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

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Abstract



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Nanomaterials are the ideal carriers for biological applications because of their high bioavailability and biocompatibility. The primary focus of many researchers employing novel techniques has been fresh discoveries. Many methods have been used to create nanoparticles, which have potential applications in various illnesses. Solid Lipid Nanoparticle (SLN) drug delivery system has outstanding results in treating chronic diseases. Actinic keratoses (AK), known as sun keratoses or senile keratoses, are benign intraepithelial neoplasms caused by abnormal keratinocyte proliferation. Hence, in the present research, Imiquimod SLNs were fabricated using various concentrations of lipids, surfactants, and cryoprotectants. Cold high-pressure homogenization was shown to produce smaller particles with improved entrapment effectiveness. The Particle size ranges from (209.6±4.57 to 502.1±8.9 nm). Formulation IMB 3 which contain 1% w/w Glycerol Monostearate and 3% w/w Glyceryl Behenate as Lipid for SLNs preparation shows better results for Mean particle size (245.1±8.45), Zeta Potential (-41±1.2), PDI (0.39±0.03), % EE (64±0.79) and Loading Capacity (48±2.6) with % cumulative drug release of 89.74±3.5 after 24 hours.

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INTRODUCTION

Actinic Keratoses (AKs) are common skin lesions that signal an increased risk of developing Squamous cell carcinoma (SCC) and other skin cancers [1]. Inflammation and oxidative stress are the two main factors that cause AK to occur. Multiple lesions are common in AK patients, indicating actinic damage to the "cancerization field." This theory implies that the skin around the AK area, which appears normal, already has genetic alterations linked to carcinogenesis [2].

The inability of cells to manage oxidative stress seems to be linked to an increased risk of carcinogenesis. Mutations that induce hypo functioning of the glutathione S-transferase, which regulates intracellular oxidation, have been linked to an increased risk of AK [3]. Hence, in the present research, Solid Lipid Nanoparticles of Imiquimod were aimed at improving the bioavailability of the drug. Different approaches have been used to develop nanoparticles, which may be used in various diseases. However, SLNs have excellent results in chronic diseases [4]. SLNs were developed by using different concentrations of Lipid (Glycerol Monostearate, Glyceryl Behenate-Compritol 888 ATO, Glyceryl Trimyristate-Trimyrustinand Diolelyl Trimethyl Ammonium Propane) and different surfactants (Poloxamer 188-Pluronic F68, Sodium Taurocholate and Polysorbate80-Tween 80) and

properties, solubility, drug and excipient interactions, melting point, etc. [5].

Formulation Methodology:

The formulation process was selected based on the particle size, PDI, and entrapment efficacy of the nanoparticles produced using widely used, and reportedly effective and reliable, methodologies with trial batches [6]. Lipid-Lipid ratio, Drug-Lipid ratio, surfactant-co-surfactant ratio, and cryoprotectant concentration were estimated before formulation.

Preparation of SLNs:

The organic phase was made by changing the ratios of Imiquimod, Glycerol Monostearate, Glyceryl Behenate, Glyceryl Trimyristate, and Diolelyl Trimethyl Ammonium Propane in ethyl alcohol (10 mL, 93 percent V/V). Variable amounts of Poloxamer 188 were dissolved in

Table 1 Formulation of Solid Lipid Nanoparticles

Formulation code	Imiquimod (%w/w)	Lipids (%w/w)			
		Glycerol Monostearate	Glyceryl Behenate	Glyceryl Trimyristate	Diolelyl Trimethyl Ammonium Propane
IMB 1	1	3	1	--	--
IMB 2	1	--	--	3	1
IMB 3	1	1	3	--	--
IMB 4	1	--	--	1	3
IMB 5	1	3	1	--	--
IMB 6	1	--	--	1	3

Surfactant: Poloxamer188, Sodium Taurocholate; Cryoprotectant: Mannitol

evaluated for particle size analysis, Polydispersity Index(PDI), Zeta potential, entrapment efficiency, percentage yield of prepared SLNs.

