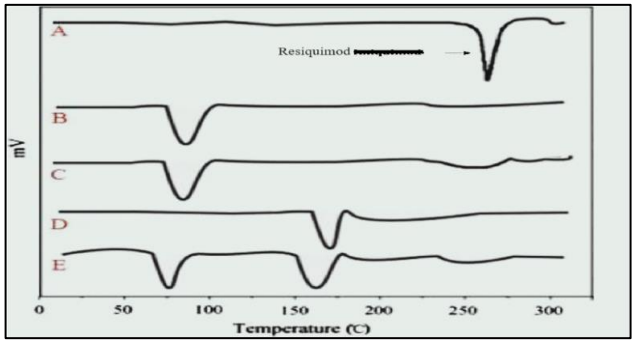

Figure 4 DSC study of Resiquimod and Physical Mixture of Drug and Various Lipids

Drug Excipients Compatibility Study

FTIR study of Drug and Excipients

The FTIR spectrum of Resiquimod was taken using the KBr disc method. An FTIR spectrum shows that the absorption peak was at 3450cm^{-1} and 475cm^{-1}. The peaks are visible and authenticated for the drug samples. The IR spectra of Resiquimod, Lipid, and Polymers. Resiquimod and other excipients' physical mixtures show unchanged peaks of Resiquimod, indicating no chemical interaction between Resiquimod, Lipid, and Excipient mixtures.