MATERIALS

The drug Imiquimod was purchased from Drugs India, Hyderabad. Glycerol Monostearate, Glyceryl Behenate, and Glyceryl Trimyristate were obtained from Fischer Scientific, Mumbai. Poloxamer188, Polysorbate 20, Polysorbate 80 from Himedia. Fructose, Maltose, Mannitol, and Sorbitol from SD Fine Chemicals, Mumbai. All the other chemicals and reagents used were of analytical grade.

METHODS

Pre-formulation Studies:

The physical and chemical properties of drugs and excipients were evaluated for organoleptic

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) [7]. 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 with Poloxamer 188 and the other without Poloxamer 188. The SLN was centrifuged for 1 hour at 50,000 rpm [Table 1]. 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 SLAs

Particle Size, PDI, Zeta Potential:

The zeta potential, PDI, and average particle size of solid lipid nanoparticles were evaluated using Zetasizer. Particle size and zeta potential were ascertained using built-in dynamic light scattering, DLS, and Laser Doppler Electrophoresis. The components were put within "folded capillary cells," and measurements of the materials' size, PDI, and zeta potential were noted. SLNs that had been lyophilized were re dispersed and examined using distilled water [8].

% Entrapment Efficiency and % Drug Loading Capacity:

A frozen centrifuge (BL135 R) was used to centrifuge a known amount of nanoparticulate dispersion at 10,000 RPM for 15 minutes. The amount of free drug detected spectrophotometrically at 265 nm in the supernatant was used to calculate the % entrapment efficiency [9].

In-vitro Drug Diffusion Profiles:

The bag diffusion method was used to conduct this research. With a threshold of 15,000 molecular weights, this bag membrane should hold onto the nanoparticle and permit the free medicines to reach the dissolving fluid [10]. This bag has been soaked in twice-distilled water. Before usage, it was left in this for 12 to 15 hours. Two hundred milligrams of lyophilization SLNs were dissolved in 3 milliliters of pH 6.8 PBS. After that, the two ends of this solution were secured with clips before being put into the membrane bag [11]. The bag is added to a conical flask with 60ml of PBS pH 6.8. Next, a thermostatic magnetic stirrer set to 38°C and 100RPM was used to secure the conical flask. Two to three milliliters of media were removed regularly and replaced with brand-new medium volumes. The filtration process uses 0.22 μm , and the UV Spectrophotometric technique is used for analysis [12].

Scanning Electron Microscopy (SEM):

The SEM samples were created by lightly scattering nanoparticles onto a double-sided carbon tape that was adhered to an aluminum stub. The stub was subsequently coated with gold using a gold sputter module in a high vacuum evaporator in an argon atmosphere to a thickness of 200 to 500. Following a scan of the samples, photomicrographs at a magnification of 27000x were taken [13].

Transmission Electron Microscopy (TEM):

The SLNs were diluted using distilled water at a ratio of 1:10. After that, one drop of the diluted solution was applied to a copper grid coated with carbon. After using filter paper to remove the extra liquid, the area was left to stand for ten meters. After applying a 1% phosphotungstic acid (PTA) stain, the grid was left to air dry for five meters. After that, photomicrographs and a Transmission Electron Microscope (TEM) image of the material were obtained [14].

RESULTS AND DISCUSSION

Pre-formulation Studies:

FTIR Study

Based on the observations of the specified criteria, it is established that the sample contains the medicine Imiquimod. The different functional groups found in the powder drug sample were identified using Fourier-transform infrared (FT-IR) spectroscopy; these findings were then confirmed by comparing them to the standard spectra of Imiquimod [Figure 1].