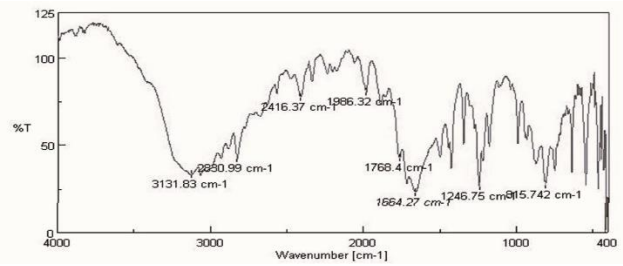

Figure 5 FTIR of Resiquimod pure drug

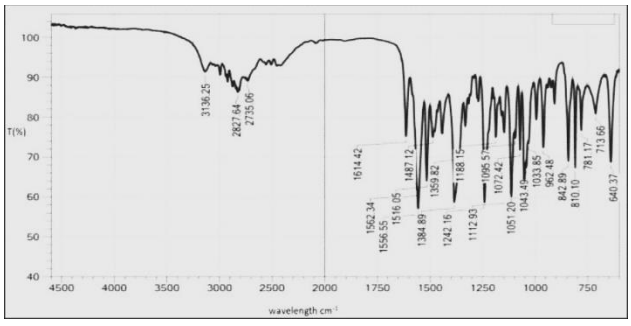

Figure 6 Glyceryl Trimyristate FTIR

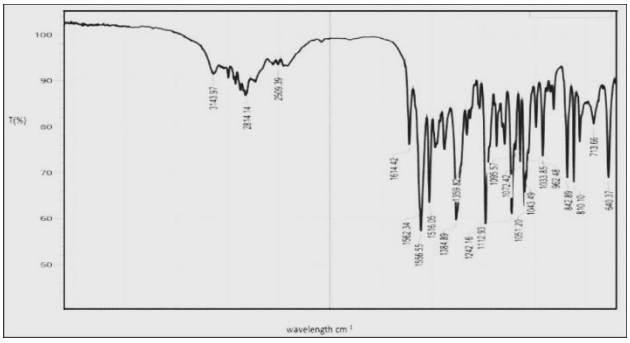

Figure 7 Dioleyl Trimethyl ammonium Propane FTIR

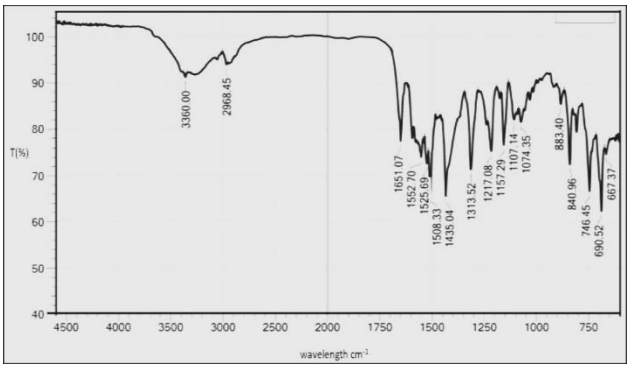

Figure 8 Polycaprolactone FTIR

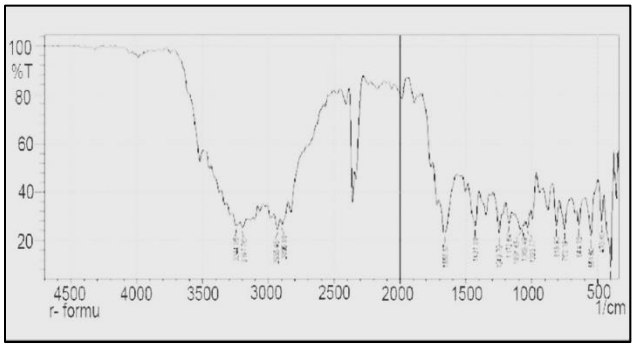

Figure 9 Drug, Lipid, and Polymer Mixtures FTIR

FTIR study of Resiquimod and Excipients shows no interaction between the drug and excipients.

Differential Scanning Calorimetry (DSC)

Using Differential Scanning Calorimetry (DSC), the thermal behavior of Resiquimod and other excipients was observed. Between 30°C and 300°C was the temperature range used. The pure resin, Lipid, and polymer DSC curves were displayed in figures 43 to 50. In support of the lipid drug mixer, no firm endothermic peaks were found comparable to the remarkable liquefying point of Resiquimod.