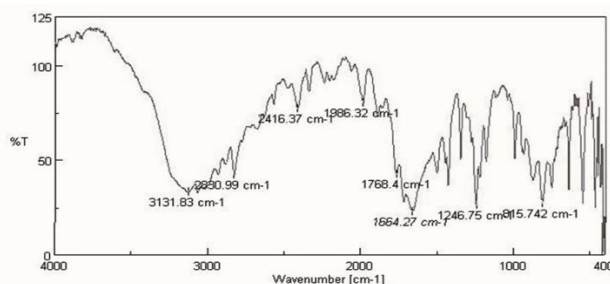


Figure 1 FTIR of Imiquimod (Drug sample)

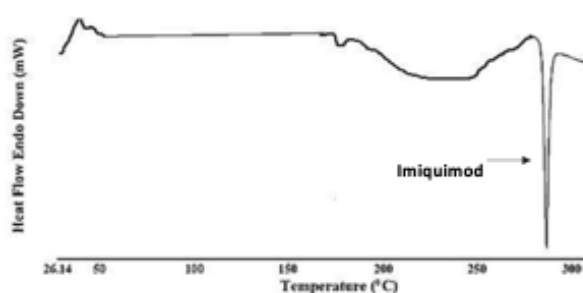


Figure 2 DSC of Imiquimod (Drug sample)

DSC Study

Differential Scanning Calorimetry (DSC) was used to ascertain Imiquimod's thermal behavior [Figure 2]. Temperatures between 30°C and 300°C were used. Imiquimod's DSC thermogram

Table 2 Physical Observation for Imiquimod and Excipients

API and Excipients	Ratio	2ndweek	4thweek	6thweek
Imiquimod	-	NC	NC	NC
Imiquimod + Glycerol Monostearate	1:1	NC	NC	NC
Imiquimod + Dioleyl Trimethyl Ammonium Propane	1:1	NC	NC	NC
Imiquimod + Glyceryl Behenate (Compritol888ATO)	1:1	NC	NC	NC
Imiquimod + Glyceryl Trimyrystate(Trimyristin)	1:1	NC	NC	NC
Imiquimod+Chitosan	1:1	NC	NC	NC

*NC=Nochange

confirms the drug sample's purity by peaking at 283°C.

Physical Interaction of Drug and Excipients Study

Compared with controls, no physical changes (colours) are observed in Imiquimod & Excipients [Table 2].

Drug Lipid Interaction Studies

The physical mixture of drugs and Lipid were examined. This should be kept in different conditions for six weeks. There seemed to be no change in any physical state compared with control [Figure 3]. An interaction study was performed using a DSC analyzer. The Pure drug Imiquimod and various Lipids were subjected to DSC evaluation. The thermal behavior of Imiquimod and different Lipids were studied. These studies show the compatibility between Imiquimod and Lipids.

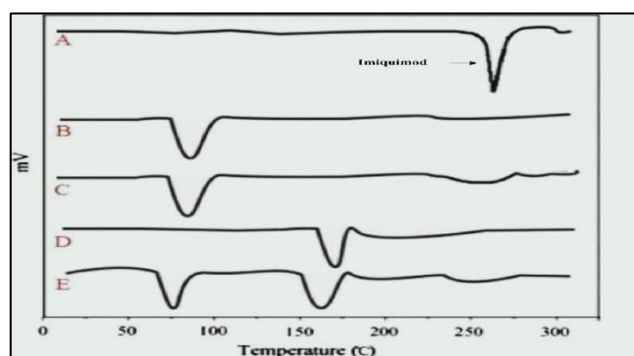


Figure 3 DSC study of Imiquimod and Physical Mixture of Drug and Various Lipids

FTIR study of Drug and Excipients

The FTIR spectrum of Imiquimod 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 Imiquimod, Lipid, and Polymers [Figure 4]. Imiquimod and other excipients' physical mixtures show unchanged peaks, indicating no chemical interaction between Imiquimod, Lipid, and Excipient mixtures.

Selection of Formulation Technique

SLN formulations were prepared by different techniques (as trials) and were compared with respect to particle size, PDI and entrapment efficiency [Table 3].

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, or large-scale manufacturing. As a result, more research was done using the high-pressure

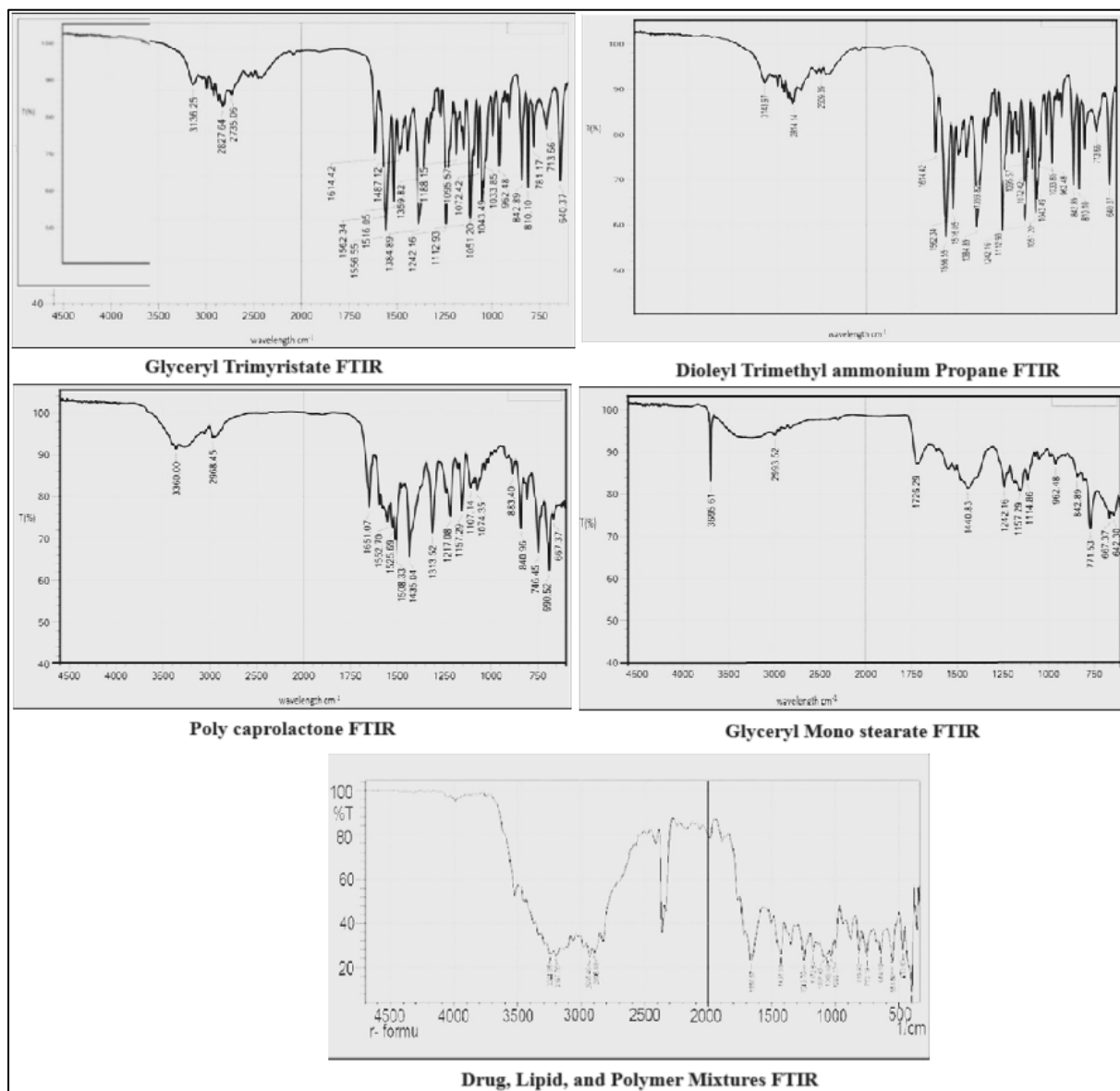


Figure 4 FTIR Spectrums of Polymer mixtures

homogenization process. This was sufficient to produce 50ml of quantity. Lyophilization has been done at a temperature of -60°C at 0.020mbar vacuum.