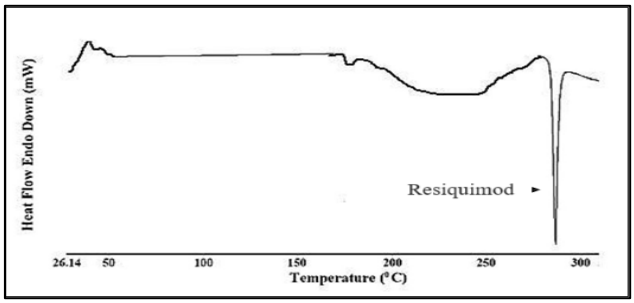

Figure 10 Pure Drug Resiquimod

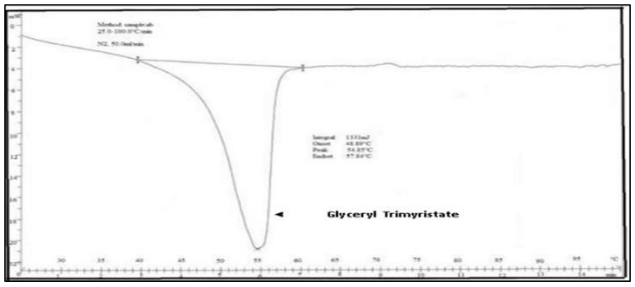

Figure 11 Glyceryl Trimyristate DSC

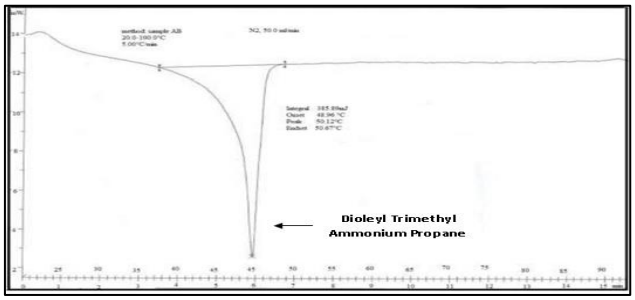

Figure 12 DSC of Dioleyl Trimethyl Ammonium Propane

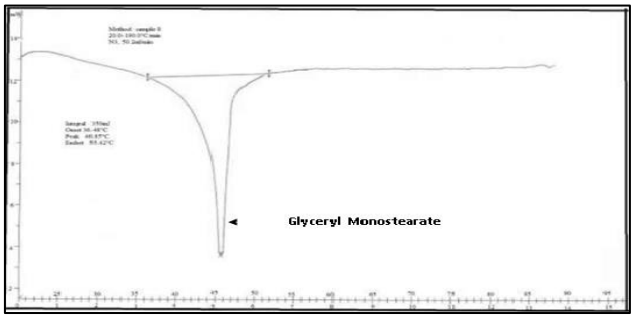

Figure 13 Glyceryl Monostearate DSC

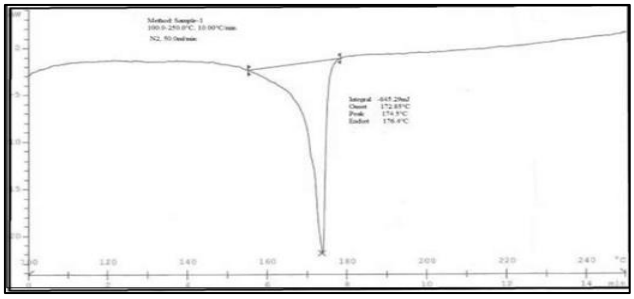

Figure 14 Glyceryl Behenate DSC

Table 3 FT-IR spectrums of drug and excipients

Characteristic Bonds	Resiquimod	Glyceryl Trimyristate	Dioleyl trimethyl ammonium propane	Polycaprolactone	Drug, Lipid, and Polymer Mixtures
N-H	3450	3136.25	3143	3360	3245
CH-Stretch	2852	2827.64	2814	2968.45	1661
C=O	1385	1614.42	1614	1651.07	1080
C=C	1090	1051.20	1051	1552.70	3245