Lipid-Lipid Ratio Estimation

This trial experiment was performed to determine the factors that affect and are necessary to formulate Imiquimod-loaded SLNs. These experiments do particle size determination by the combination with Glycerol Monostearate, Glyceryl Behenate (Compritol 888ATO), Glyceryl Trimyristate (Trimyristin) and Diolely Trimethyl Ammonium Propane. Using higher partition coefficients lipids were chosen by combining lipids in different ratios, and SLNs were formed

[Table 4]. 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 Imiquimod.

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 [Table 5]. Results show that if the concentration of lipids increases, the encapsulation efficiency may increase by up to 1:6. After the ratio of 1:6, no significant increases in particle size and % EE were observed.

Table 3 Selection of formulation technique

Batch No.	Technique	Particles Size*	PDI*	%EE*
ISLN01	Solvent evaporation	623.6±6.5	0.850±0.14	32.45±2.8
ISLN02	ColdHigh- Pressure Homogenization	370.6±5.2	0.460±0.11	66.74±2.1

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

Table 4 Estimation of Lipid Lipid ratio based on Particle Size of SLNs

Formulation Code	Glycerol Monostearate (%)	Glyceryl Behenate (%)	Glyceryl Trimyrystate (%)	Dioley Trimethyl Ammonium Propane (%)	Particles Size (nm)
IMLL - 1	1	1	--	--	525
IMLL - 2	--	--	1	1	425
IMLL - 3	1	2	--	--	321
IMLL - 4	--	--	1	2	225
IMLL - 5	2	1	--	--	369
IMLL - 6	--	--	2	1	587

Table 5 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	0.667	475±2.85	71.48±5.48
IMDL - 2	1:2	0.578	380±3.74	95.49±6.48
IMDL - 3	1:3	0.689	780±4.79	89.48±6.48
IMDL - 4	1:4	0.420	445±2.85	78.48±6.48
IMDL - 5	1:5	0.741	423±3.96	97.19±6.49
IMDL - 6	1:6	0.850	482±4.75	74.58±4.59

Table 6 Surfactant and Co-Surfactant Ratio Estimation

Formulation code	Poloxamer188 (%)	Sodium Taurocholate (%)	Zeta Potential (mV)	Avg. Particle size (nm)	PDI
IMSS - 1	0.5	0.5	-39	347	0.899
IMSS - 2	1	0.5	-40	329	0.789
IMSS - 3	0.5	1	-54	350	0.587
IMSS - 4	1.5	1	-39	380	0.874
IMSS - 5	1	1.5	-54	390	0.597
IMSS - 6	2	1.5	-44	410	0.482

Surfactant and Co-Surfactant Ratio Estimation

The concentration of Surfactant and Co surfactant is used for the stabilization. These may be required to stabilize the lipids [Table 6]. Formulation IMSS-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 took place in distilled water to

protect SLNs. Lyophilization was done with ultrasound for mean particle size analysis. The mean particle size of lyophilized formulations is ten times greater than those without cryoprotectants [Table 7].

Particle Size Analysis, Zeta Potential and PDI

Particle size, zeta potential, and PDI analyses of the prepared formulation were carried out. The particle sizes were within normal ranges, ranging from 209.6±4.57 to 502.1±8.9 nm. These particles were in an acceptable nanometer range. PDI ratio for the mass of the given samples was found to be below 0.51 for all SLN formulations. For pharmaceutical stability, zeta potential has +ve

Table 7 Cryoprotectant based on Mean Particle Size and PDI

S. No	Cryoprotectant	Mean Particle size (nm)	Particle size distribution (nm \pm SD)	Intensity (%)	PDI
1	Fructose	635.8	755.8 \pm 75.89	68.48	0.489
2	Maltose	540.15	670.6 \pm 66.36	98.56	0.647
3	Mannitol	370.5	460.9 \pm 75.65	110.5	0.374
4	Sorbitol	399.5	608.4 \pm 99.8	88.4	0.456
5	without Cryoprotectant	570.1	192.3 \pm 48.63	75.4	0.357

Table 8 Evaluation of Imiquimod-loaded SLN Formulations

Formulation code	Mean Particle Size (nm) \pm SD	Zeta Potential (mV) \pm SD	PDI \pm SD	% Encapsulation Efficiency \pm SD	Loading Capacity (%) \pm SD	% yield \pm SD
IMB 1	209.6 \pm 4.57	-35 \pm 2.0	0.17 \pm 0.01	71 \pm 0.75	39 \pm 2.2	79 \pm 2.58
IMB 2	321.4 \pm 7.48	-35 \pm 1.1	0.28 \pm 0.04	64 \pm 0.67	19 \pm 6.5	65 \pm 3.69
IMB 3	245.1 \pm 8.45	-41 \pm 1.2	0.39 \pm 0.03	64 \pm 0.79	48 \pm 2.6	73 \pm 1.47
IMB 4	421.6 \pm 7.85	-42 \pm 2.3	0.14 \pm 0.09	65 \pm 0.64	15 \pm 4.5	73 \pm 3.21
IMB 5	301.8 \pm 6.48	-44 \pm 2.5	0.51 \pm 0.02	60 \pm 0.36	51 \pm 3.0	68 \pm 3.65
IMB 6	502.1 \pm 8.9	-45 \pm 2.0	0.37 \pm 0.03	72 \pm 0.49	48 \pm 7.2	69 \pm 7.41

Table 9 In-vitro % CDR of Imiquimod-loaded SLN formulations

Formulation Code	% Cumulative Drug Release of Imiquimod SLNs					
	IMB-1	IMB-2	IMB-3	IMB-4	IMB-5	IMB-6
2	8.65 \pm 4.8	11.49 \pm 5.8	10.38 \pm 6.8	12.85 \pm 0.5	8.69 \pm 4.5	21.65 \pm 2.5
4	12.49 \pm 3.9	18.65 \pm 4.5	18.58 \pm 5.4	15.47 \pm 4.5	17.58 \pm 7.5	22.49 \pm 1.1
8	32.48 \pm 7.5	31.15 \pm 6.6	31.85 \pm 7.2	29.45 \pm 5.3	33.19 \pm 6.4	41.48 \pm 3.8
12	42.15 \pm 3.6	44.25 \pm 7.8	49.49 \pm 4.7	47.35 \pm 2.5	44.16 \pm 3.6	46.19 \pm 4.8
16	54.19 \pm 2.5	55.28 \pm 5.7	57.48 \pm 3.2	54.19 \pm 5.8	53.74 \pm 1.9	54.58 \pm 4.8
20	65.19 \pm 5.8	66.48 \pm 3.6	64.55 \pm 3.7	62.06 \pm 2.5	70.10 \pm 6.3	75.19 \pm 2.7
24	82.48 \pm 3.6	87.49 \pm 5.4	89.74 \pm 3.5	84.19 \pm 8.9	82.17 \pm 7.7	78.19 \pm 4.6

and -ve value. For good stability, zeta potential has -35 to -45mV. Formulation IMB 3 has close zeta potential (-41mV).