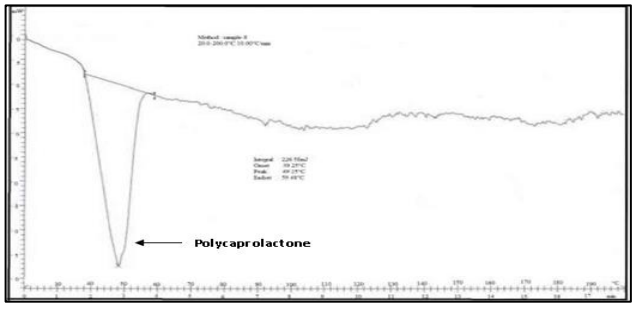

Figure 15 Polycaprolactone DSC

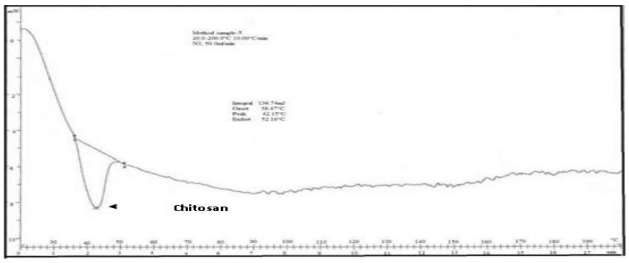

Figure 16 DSC of Chitosan

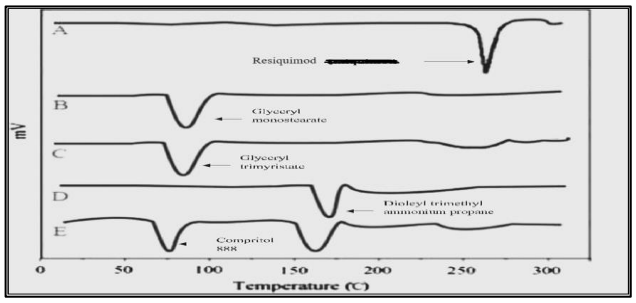

Figure 17 DSC Thermogram of Resiquimod and Physical Mixture of Drug and Lipids

Scanning Electron Microscopy

The identification of Resiquimod-loaded SLNs (RSB 3) was done using SEM. SLN will soon be identifiable as a smooth, round shape with no cracks. The image demonstrates that the solvent has been completely removed from the produced SLN and that the formulation process was adequate based on the 200 nm particle size.

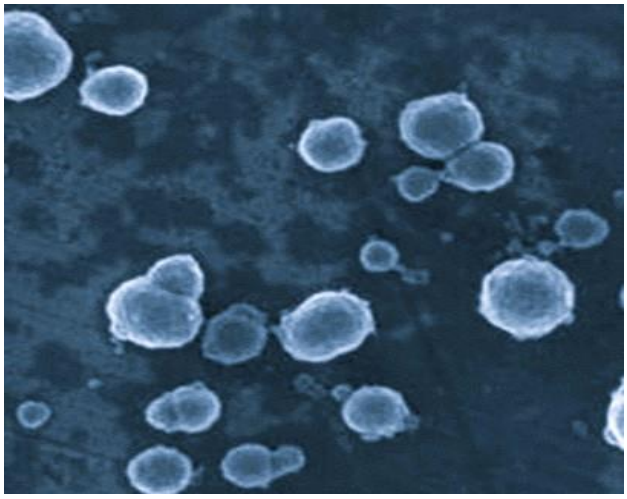

Figure 18 Images from Scanning Electron Microscopy of SLNs (RSB 3)

Development of SLNs

Selection of Formulation Technique

Particle size, PDI, and entrapment efficiency of SLN formulations were compared using various approaches (as trials). Table 4 displays the acquired results.

Cold high-pressure homogenization was shown to produce smaller particles with improved entrapment effectiveness.The difference in particle size and entrapment efficiency achieved with the two approaches was statistically significant (0.01). However, the difference in PDI was not. The findings were consistent with those reported in the literature. Because the high-pressure homogenization technique does not employ organic solvent, there is no risk of leftover organic solvent. With the solvent evaporation process, organic solvents have a significant toxicological drawback. Another advantage of high-pressure homogenization is that it can readily be scaled up, allowing for laboratory, pilot,

Table 4 Selection of formulation technique

S. No	Batch No.	Technique	Particles Size*	PDI*	% EE*
1	ISLN01	Solvent evaporation	622.5 ± 6.4	0.849 ± 0.13	31.44 ± 2.7
2	ISLN02	Cold High- Pressure Homogenization	369.5 ± 5.1	0.459 ± 0.12	65.73 ± 2.2

PDI: Polydispersity Index, *Data expressed as mean ± SD (n = 3)

Table 5 Estimation of Lipid_Lipid ratio based on Particle Size of SLNs

Formulation Code	Glycerol Monostearate (%)	Glyceryl Behenate (Compritol 888ATO) (%)	Glyceryl Trimyristate (Trimyristin) (%)	Dioleyl Trimethyl Ammonium Propane (%)	Particles Size(nm)
IMLL - 1	1	1	--	--	524
IMLL - 2	--	--	1	1	424
IMLL - 3	1	2	--	--	322
IMLL – 4	--	--	1	2	224
IMLL - 5	2	1	--	--	368
IMLL - 6	--	--	2	1	586

or large-scale manufacturing. As a result, more research was done using the high-pressure homogenization process. This was sufficient to produce 50ml of quantity. Lyophilization has been done at a temperature of -60^{0}C at 0.020mbar vacuum.