In-Vitro Release Studies

In vitro drug release of imiquimod-loaded SLNs evaluated at pH 6.9. This was done using the bag diffusion method. Studies show that there was no difference in drug solubility in the buffer. % Cumulative drug release for Imiquimod loaded SLNs formulations range 78.19 \pm 4.6 to 89.74 \pm 3.5 after 24 hours [**Table 8**, **Table 9** & **Figure 5**]. Due to possible degradation, the lower percentage of encapsulated drugs in SLNs.

The results for Mean particle size (245.1 \pm 8.45), Zeta Potential (-41 \pm 1.2), PDI (0.39 \pm 0.03), % EE (64 \pm 0.79), and Loading Capacity (48 \pm 2.6) are better for IMB 3, which contains 1% w/w Glycerol

Monostearate and 3% w/w Glyceryl Behenate as Lipid for SLNs preparation, out of all the formulations above. In a 24-hour *in vitro* drug release research conducted using the Bag Diffusion method, IMB 3 demonstrates the most significant cumulative drug release percentage of 89.74 \pm 3.5. Based on all the results and data, IMB 3 was chosen as the optimal formulation for additional research.

Scanning Electron Microscopy

Imiquimod-loaded SLNs (IMB 3) were identified using SEM. SLN will soon be identifiable as a smooth, glossy circle without cracks [**Figure 6**]. The image demonstrates the total elimination of the solvent from the produced SLN and verifies the effectiveness of the formulation process with particles as small as 200 nm.

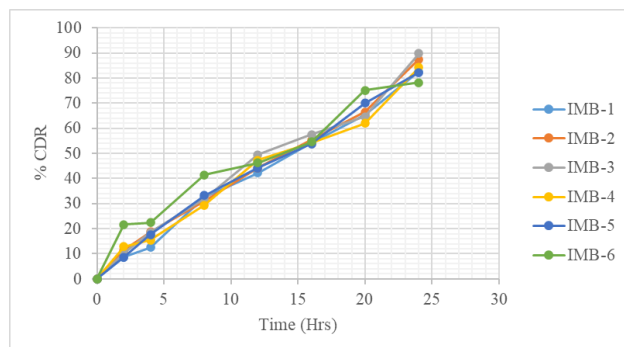


Figure 5 *In-vitro* % CDR of Imiquimod-loaded SLN formulations

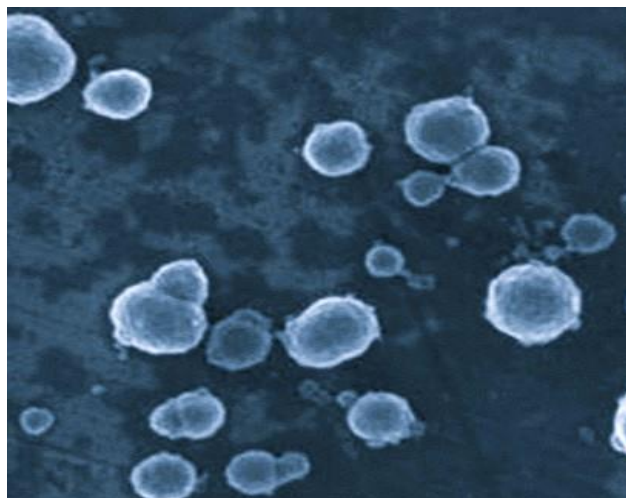


Figure 6 Scanning Electron Microscopy images of SLNs (IMB 3)

CONCLUSION

Different concentrations of lipids and surfactants were used to design SLNs. Mannitol showed much effectiveness as a cryoprotectant. Scanning Electron Microscopy (SEM) images revealed that the Nanoparticles in SLN droplets were intact, non-aggregated, and nearly spherical. The particle size range for SLNs loaded with Imiquimod is 209–502 nm. This could contribute to the SLNs' longer blood circulation time. The PDI was discovered to be less than 0.6. In SLN formulations, the negative charge of the zeta potential may demonstrate adequate stability. The encouraging results represent a novel contribution of Imiquimod SLNs in Topical Drug Delivery.

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