Lipid-Lipid Ratio Estimation

This trial experiment was performed to determine the factors that affect and are necessary for formulating Resiquimod SLNs. Particle size determination is done in these experiments by the combination of Glycerol Monostearate, Glyceryl Behenate (Compritol 888ATO), Glyceryl Trimyristate (Trimyristin) and Dioleyl Trimethyl Ammonium Propane. By using higher partition coefficients, lipids were chosen. By combining lipids in different ratios, SLNs were formed. Particle size has been measured. A combination of Glycerol Monostearate and Glyceryl Behenate (Compritol 888ATO) has shown the smallest mean particles. These may be selected for encapsulation of Resiquimod.

Drug-Lipid Ratio Estimation

The Drug-Lipid Different Ratio was used for the Particle Size and % EE Effects study. It has encapsulated the SLN's efficiency. Results show that if the concentration of lipids increases, it may increase the encapsulation efficiency up to 1:6. After the ratio of 1:6, no significant increases in particle size and % EE were observed. Microemulsion droplets are surrounded by surfactant, and co-surfactant is surrounded by interfacial film. It may affect the particle size and % EE. This concentration has been increased from 1:1 to 1:6. These may also increase the particle sizes. There was no significant increase in %EE. The drug: Lipid was increased up to 1:6. It may also increase the drug lipid ratio. These may not increase the encapsulation efficiency. Due to this saturation of the lipid matrix, a higher loading level occurs.

Surfactant and Co-Surfactant Ratio Estimation

Screening of Poloxamer 188 and Sodium Taurocholate with surfactant and co-surfactant may keep the concentration constant from 0.5 to 3% of co-solvents. SLNs were prepared using different surfactant concentrations for particle size, Zeta potential, and PDI values.

A concentration of Surfactant and Co surfactant is used for the stabilization. These may be required to stabilize the lipids. Formulation RSS-2 shows the lowest particle size, polydispersity index (PDI), and Zeta Potential. All the formulations had a zeta potential of less than 54mV.

Estimation of Cryoprotectant Concentration

Fructose, Maltose, Mannitol, and Sorbitol were used for the Cryoprotectant investigation in the Lyophilization process for aggregation of SLNs. Redispersion occurred in distilled water to protect SLNs. Lyophilization was done with ultrasound for

Table 6 Estimation of Drug-Lipid Ratio

Formulation code	Drugs Lipid Ratio (%w/w)	PDI	Particle size± S D (n=3)	% EE ± SD
IMDL - 1	1:1	0.666	474 ± 2.84	72.47 ± 5.47
IMDL - 2	1:2	0.577	379± 3.73	94.48 ± 6.47
IMDL - 3	1:3	0.688	779± 4.78	88.47± 6.47
IMDL - 4	1:4	0.419	444± 2.84	77.47± 6.47
IMDL - 5	1:5	0.742	422± 3.95	96.18± 6.48
IMDL - 6	1:6	0.851	481± 4.74	73.57± 4.58

Table 7 Surfactant and Co-Surfactant Ratio Estimation

Formulation code	Poloxamer 188 (%)	Sodium Taurocholate (%)	Zeta Potential (mV)	Avg. Particle size (nm)	PDI
IMSS - 1	0.5	0.5	-39	347	0.898
IMSS - 2	1	0.5	-40	329	0.788
IMSS - 3	0.5	1	-54	350	0.586
IMSS - 4	1.5	1	-39	380	0.873
IMSS - 5	1	1.5	-54	390	0.596
IMSS - 6	2	1.5	-44	410	0.481

Table 8 Cryoprotectant based on Mean Particle Size and PDI

S. No	Cryoprotectant	Mean Particle size (nm)	Particle size distribution (nm ± SD)	Intensity (%)	PDI
1	Fructose	634.8	754.9 ±74.88	67.49	0.488
2	Maltose	519.15	669.7 ± 65.35	97.57	0.646
3	Mannitol	369.5	451.8 ± 74.64	112.4	0.373
4	Sorbitol	398.5	609.5 ± 98.7	87.5	0.455
5	without Cryoprotectant	569.1	193.4 ± 47.62	74.5	0.356

mean particle size analysis. The mean particle size of lyophilized formulations is ten times greater than those without cryoprotectants.

The particle size is more significant in Fructose than in any other substance without Cryoprotectant. Size has been increased from 369.5 to 634.8 nm. The most effective Cryoprotectant was tested in mean particle size and used for further study.

Formulations of SLNs

Different types of lipid proportions were used for the preparation of SLNs. This may act as a biological barrier through diffusion for the release of drugs. The freeze-drying process is used for the SLN preparation. In which mannitol was used as a stabilizer. A cold, high-pressure homogenization technique is used for the preparation of SLNs. Drugs and different concentration lipids were used to prepare SLNs. Surfactants that were used for the preparations of SLNs were Poloxamer 188 and Sodium Taurocholate. This was sufficient to produce 50ml of quantity. Lyophilization has been done at a temperature of -60^{0}C at 0.020mbar vacuum.

Evaluation of Resiquimod Loaded SLNs

Particle Size Analysis, Zeta Potential and PDI

Zeta potential, PDI, and particle size were evaluated for the prepared formulation.

Normal ranges for the particle sizes were formed, ranging from 209.6±4.57 to 502.1±8.9 nm.

These particles' nanometre range was acceptable. For all SLN formulations, the PDI ratio for the mass of the provided samples was less than 0.51.

Zeta potential for pharmaceutical stability has values of +ve and -ve. The ideal zeta potential is between -35 and -45 mV. The zeta potential of formulation RSB 3 is near (-41mV).

Table 9 Formulation of Solid Lipid Nanoparticles

Formulation code	Resiquimod (% w/w)	Lipids (% w/w)			
		Glycerol Monostearate	Glyceryl Behenate (Compritol 888ATO)	Glyceryl Trimyristate	Dioleyl Trimethyl Ammonium Propane
RSB 1	1	3	1	--	--
RSB 2	1	--	--	3	1
RSB 3	1	1	3	--	--
RSB 4	1	--	--	1	3
RSB 5	1	3	1	--	--
RSB 6	1	--	--	1	3

Surfactant: Poloxamer 188, Sodium Taurocholate Cryoprotectant: Mannitol

Table 10 Evaluation of Resiquimod-loaded SLN Formulations

Formulation code	Mean Particle Size (nm) ± SD	Zeta Potential (mV) ± SD	PDI± SD	% Encapsulation Efficiency ± SD	Loading Capacity (%) ± SD	% yield ± SD
RSB 1	208.7±5.58	-36±3.0	0.16±0.02	72±0.76	38±2.3	78±2.57
RSB 2	322.5±8.49	-36±1.1	0.27±0.05	65±0.68	18±6.4	64±3.68
RSB 3	244.2±9.46	-42±1.3	0.38±0.04	65±0.78	47±2.5	72±1.46
RSB 4	422.7±8.86	-43±2.4	0.15±0.08	66±0.65	14±4.6	72±3.22
RSB 5	302.9±7.49	-45±2.6	0.52±0.03	61±0.37	52±3.1	67±3.64
RSB 6	503.2±9.48	-46±2.0	0.36±0.04	73±0.48	47±7.3	68±7.42

Table 11 *In vitro* % CDR of Resiquimod loaded SLN formulations

Formulation Code	% Cumulative Drug Release of Resiquimod SLNs					
	RSB-1	RSB-2	RSB-3	RSB-4	RSB-5	RSB-6
2	8.66±4.8	12.48±5.7	11.37±6.7	13.84±0.4	9.68±4.4	22.64±2.4
4	11.51±3.9	19.64±4.4	19.57±5.3	16.46±4.4	18.57±7.4	23.48±1.2
8	31.49±7.5	32.14±6.5	32.84±7.3	31.44±5.2	34.18±6.3	42.47±3.7
12	41.16±3.6	45.24±7.7	48.48±4.6	46.34±2.4	45.17±3.5	47.18±4.7
16	53.21±2.5	56.27±5.6	58.47±3.3	55.18±5.7	54.73±1.8	55.57±4.7
20	64.21±5.8	67.47±3.5	65.54±3.6	63.05±2.4	71.11±6.4	76.18±2.8
24	81.49±3.6	88.48±5.5	88.73±3.4	85.18±8.8	83.16±7.6	79.18±4.7

In Vitro Release Studies

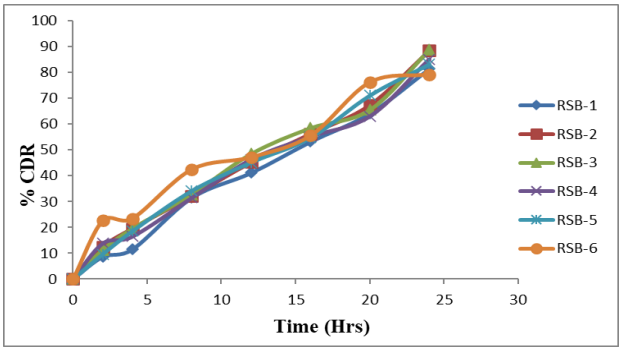

Figure 19 *In vitro* % CDR of Resiquimod loaded SLN formulations

At pH 6.9, the in vitro drug release of SLNs loaded with Resiquimod was assessed. Bag diffusion was used to accomplish this. Research indicates that the solubility of the medication in the buffer was the same. %) For formulations of SLNs loaded with Resiquimod, the cumulative drug release ranged from 78.19±4.6 to 89.74±3.5 during 24 hours. The decreased proportion of medication encapsulated in SLNs could be attributed to degradation.

RSB 3, which contains 1% w/w Glycerol Monostearate and 3% w/w Glyceryl Behenate as Lipid for SLNs preparation, achieves improved results for Mean Particle Size (244.2±9.46), Zeta Potential (-42±1.3), PDI (0.38±0.04),% EE

(65±0.78), and Loading Capacity (47±2.5). In vitro drug release investigation employing the Bags Diffusion method, RSB 3 displays the most significant % cumulative drug release (88.73±3.4) after 24 hours. From the data from the above results, RSB 3 was chosen as the optimal formulation for further investigation.

CONCLUSION

Based on the current outcomes, it is possible to infer that Resiquimod's solid lipid nanoparticles were successfully developed and optimized. Formulated SLNs can be employed for topical drug administration in the form of hydrogels to treat skin disorders such as actinic keratoses. Different concentrations of lipids and surfactants were used to design SLNs. Mannitol was showing much effectiveness as a Cryoprotectant. Drug-excipient Compatibility studies of drug, Lipid, polymer, and their combinations using FTIR and DSC confirm no chemical interaction between drug and excipient. Scanning Electron Microscopy (SEM) pictures showed that the nanoparticles in SLN droplets were intact, non-aggregated, and approximately spherical. Resiquimod-loaded SLNs have particle sizes ranging from 208 to 503 nm. This may aid in prolonging the circulation period of SLNs in the blood. The PDI was found to be less than 0.6. The negative charge of zeta potential in SLN formulations demonstrated adequate stability. When Compared To All Formulations, RSB3 Formulation Is Good; It Shows High Drug Release The results are encouraging and represent a novel contribution of Resiquimod SLNs in Topical Drug Delivery.

ACKNOWLEDGEMENT

I would like to thanks to Principal and Guide of Vasavi Institute of Pharmaceutical Sciences, Vasavi Nagar, Peddapalli (V), Sidhout (M), YSR Kadapa-516 247, Andhra Pradesh, India

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Funding Support

The authors declare that they have no funding for this study.

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